REMOVAL OF TETRACYCLINES IN WASTEWATER: ACCUMULATION AND DISTRIBUTION OF CHLORTETRACYCLINE IN BULK WATER AND BIOMASS COMPARTMENTS IN ACTIVATED SLUDGE

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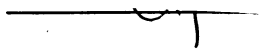

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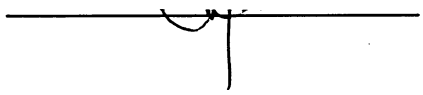

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DEDICATION

To all those who strive to make this world a better place, who have devoted their lives to a greater cause and aspire for justice, I dedicate this thesis.

To Ahmad and Tala for being part of my life.

ABSTRACT

Removal of Tetracyclines in Wastewater: Accumulation and Distribution of Chlortetracycline in Bulk Water and Biomass Compartments in Activated Sludge

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A study was conducted to examine the removal of chlortetracycline and its distribution and accumulation in three compartments; bulk water, extracellular polymeric substance (EPS) and the microbial cells in activated sludge. Also the effect of different environmental conditions on the distribution and accumulation in the three compartments was investigated. Effluent samples collected from a municipal activated sludge treatment system were set up in batch experiments to test the distribution and accumulation of chlortetracycline under aerobic and anoxic conditions for 14 days. In addition, the impact of the activity of the microbial community on the amassing of the antibiotic in the biomass was examined. The effect of divalent cations on import and accumulation of chlortetracycline was tested.

Sorption is believed to be the main removal pathway in wastewater treatment systems for tetracyclines in general and chlortetracycline in particular. In this study that notion was confirmed, and it was found that the removal via sorption under anoxic condition (43.2%) is almost double of that under aerobic conditions (27.0%). The amount of what accumulated in the cells compared to that sorbed in the EPS is twice as much in the former and triple as much in the latter. These findings suggest that changes in the structure and charge of the EPS could be the reason of higher accumulation in the polymeric substance.

The impact of microbial activity on the sorption and distribution of the chlortetracycline in the three compartments showed almost a similar behaviour to that under aerobic and anoxic conditions. It was clear that the more viable the microbial community, the less the antibiotic accumulated in the both biomass compartments; the EPS and microbial cells. Biomass with inhibited respiration accrued 90% of the initial concentration; where as the active microbial community was more resistant and only 24.2% of the initial concentration accumulated within the cells. The findings suggest that the antibiotic makes its way to the cells thus bypassing the EPS, and is trapped in the EPS as it is pumped out of the cells in an energy dependent mechanism.

The presence of ethylenediaminetetraacetic acid (EDTA) which is a strong chelator had no import effect. Nevertheless it did indicate that the accumulation in the EPS could be attributed to the presence of cations since there was a high negative correlation (-0.98) between the disappearance of the antibiotic from the EPS compartment and the EDTA concentration used in incubation.

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1.0 INTRODUCTION

In recent years, there has been growing concern about the occurrence of pharmaceuticals in the aquatic environment. Moreover, several studies have reported the occurrence of a great variety of pharmaceuticals in surface waters (Hirsch et al, 1999; Huber et al, 2003; Miao et al, 2004). This would affect water quality and can accelerate the evolution of antimicrobial-resistant bacteria. These concerns have led to the need for a more reliable removal method of antimicrobials from wastewater discharge in order to eliminate, if possible, their presence in water supplies.

Amongst pharmaceuticals, veterinary products have been the subject of growing attention because of their potential to pollute both aquatic and soil environments. Direct release of these pharmaceuticals to the environment occurs in agricultural practice with the discharge of excess feed pellets and animal wastes in aquaculture and by land application of manure containing un-metabolized drugs from animal husbandry. The presence of veterinary antibiotics and other pharmaceuticals in soils, sediments and aquatic environments is of concern because these bioactive compounds may promote the development and spread of antibiotic-resistance among bacterial populations or induce biological responses in non-target organisms. Accurate assessments of organism exposures to pharmaceuticals are hampered currently by a lack of applicable environmental fate models. The more we know about the sinks of these antibiotics and their partitioning to biomass within the biological wastewater treatment systems, the more we are able to develop accurate models for their environmental fate.

The information about the nature and physio-chemical properties concerning the behaviour of tetracyclines in general and chlortetracycline in specific is available, but must be gleaned from diverse fields. For example, publications on the technical approaches to remove heavy metals and organic pollutants from water streams by immobilized organisms in bioreactors contain a large body of data about biofilms as sorbents. Unfortunately, most of these studies do not distinguish between different

sorption sites in the biomass; usually assuming but not verifying that sorption occurs at cell walls. In addition, there is no indication of a concept of structured matrix which includes the diverse compartments with different sorption properties. The study of the reaction of the microbial community embedded in an extracellular polymeric substance to antibiotics in general and tetracyclines in particular takes a different angle depending on the field. In the medical field where biofilm interactions with antibiotics is widely studied, it is mainly concerned with the antibiotics penetration to the cells and the role the extracellular polymeric substance play in impeding its penetration to the microbial cells, and the degree of resistance to the drug. Whereas when addressing the interaction from an industrial perspective namely for improving the removal efficiency of biological wastewater treatment systems, hence maintaining a good water quality level, that very problematic issue from the medical standpoint becomes the quality to be emphasised on to achieve better removal. Because the more the bacteria are able to resist the inhibitory effect of these antibiotics, the better it is able to degrade it.

In this study the removal and behaviour of chlortetracycline, a veterinary antibiotic which is heavily used in North America (Meyer et al, 2000), were investigated in biological wastewater treatment systems. Since sorption is the main pathway for the removal of the antibiotic, not biodegradation, its accumulation in both the microbial cells and the extracellular polymeric substance (EPS) was quantified, and examine the effect of different environmental conditions over the accumulation distribution and removal of the antibiotic from the bulk water was examined. No study before has tried to look into the sorption compartments of the antibiotic in sludge. Sorption was usually studied from the kinetics perspective, and the bulk of the research was mainly either examining tetracyclines sorption to manure, soil or sediments.

To test the adsorption effect and to quantify the distribution of chlortetracycline in three main compartments; the bulk water, the EPS and the microbial cells, batch experiments containing activated sludge from a municipal wastewater treatment plant were set up in the lab. The removal of the antibiotic was tested under aerobic and anaerobic conditions. The effect of the microbial community activity and inhibition on the removal of

chlortetracycline was also examined. In addition, the role of divalent cations and their effect over import and the accumulation distribution in the EPS and the microbial cells were investigated.

2.0 LITERATURE REVIEW

2.1 Pharmaceuticals and Personal Care Products (PPCPs) as Emerging Pollutants

Freshwater is one of earth's most valuable supplies, yet it is subject to an increasing demand due to the continued exponential growth in the human population. Thus, protecting the integrity of water resources has become one of the most essential environmental issues of the millennium. Pharmaceuticals and personal care products (PPCPs) which include drugs used in human and veterinary medicine, as well as soaps, skin care products, insect repellents, sunscreens, and cosmetics that contain a great variety of other chemicals have been the subject of increasing concerns in past decades (Daughton and Jones-Lepp, 2001). Although PPCPs offer improvements in industry, agriculture, medical treatment, and even common household conveniences, their potential adverse human and ecological health effects resulting from the production, use, and disposal of these compounds are still under close investigation and study (Daughton and Ternes, 1999).

Certain PPCP chemicals may have harmful environmental effects even at very low levels. Furthermore, some may be persistent in the environment. Even among non-persistent chemicals, many are used continuously at such rates that their concentrations in the environment may increase rather than attenuate (McBride and Wyckoff, 2002). The fact that PPCPs can be introduced on a continual basis in the aquatic environment via treated and untreated sewage essentially imparts a quality of "persistence" to compounds that otherwise may not possess any inherent environmental stability simply because their removal/transformation through biodegradation, hydrolysis, and photolysis is continually countered by their replenishment, establishing a pseudo-steady-state in a manner analogous to a "bacterial chemostat" (Daughton and Jones-Lepp, 2001).

Therefore, since PPCPs can be designed to stimulate or inhibit physiological responses in humans, animals and plants, they can have unforeseen adverse effects on non-target

ecological species when released in the environment. Several drug products are also used to inhibit physiological actions such as steroids which are used to reduce inflammation. In addition, there are many other PPCPs that may not affect any physiological functions such as cosmetics, fragrances, and sun screens. Continuous releases and chronic exposure can result in subtle effects to aquatic species (Breton and Boxall, 2003).

Another concern is whether these substances present in water, albeit in low concentrations, pose a risk to human health after ingestion of contaminated drinking water over a lifetime. Household chemicals, pharmaceuticals, and other consumables as well as biogenic hormones are released to the environment mainly via surface and ground waters from human and animal use largely through sewage treatment works systems (STWs), failed septic fields, leaking underground sewage transporting systems, and runoffs; either directly by bathing/washing/swimming (via discharge of externally applied PPCPs, such as fragrances or sun-screen agents, or those excreted in sweat) or indirectly by excretion in the faeces or urine of un-metabolized parent compounds. Bioactive metabolites (including re-convertible conjugates) are also excreted (Halling-Sorensen et al, 1998; Daughton and Jones-Lepp, 2001). Veterinary pharmaceuticals used in animal feeding operations may be released to the environment with animal wastes through overflow or leakage from storage structures or land application (Meyer et al; 2000). As a result, there are a wide variety of transport pathways for many of the different chemicals to enter and persist in environmental waters (Fig 2.1). Disposal via municipal refuse serves as another route of introduction to the environment (e.g., via leaching to groundwater). Other routes to the environment include storm overflow events.

Many PPCPs are extremely bioactive compounds and are unwittingly introduced to the environment as complex mixtures as mentioned above, especially through both treated and raw sewage effluent. Yet drugs differ from other organic contaminants such as agrochemicals in that they often have multiple functional groups (including ionizable groups and more frequent and extensive fluorination) and often lower effective doses (sub mg/kg), thus complicating fate / transport modeling and lending an extra dimension to the analytical techniques required for monitoring.

A pharmaceutical's structure spans the spectrum from very simple low-molecular weight to large, complex molecules. In contrast to the conventional persistent, bioaccumulative, and toxic pollutants, most drugs are usually neither bioaccumulative nor volatile. However, personal care products, such as the musk fragrances and sun screen agents, tend to be more lipophilic (Daughton and Jones-Lepp, 2001).

Yet what is known about the extent of environmental prevalence, transport, and ultimate fate of many of these chemicals after their intended use is still limited, particularly hormonally active chemicals (Kolpin et al, 2002), personal care products, and pharmaceuticals that are designed to stimulate a physiological response in humans, plants, and animals (Daughton and Ternes, 1999; Jorgensen and Halling-Sorensen, 2000). One reason for this general lack of data is that, until recently, there have been few analytical methods capable of detecting these compounds at the low concentrations expected in the environment (Sedlak et al, 2000).

Potential impact of the environmental presence of these compounds include abnormal physiological processes and reproductive impairment (Harrison et al, 1997), increased incidences of cancer (Davis and Bradlow; 1995), the development of antibiotic-resistant bacteria (Gilliver, 1999), and the potential increased toxicity of chemical mixtures (Stumper and Jobling, 1995). For many of the PPCPs, the potential effects on humans and aquatic ecosystems have not been clearly understood until the present (Halling-Sorensen et al, 1998; Daughton and Ternes, 1999). Therefore, and in light of the above, the environmental effects of these chemicals may be long lasting and possibly enhanced by the cumulative effects that could result when PPCPs are combined in the environment (Daughton and Ternes, 1999). The environmental effects of most PPCP chemicals are unknown, thus their cumulative and combined effects will not be identified nor measured until sometime in the future.

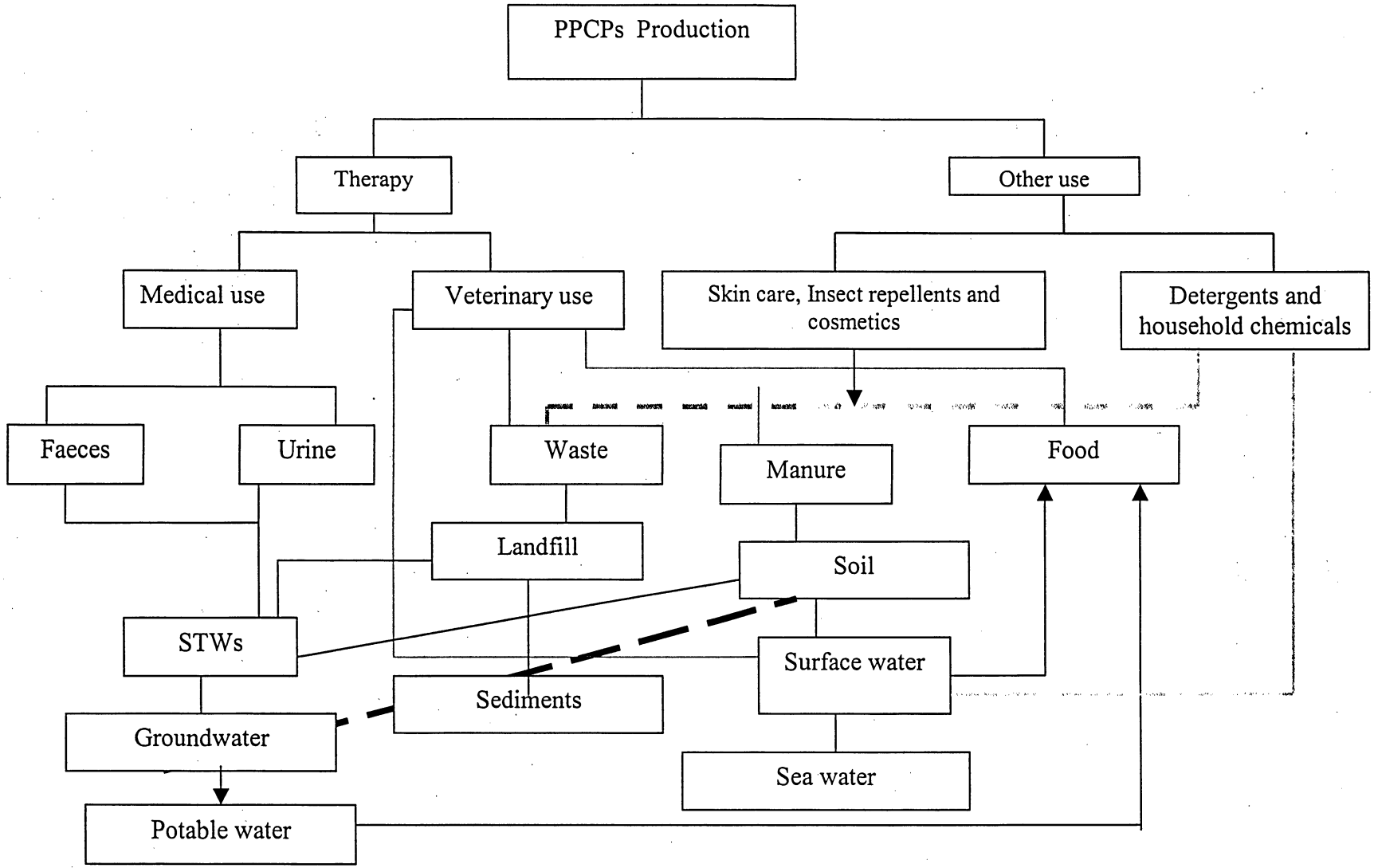


Fig 2.1: Sources, distribution, and sinks of PPCPs in the environment (adapted from Kümmerer; 2004)

2.2 Pharmaceuticals Pathways in the Environment

Many drugs used by humans make their way into streams, primarily by way of sewage. The types of human pharmaceuticals that have been identified in surface waters include birth-control hormones, antibiotics, blood lipid regulators, analgesics, non-steroidal anti-inflammatory drugs, beta blockers, antidepressants, anti-cancer drugs, tranquilizers, and X-ray contrast media (Daughton and Ternes, 1999). Yet the alarming findings are that limited studies indicate these drugs and other PPCPs are not effectively removed by sewage treatment plants (Daughton and Ternes, 1999).

On the other hand, veterinary pharmaceuticals (VPs) such as steroids, hormones, antibiotics, and other veterinary pharmaceuticals are physiologically highly active substances used in agriculture, in stock yards, and in feed lots with large herds of animals to combat parasites, and to prevent and treat a bacterially transmitted diseases, and accelerate meat production (Boxall et al., 2003; Kay et al, 2005). In the European Union (EU), antibiotics and anthelmintics (parasiticides) are the most important groups of VPs, both with a market value of more than 200 million Euros in 1999. Of the total usage of 5000 tons of antibiotics, 3500 tons have been used for therapeutic purposes while the remaining 1500 t is added to the feed in order to promote the growth of farm animals (Tolls, 2001). In the United States, farm animals are estimated to consume 70% (11200 tons) of all antibiotics administered (Wilson et al, 2004). Following administration, part of these compounds would end up in the environment as constituents of urine, feces, or manure. Because large quantities of these drugs may be excreted as the parent compound and/or metabolites and enter the environment due to the spreading of manure and slurry on agricultural land, or direct deposition by grazing livestock, or discharged into streams by runoff (Halling-Sørensen et al., 1998), any of these routes may result in impacts to surface water and groundwater (Fig 2.2).

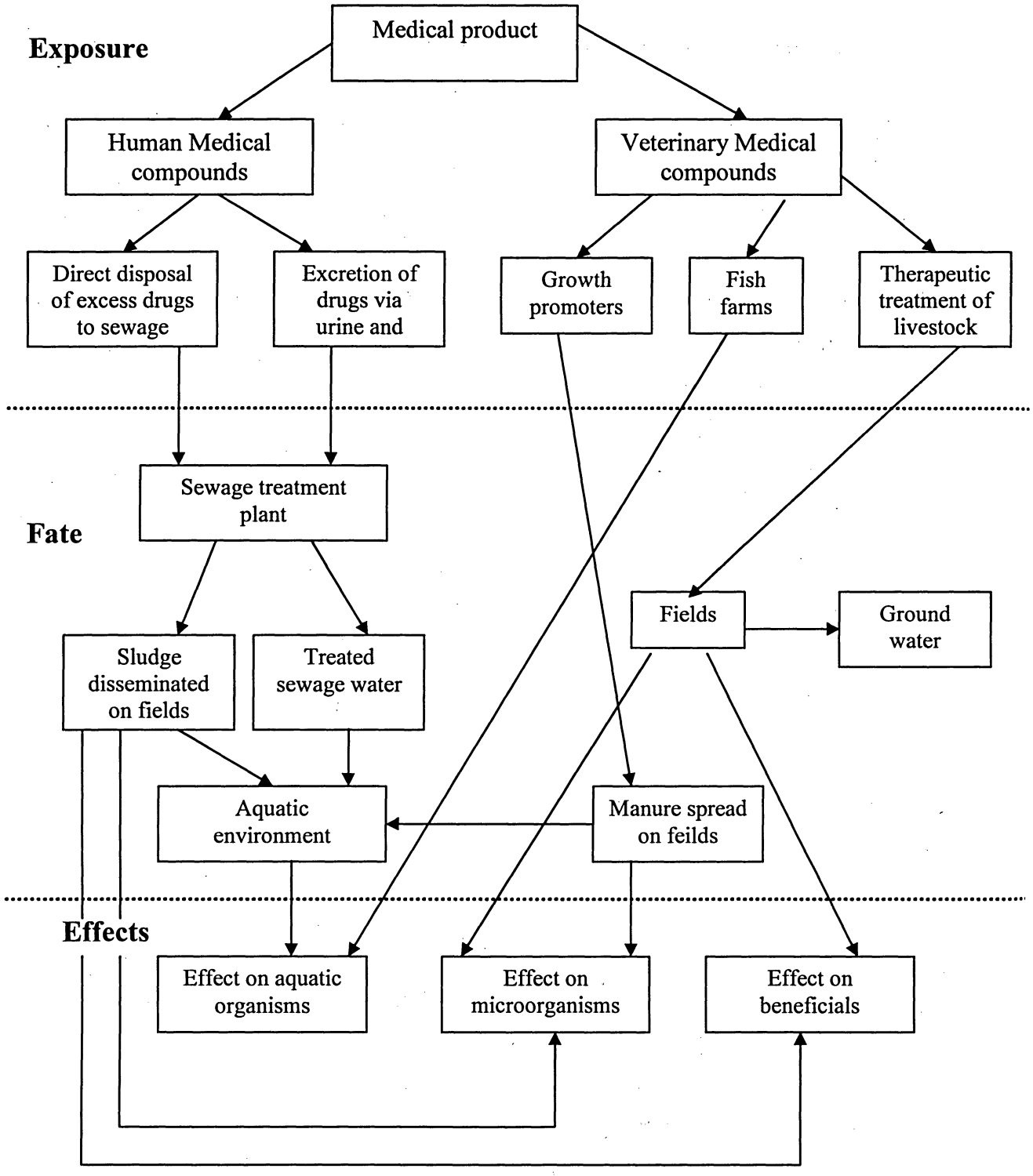


Fig 2.2: Anticipated exposure routes of both veterinary and human medicinal substances in the environment. (Adapted from Halling-Sörensen et al, 1998)

2.3 Fate of Pharmaceuticals

Human Pharmaceuticals enter the sanitary sewer from two sources: (1) excretion of partially-metabolized pharmaceuticals by the human body and (2) disposal of unused or expired medications in the sewer. The two largest sources of pharmaceuticals entering the sewer systems are hospitals and residential (Hirsch et al, 1998; Kanda et al, 2003; Miao et al, 2004). It is believed that the frequent use of over-the-counter medications, in addition to the direct discharge of outdated medication to household drains, contribute tremendously to the continual release of pharmaceuticals in the environment which serves to sustain exposures to aquatic species (Boyd et al, 2003; Carballa et al, 2004). It is reported that approximately 60% of prescription drugs in North America is flushed down the drain; a 2002 survey in Canada showed that 19% of individuals disposed of non-prescription drugs and 20% disposed of prescription drugs to the sewer, whereas 50% of individuals disposed of non-prescription and 39% disposed prescription drugs to the garbage (Duaghton, 2003). Whereas in Europe one third of the total volume of pharmaceuticals sold in Germany and about 25% of that sold in Austria is disposed of with household wastes or down the drain (Kummerer, 2004).

After administration, pharmaceuticals and their metabolites are excreted from the body in urine and feces. For human pharmaceuticals, the excreted substances generally enter a sewage treatment plant (STP) where treated effluents are released to surface water. However, there are several reasons, such as the low concentrations of individual pharmaceuticals (possibly below the catabolic enzyme affinities of sewage microbiota), coupled with metabolic "novelty" to microorganisms (possibly an issue with newly introduced drugs), that could lead to incomplete removal from STPs (Daughton. and Jones-Lepp, 2001; Sacher et al, 2001; Breton and Boxall, 2003). On the other hand, lipophilic pharmaceuticals could partition to solids (sludge) (Halling-Sorensen et al, 1998; Kim, et al 2005). Therefore, removal efficiencies from STPs can span the entire spectrum from the complete to the ineffective. In general STPs are designed to remove conventional pollutants such as suspended solids and biodegradable organic materials, but they are not designed to remove low concentrations of synthetic pollutants such as pharmaceuticals from influents (Al- Ahmad et al, 1999; Daughton and Ternes, 1999;

Sedlak et al., 2000). The reported removal efficiency of STPs is approximately 60% (Ternes,1998; Daughton and Ternes,1999). And because many synthetic compounds are designed to be resistant to biological degradation, the removal efficiencies for pharmaceuticals appear to be chemical-specific (Kümmerer et al, 2004). Consequently, pharmaceuticals are inevitably released in the environment. In addition sewage is in some cases discharged directly to streams without treatment. For example, in Canada, it is estimated that 3.25 billion litres per day of untreated sewage are discharged in surface waters and the ocean (Bonner and Wristen; 1999).

Conversely, once administered to an animal, veterinary pharmaceuticals and their metabolites will also be excreted and released, sometimes directly to land or stored and applied to land at a later stage. Disposal of sewage sludge, farmyard manure and/or slurry to land, in landfills and manure pits, is a potential source of these pharmaceuticals in the environment (Boxall et al., 2002). The main pathways would be surface water by drain flow and/or runoff or to groundwater by leachate percolating through soil (Kay et al, 2005).

2.4 Degradation Pathways

Once pharmaceuticals are released to the environment after excretion from humans or dosed animals or via any of the above mentioned pathways, they are subject to various processes such as sorption, abiotic transformation and biotic transformation in natural and engineered aquatic environments. These processes directly influence the fate and transport of these compounds in the environment as well as their biological activities. At present, literature regarding the fate and transport of pharmaceuticals in the aquatic environment is very limited, although more studies are being reported recently due to the increasing concerns about these compounds. In this review (as drawn from the literature) a particular focus is the antibiotic group tetracyclines, since they are important in both human medicine and animal husbandry; with the latter use being the focus of this study.
Generally speaking the degradation of most xenobiotics is faster and more complete under aerobic as compared to anaerobic conditions. Correspondingly, degradation of

oxytetracycline (for example) when compared to other antibiotics such as tylosin in activated sludge, soil or surface water was observed to be similar or slightly lower under anaerobic as compared to aerobic conditions (Ingerlev et al, 2001; Thiele-Bruhn, 2003). The Following are the different degradation pathways for pharmaceuticals.

2.4.1 Sorption

Adsorption of pharmaceuticals to activated sludge, anaerobic biomass, and consequently to soils is crucial in determining the possibility of detecting these compounds in the effluents, since almost all STPs are based on the activated sludge process, while the excess sludge is most commonly stabilised anaerobically. In due course it is important to decide the ultimate fate of these organic chemicals (Drillia et al, 2005). The extent of adsorption is related to various soil properties, including organic matter content, type and amount of clay content, ion exchange capacity and pH (Tolls; 2001). Moreover, various physicochemical parameters of the compounds in question, such as water solubility and the octanol–water partition coefficient, also play an important role in determining the extent of adsorption (Singh et al, 1990; Barriuso et al, 1992).

Previous studies indicate that strong adsorption of tetracyclines (tetracycline, chlortetracycline and oxytetracycline) to clay materials (Kulshretha et al, 2004), soil and sediments (Pouliquen and Lebris, 1996; Rabolle and Spliid, 2000) occurs over a wide range of environmental conditions. Although not as strong as tetracyclines, significant sorption of some macrolides such as tylosin and avermectin (Rabolle and Spliid, 2000) and fluoroquinolone (Nowara et al, 1997) antibiotics to soil clay minerals has been reported. Sulfonamides exhibit weak adsorption to soil (Thiele, 2000) and activated sludge (Ingerslev and Halling-Sorensen, 2000). Conversely, there are few studies on the sorption of aminoglycoside and β-lactam antibiotics. Aminoglycosides are comprised by two or more sugars or amino sugars attached to an aminocyclitol ring, resulting in a high polarity of the compounds. The amino groups can be positively charged by protonation under acidic conditions. The positive charge may facilitate adsorption to soil clay minerals that typically posses negative charge. β-Lactam antibiotics are highly polar

compounds. Sorption of β-lactams to soil is expected to be weak due to their high polarity and carboxylic acid functional groups (Huang et al, 2001; Thiele-Bruhn, 2003).

2.4.2 Abiotic Transformation

Abiotic transformations in surface waters may occur via hydrolysis and photolysis. Pharmaceuticals, usually designed for oral intake, are as a rule resistant to hydrolysis, which suggests the mechanism of direct and indirect photolysis is the primary pathway for their abiotic transformation in surface waters (Andreozzi et al, 2003). Among the mainly used antibiotics, β-lactams, macrolides and sulfonamides are susceptible to hydrolysis. However, hydrolysis of macrolides and sulfonamides in neutral pH range is very slow and can be considered negligible (Volmer and Hui, 1998). Among the above mentioned antibiotics, β-lactams generally undergo hydrolysis fairly quickly under mild acidic and base conditions (Waley, 1975). Another abiotic transformation is the photo-degradation that can have an impact on the persistence of organic pollutants in the surface layers of water bodies that receive considerable amounts of sunlight. Pharmaceuticals such as quinolones and tetracyclines are susceptible to photo-degradation (Torniainen et al, 1996). In view of the fact that light will not penetrate soil and sludge, photo-degradation is not important in these matrices. Instead, biodegradation and abiotic degradation, such as sorption and hydrolyses should be considered, if such compounds are present. In the aqueous environment all of the processes should be considered more or less effective depending on the water quality. In the marine aquatic environment, pharmaceuticals will only degrade very slowly (Oka et al, 1989).

2.4.3 Biotic Transformation

The persistence of antibiotics in the terrestrial and aquatic environment ranges from less than one day to weeks or even months depending primarily on the temperature and the chemical structure of the antibiotic. According to the rate of degradation and the sorptive properties, the parent substance or its metabolites present in the soil may reach the aquatic environment through surface run-off or leaching through the soil profile (Rabolle and Spliid, 2000).

The stability of a number of agricultural antibiotics in soil was studied by Gavalchin and Katz (1994). It was observed that the persistence of antibiotics increases in the following sequence: chlortetracycline > bacitracin > erythromycin > bambermycin > tylosin > penicillin and streptomycin. The loss of antibiotic was attributed to biodegradation although other reactions are also possible. Tetracyclines (e.g. oxytetracycline), and other antibiotics, were found to be persistent in marine aquaculture sediment (Simon; 2005). In liquid manure, considerable degradation of tetracycline was reported (Kuhne et al., 2000). In manure-containing systems, rapid loss of tylosin from the aqueous phase was observed under both aerobic and anoxic conditions (Loke et al, 2000). The loss of tylosin was caused by a combination of sorption, abiotic transformation and biodegradation (Rabolle and Saliip, 2000). Low biodegradability of β-lactam, fluoroquinolone and sulfonamide antibiotics was reported by Al-Almad et al. (1999). Partial biodegradation was observed with the β-lactam antibiotics and no biodegradation was observed with ciprofloxacin and sulfamethoxazole (Al-Almad et al, 1999). The biodegradation of several sulfonamides was examined in activated sludge (Ingerslev and Halling-Sorensen, 2000). Significant biodegradation occurred only after a considerable lag time and the use of unusually high sulfonamide concentrations to stimulate particular degraders. Thus it is suggested that biodegradation of sulfonamide in sewage treatment systems may be negligible. Based on the above mentioned, the degradability of antibiotics discussed follows the trend, Aminoglycosides - β-lactams - and some macrolides > quinolones, sulfonamides and tetracyclines.

To understand the full picture, it is important to look at both the parent compounds and their degradation products. For example, in groundwater, pesticides are known to transform into compounds that are much more persistent and mobile than the parent compounds (Kolpin et al, 2002). In some cases, these metabolites are less toxic, but in others, they are just as toxic as or more toxic than the parent compound. In general, human antibiotics are metabolized to more polar compounds, and the more polar metabolites pass through water treatment plants with high probability (Ternes, 1998).

2.5 Antibiotics in Biological Wastewater Treatment Systems

Due to the low capacity of STPs to efficiently remove and degrade some human and veterinary antibiotics, they have become a main focal point in numerous studies (Baronti et al, 2000; Halling-Sørensen, 2003; Loke et al, 2003; Carballa, 2004; Kulshretha et al, 2004). A significant research effort has been carried out to study the fate of antibiotics in these systems (Ternes, 1998; Bernard and Gray, 2000; Ingerslev et al, 2000; Sedlak et al 2000; Halling-Sørensen, 2001; Ingerslev et al, 2001; Andreozzi et al, 2003).The main results show that the removal efficiency depends on the antibiotics, which can be adsorbed on cell surfaces or absorbed if hydrophobic, and their susceptibility to aerobic and anaerobic degradation (Ingerslev et al, 2000; Halling-Sørensen, 2001). In addition, biodegradation of the compounds will depend on the characteristics and the operating conditions of the treatment plant (Kim et al 2005).

2.6 Tetracyclines

Tetracyclines (TCs) constitute a family of antibiotics which are produced by *Streptomyces* and exhibit broad-spectrum antimicrobial activity against a variety of disease-producing bacteria ranging from gram-positive to negative bacteria, and are especially effective against *Staphylococcus* , *Streptococcus* , *Pneumococcus* , *Gonococcus* , *Cholera Dysentery bacillis*, *Pertussis* , *Rickettsia* , *Chlamydia* and

Mycoplasma (Fig. 2.3). TCs are actively transported into the cells of susceptible bacteria and exert a bacterio-static effect by inhibiting protein biosynthesis after binding to the 30S ribosomal subparticle (Schnappinger and Hillen, 1996; Chopra and Roberts, 2001). Since the first member of the tetracycline family, chlortetracycline (CTC) was discovered in 1948 (Chopra and Roberts; 2001), eight TCs are now commercially available, of which oxytetracycline (OTC), tetracycline (TC), CTC, and doxycycline (DC) are commonly used as veterinary medicines. They are applied to food-producing animals including honeybees because of the broad-spectrum characteristic of the antibiotics and their economic advantages in avoiding wide-spread infections (Kazemifard, 1997; Oka et al, 2000), they are also used as growth promoters and prophylactics in swine and cattle production (Boxall et al, 2003; Kay et al, 2005). Currently Chlortetracycline and oxytetracycline are 2 of only 10 antibiotic compounds licensed for use as growth promoters for livestock in the United States (Beaudry and del Castillo, 2005). Whereas in 1991, about 12.2% of marketed pigs in Ontario, Canada, had been exposed to tetracyclines in their food, and 20% had been injected with tetracyclines during the fattening period (Dunlop et al, 1998).

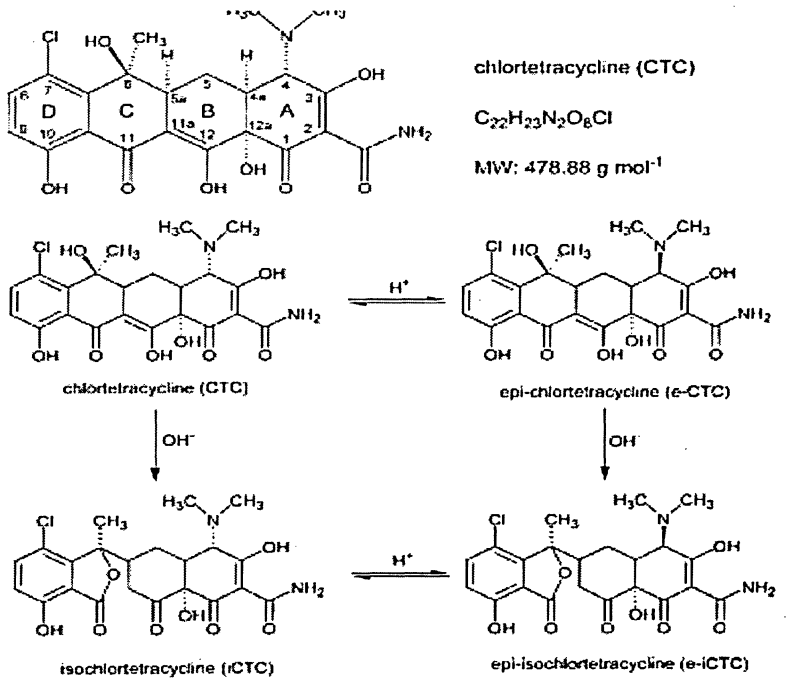


Fig.2.3: Chemical structure of chlortetracycline and its pH dependent epimerization and isomerization reactions (Eichhorn and Aga, 2004).

TCs have similar chemical and physicochemical properties. They are amphoteric compounds with characteristic pH values and form crystalline hydrates and salts with acids and bases (Pena et al, 1998). Their UV spectra show strong absorption around 270 and 360 nm in neutral and acidic solutions (Oka et al, 2000). TCs are soluble in acids, bases, alcohols, and polar organic solvents and are extractable with several organic solvents such as *n*-butanol and ethyl acetate. Stability of the TCs is poor under strong acidic and alkaline conditions and form reversible epimers, 4-*epi*-TCs, anhydro-TCs, and iso-TCs under weakly acidic (pH 3) and strongly acidic (below pH 2), alkaline conditions, respectively (Kazemifard, 1997; Pena et al, 1998). TCs produce strong fluorescence with metal ions or under basic conditions (Kohn, 1961; Piger and Schlatter, 1976). They form chelate complexes with metal ions at ß-diketones (C_{10} –C_{12}) and carboxyamide (C_2) (Lee and Evrett, 1981; Oka et al, 2000) and bind with proteins and silanol groups in the stationary phase (Fedeniuk and Shand, 1998; Oka et al, 2000).

Due to the antibacterial activity of tetracyclines, biodegradation brought about by bacteria can be anticipated to play a negligible role in the elimination of the antibiotic. To date, no proof of microbial degradability of tetracyclines in the environment has been reported (Alexy et al, 2004). Chemical transformation processes, in turn, such as isomerization and epimerization have been reported, giving rise to structurally related compounds likewise exhibiting resistance to breakdown. For instance, chlortetracycline (CTC) is converted to *iso*-chlortetracycline (iCTC) under alkaline conditions (see Figure 2.3), while the epimerization has been found to be catalyzed in acidic solutions in a pH range from 2 to 6 (Eichhorn and Aga, 2004; Søeborg et al, 2004; Diana et al, 2005).

2.6.1 Degradation of Tetracyclines

Tetracyclines are relatively persistent antibiotics; several studies showed that they are not readily biodegradable (Ingerslev et al, 2001; Alexy et al, 2004), that they tend to be persistant in manure (De Liguro et al, 2003) and have been observed to accumulate in soils after application of manure (Hamscher et al, 2002) where they were detected in concentrations of up to 0.2 µg per kg in soil (Kümmerer, 2003) and in sediments in

aquaculture operations (Capone et al 1996). Chlortetracycline (CTC), oxytetracycline (OTC), and tetracycline (TC) have been measured in surface waters in the United States at maximum concentrations of 0.69, 0.34, and 0.11 μg/L, respectively (Kolpin et al, 2002). Yet there have been some studies that show TCs may degrade in manure; Kuhne et al (2000) reported that there was a remarkable decrease of tetracycline concentrations during the examination period and that the decrease appeared to be exponential. The same study reported that the faster degradation of tetracycline in liquid manure compared with Ringer's solution was thought to be due to alkaline pH values. During manure storage, pH values increased significantly from 7.6 to 8.7, whereas Ringer's solution had pH-values of 6.2 to 6.4 during the experiment. They concluded that degradation is both pH and temperature dependant. The findings by Kuhne et al (2000) which contradict what is widely reported in the literature pertaining to TCs degradation, could possibly be due to the fact that the study did not investigate the inactivation of tetracyclines by their binding to macromolecules or chelates.

2.6.2 Resistance to Tetracyclines

Antimicrobial resistance is a growing public health concern and has been a subject of debate for decades. Given that TCs tend to be persistent, the main concern about their continual introduction to the environment and their use in animal diets is that their use may lead to the emergence of drug resistance among pathogenic microbes due to prolonged exposure to low levels of tetracyclines (Witt et al, 1999; Boxall et al, 2003). In view of the fact that the selection pressure of antibiotics present above a certain concentration against the microbial biocoenosis is an important factor in the selection and spread of resistant bacteria (Levy, 1998), the transfer of resistance genes as well as the already resistant bacteria themselves is favoured particularly by the presence of antibiotics over a long period and at sub-therapeutic concentrations. This has been confirmed by several authors who have studied the transfer of genes between bacteria in sediment, soil, water, and wastewater (Mach and Grimes, 1982; Top et al, 1994). Hence, if antibiotics are improperly used in veterinary and human medicine (Teuber, 1999; Salyers, 2002) or as growth promoters their prevalence could increase the speed with

which resistant bacterial strains are selected (Khachatourians, 1998; Aarestrup et al, 2001).

Acquired resistance can be transferred to other bacteria living in other environments such as ground water or drinking water. Thus far several studies indicate that Tetracycline-resistant bacteria have been identified in waste water (Guardabassi et al, 2000; Guillaume, 2000; Schwartz, 2003). It has also been shown that tetracycline-resistant bacteria in swine outflow can pass this resistance on to bacteria commonly found in soil (Haack and Andrews, 2000). The development of TC resistance in soil bacteria exposed to TC suggests that indigenous soil microorganisms may serve as reservoirs for the propagation and possibly the amplification of antibiotic resistance, and potentially pose a direct hazard to public health. Finally, studies have documented the transport of tetracycline-resistant genes in groundwater under swine production facilities (Chee-Sanford et al, 2001).

There are three different specific mechanisms of tetracycline resistance that have been identified so far: tetracycline efflux, ribosome protection and tetracycline modification (Schnappinger and Hillen, 1996; Chopra and Roberts, 2001; Thiele-Bruhn, 2003) (see Fig. 2.4). Tetracycline efflux is achieved by an export protein from the major facilitator superfamily (MFS). The export protein functions as an electro-neutral antiport system which catalyzes the exchange of tetracycline-divalent-metal-cation complex for a proton (Schnappinger and Hillen, 1996). In Gram-negative bacteria the export protein contains 12 TMS (transmembrane fragments) whereas in Gram-positive bacteria it displays 14 TMS. Ribosome protection is mediated by a soluble protein which shares homolgy with the GTPases participating in protein synthesis, namely EF-Tu and EF-G (Chopra and Roberts, 2001). The third mechanism involves a cytoplasmic protein that chemically modifies tetracycline. This enzymatic reaction takes only place in the presence of oxygen and NADPH and does not function in the natural host (Bacteroides) (McMurry et al, 1980; Chopra and Roberts, 2001).

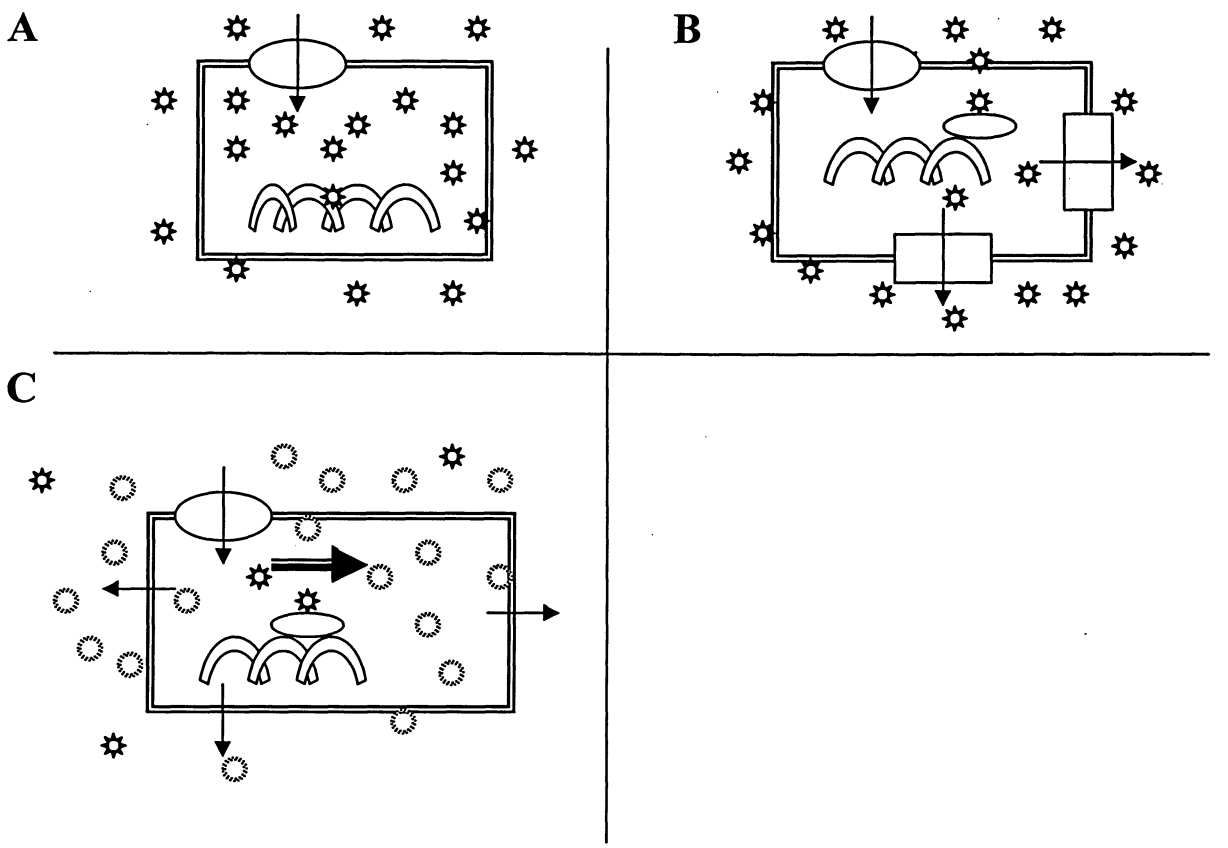


Fig. 2.4: Different mechanisms of tetracycline resistance.

(A) Susceptible bacteria accumulate tetracycline (☼) to an internal concentration high enough to allow tetracycline to bind to ribosomes and stop protein synthesis.
(B) Bacteria carrying an efflux type of resistance gene produce a cytoplasmic membrane protein (rectangular box), which pumps tetracycline out of the cell. This keeps the intracellular level low to allow protein synthesis to proceed.
(C) Bacteria carrying a tetracycline modification resistance gene produces an enzyme that chemically modifies tetracycline (☼) to an inactive form (○), which diffuses freely out of the cell. (adapted from Speer et al, 1992).

The two first mechanisms are the most widespread and most of their genes are normally acquired via transferable plasmids and/or transposons. These two mechanisms were observed both in aerobic and anaerobic gram-negative or gram-positive bacteria demonstrating their wide distribution within the bacterial kingdom. To date, about sixty-one tetracycline resistance genes have been sequenced and thirty-two classes of genes identified in non-producers and producers (*Streptomyces*) (Chopra and Roberts, 2001). Each new class is identified by its inability to hybridize with any of the known tet genes under stringent conditions (Levy et al, 1989).

2.6.3 Accumulation of Tetracyclines in the Environment

Besides causing development of antibacterial resistance, the continuous discharge of generally undegraded antibiotics in the environment will result in their accumulation and their concentration will increase in the future. A recent study showed that as high as 4 μg/L tetracycline and 1.2 μg/L chlortetracycline have been detected in municipal wastewater (Karthikeyan and Bleam, 2003). Further more, a reconnaissance study by the United States Geological Survey (USGS) reported detectable levels of tetracyclines in several rivers and streams in many parts of the U.S. (Koplin et al, 2004). Given that the antimicrobials induce toxic effects in aquatic plants and microorganisms at microgram per litre concentrations, the data presented indicate that the concentrations detected are within the toxic range (Kümmerer et al, 2000; Miao et al, 2004).

When evaluating the effect of continuous accretion of antibiotics on microbial communities, it is important to keep in mind that target organisms vary between antibiotics. For instance, antibacterial agents or their residues may reduce or alter the capacity of activated sludge in the STP to degrade other organic xenobiotics or to nitrify ammonia due to their bacterial potency. Gram-negative bacteria, e.g., *Nitrosomonas* sp., run the nitrification process. Only Gram-negative affecting antibacterial agents, such as tetracyclines are, therefore, expected to inhibit the nitrification. This may result in a shift in the microbial sludge population enabling unaffected species to create a dominant species and potentially alter the activity of the sludge, *i.e.*, reduce the nitrification capacity (Halling-Sørensen, 2001). On the other hand, if the activated sludge is used as soil conditioner, antibacterial agents and residues sorbed to the sludge may also affect the soil bacterial community. These indigenous communities of bacterial and fungal populations are very complex and they have the important task of cycling nutrients. Some processes are driven by just a few species, where others, such as the decomposition of organic matter, are driven by teamwork between many types of micro-organisms. Proper cycling of nutrients is critical for quality soils and essential for maintaining sustainable use of agricultural lands. Consequently, when sludge containing tetracyclines is applied to soil that could have an effect on microbial community structure, not only through its

direct bacteriostatic effect, but also indirectly by influencing microbial interactions among different populations (Rysz and Alvarez, 2004).

2.6.4 Adsorption as a Main Pathway for Removal

Sorption of pharmaceuticals depends on the extent of neutral and ionic species present and the characteristics of the target particles. Sorption may have an impact on the spread and (bio)availability of pharmaceuticals in the environment as particle bound transport, and their removal during wastewater treatment. Thus, when considering the problem of accumulation of antibiotics in the environment, one has to shed light on the sorption of these chemicals and the role it plays in their fixation and immobilization. Tetracyclines are known to be highly sorbed to clay materials, soil, and sediments (Sithole and Guy, 1987a; Pouliquen and LeBris, 1996; Rabolle and Spliid, 2000). In fact the K_d soil-water of tetracycline is 1140-1160 L/kg (Sithole and Guy, 1987b).

Sithole and Guy (1987a,b) proposed three major sorption mechanisms for TC: complexation by divalent cations, ion exchange, and hydrogen bridging from acidic groups of humic acids to polar groups of the TCs. Adsorption of TCs to various exchange sites is characterized by two processes of different kinetics that can be interpreted as a fast initial adsorption to outer surfaces, followed by a penetration into inter-layers of clay minerals and micro-pores (Sithole and Guy, 1987b; Thiele-Bruhn, 2003). Yet the occurrence of tetracyclines in surface waters suggests that their sorption to solids is not irreversible and that there are conditions that could favour their mobility in the environment (Kim et al, 2005).

The sorption isotherm of tetracycline in soil displays Langmuir-type shape, indicating that sorption occurs at a limited number of sorption sites on the surface of clay minerals (Sithole and Guy, 1987a). Sithole and Guy (1987a) varied the surface accessible to tetracycline by performing sorption experiments with bentonite exchanged with trimethyl-dodecyl-ammonium (C_{12}-TMA) ions and with bentonite coated with tannic acid. In the former case, the clay coagulated, resulting in a significantly reduced surface

area and concurrently a low value of $C_{s,max}$. In addition, they found that the $C_{s,max}$ for sorption of tetracycline to the clay mineral bentonite is 2.5 times higher when the cation exchange sites are occupied by Ca^{2+} instead of Na^{+} (at pH 6.1) (Sithole and Guy, 1987a). Moreover, the sorption coefficients for oxytetracycline toward sand were much lower than those found for sediments that contain a significant portion of silt and clay (Pouliquen and LeBris, 1996). Hence, sorption of tetracycline appears to be strongly related to the particle size of the solids, which in turn is related to the specific surface. In addition to adsorption, it is assumed that diffusion into porous soil particles also contributes to the fixation. Apparently the hydrophobic interactions are not effective in counteracting the effect of the reduced surface area and thus do not play a major role in tetracycline sorption. X-ray diffraction analysis of clay minerals showed that sorption of tetracycline widened the clay interlayer spacing, indicating that the interlayer of expanding clays is also involved in sorption (Nowara et al, 1997).

Another factor affecting the sorption and fixation of TCs is the pH of the medium. Infra red (IR) spectra of tetracycline sorbed to montmorillonite at various pH values suggest interaction of tetracycline with Ca^{2+} at the clay surfaces to be the prevalent sorption mechanism at an intermediate pH (6.1). Therefore, the high sorption coefficients of the Tetracyclines at typical soil pH values appear to be primarily due to interactions of anionic TC species at the clay surfaces, either the basal planes or the interlayer spaces exposed in expandable clays (Tolls, 2001).

2.6.5 Influence of Divalent Cations

Solids and biomass are the main sinks of TCs in the different environmental compartments since TCs tend to sorb strongly to solid matter. Cationic interactions play a major role in the sorption mechanism of TCs. Generally, cation exchange is thermodynamically more favourable than hydrophobic partitioning-type processes (Horvath et al, 1976); therefore, cation exchange may dominate even when only a small fraction of the aqueous-phase species exists as a cation (Fabrega et al, 1998). Cation exchange has been shown to dominate for other organic bases well above solution pH =

pK_a + 2 (Zachara et. al, 1986). In addition, the greater sorption by the lower pH soils where TCs exist as a cation suggests cation exchange as a significant sorption mechanism.

The tetracycline species under environmental pH conditions (4 to 8) are Zwitterionic in character with increasing negative charge above pH 6 (Sithole and Guy; 1987a).The four forms of tetracycline that are possible by increasing the pH are:

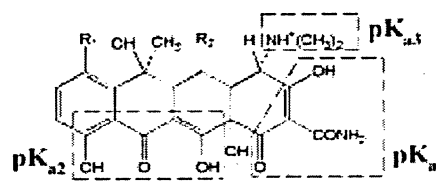
- (00 +) all basic groups are protonated and species is positively charged. This form predominates below pH 3.3;
- (- 0 +) the species that results from the loss of the proton from the tricarbonyl methane system;
- (0 - +) the species that results from the loss of the proton from the phenolic diketone moeity; and
- (000) the species that would result from the proton loss from the dimethylammonium cation.

In environmental pH regime (pH 4 to 8), the Zwitterionic species (- 0 +) predominates and reaches a maximum concentration at pH 5.5 (Sithole and Guy, 1987a). Also, although the net charge of a Zwitterion is neutral (+ - 0) and the (+ - -) species has a net negative charge, the negative and positive charges are spatially separated and may act independently similar to the reactivity of soil cation and anion exchange sites (Hyun et al, 2003). Therefore, cation exchange may have a significant contribution to the overall sorption of TCs at pH values well above 5.5 (Sassman et al, 2005).

The chemistry of tetracycline antibiotics suggests that cation exchange with soil and sediment clay components is an important sorption mechanism for these antibiotics. As shown in Figure (2.5), tetracycline antibiotics are positively charged in strongly acidic solutions and anionic under alkaline conditions. Thus, cationic tetracycline species could neutralize negative charge sites under acidic conditions (high sorption) but would be repulsed from clay surfaces at a high pH. In general, oxytetracycline sorption capacities of illite and bentonite were decreased when solution pH was increased from strongly acidic to weakly alkaline conditions (Figueroa et al, 2004). Sithole and Guy (1987b) found that the adsorption starts to decrease over the pH range of 4 to 7 (as the medium gets less acidic) and remains constant over the range 7 to 8. This suggests that the binding

is to acidic sites on the organic material. In the same study, it was reported that the adsorption of tetracycline onto the humic acid and peat particulates required about 18 h to attain equilibrium which in turn indicates that the binding of tetracycline to the organic particulates may involve the slow diffusion of the tetracycline in the peat and humic acid matrix (Sithole and Guy, 1987b).

At alkaline pH values (pH>7) where the hydroxyl groups (pK_{a2}) become increasingly more negative and the C-4 nitrogen (pK_{a3}) begins to deprotonate, TCs can complex with metals as has been observed spectroscopically for several metals including 1:1 and 2:1 metal-TC complexes with Ca and Mg, respectively (Lambs et al, 1988; Wessels, et al, 1998; Tongaree et al, 1999).



Antibiotic	R1	R2	pKa1	pKa2	pKa3
OTC	H	OH	3.57	7.49	9.88
TET	H	H	3.3	7.7	9.7
CTC	Cl	H	3.6	7.52	9.88

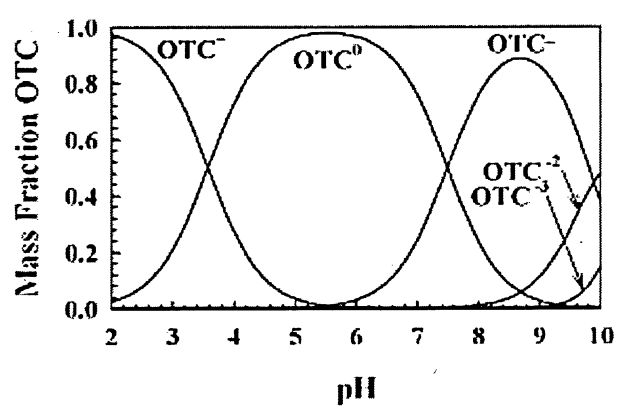


Fig. 2.5: Tetracycline chemistry and solution speciation.
All Tetracyclines have the common base structure which is shown with environmentally relevant proton exchange sites. R_1 and R_2 groups are reported for oxytetracycline (OTC), tetracycline (TET), and chlortetracycline (CTC). The speciation diagram was calculated for oxytetracycline but is similar for TET and CTC due to the closeness of pKa values all of these compounds. (Figueroa et al, 2004)

2.7 Tetracycline Transport at the Bacterial Scale

2.7.1 Uptake of Tetracyclines

Tetracycline is a broad-spectrum antibiotic which inhibits protein synthesis (Chopra and Roberts, 2001). Since the targets of tetracycline are intracellular ribosomes, tetracycline must cross the cell membrane of bacteria to gain access to the target. Bacteria susceptible to tetracycline exhibit a mono-phasic, rapid uptake of the antibiotic (Argast and Beck, 1985) which is partially energy-dependent (McMurry et al, 1980). The passive accumulation of tetracycline can be explained by binding to cell components such as phospholipids or proteins (Argast and Beck, 1984). Nikaido and Thanassi (1993) presented a model that details the uptake of TCs as shown in Fig. (2.6). Tetracycline is assumed to pass the outer membrane of gram-negative bacteria through the porins (Thanassi et al, 1995), probably chelating a M^{2+} ion as $[M\text{-}TC]^{+}$. This assumption has been confirmed by the observation that porin-deficient mutants are less susceptible to tetracycline (Pugsley and Schnaitman, 1978). The cationic $[M\text{-}TC]^{+}$ is attracted by the Donnan potential across the outer membrane causing the accumulation in the periplasm, where the $[M\text{-}TC]^{+}$ complex might dissociate, yielding uncharged tetracycline. This weakly lipophilic compound is able to diffuse through lipid bi-layers and does not depend on a protein channel (Argast and Beck, 1984). Consequently, tetracycline is expected to penetrate the cell in its electrically neutral form.

In the cytoplasm it may be converted to an ionic compound again since the internal pH and the M^{2+} concentration are higher than in the periplasm (Yamaguchi et al, 1991b; Nikaido and Thanassi, 1993; Thanassi et al, 1995). The pH difference dependens on the proton motive force and explains the energy dependence of tetracycline accumulation. In agreement with this model, the uphill accumulation of tetracycline is only driven by ΔpH and not by the trans-membrane electrical potential ($\Delta\psi$) (Yamaguchi et al, 1991a) and is strongly temperature-dependent (Argast and Beck, 1985). In addition, the antibacterial activity is influenced by pH and by the Mg^{2+} concentration in the extracellular medium (Schnappinger and Hillen, 1996). It is observed that; the higher the pH and the higher the

magnesium concentration, the higher the concentration of the membrane-impermeable chelate complex. Consequently, tetracycline accumulates in a compartment having a higher pH and a higher magnesium concentration (Yamaguchi et al, 1991b). Thus, the uphill concentration of tetracycline into the cytoplasm can, at least for *Escherichia coli*, be explained by the Donnan-potential-facilitated uptake through the outer membrane, by the pH gradient-driven diffusion across the cytoplasmic membrane, and by the protonation and complexation behaviour of tetracycline (McMurry et al, 1983; Yamaguchi et al, 1991b). Sigler et al (2000) found that the diffusion through the cytoplasmic membrane is the rate-limiting step of uptake of TC by gram-negative bacteria.

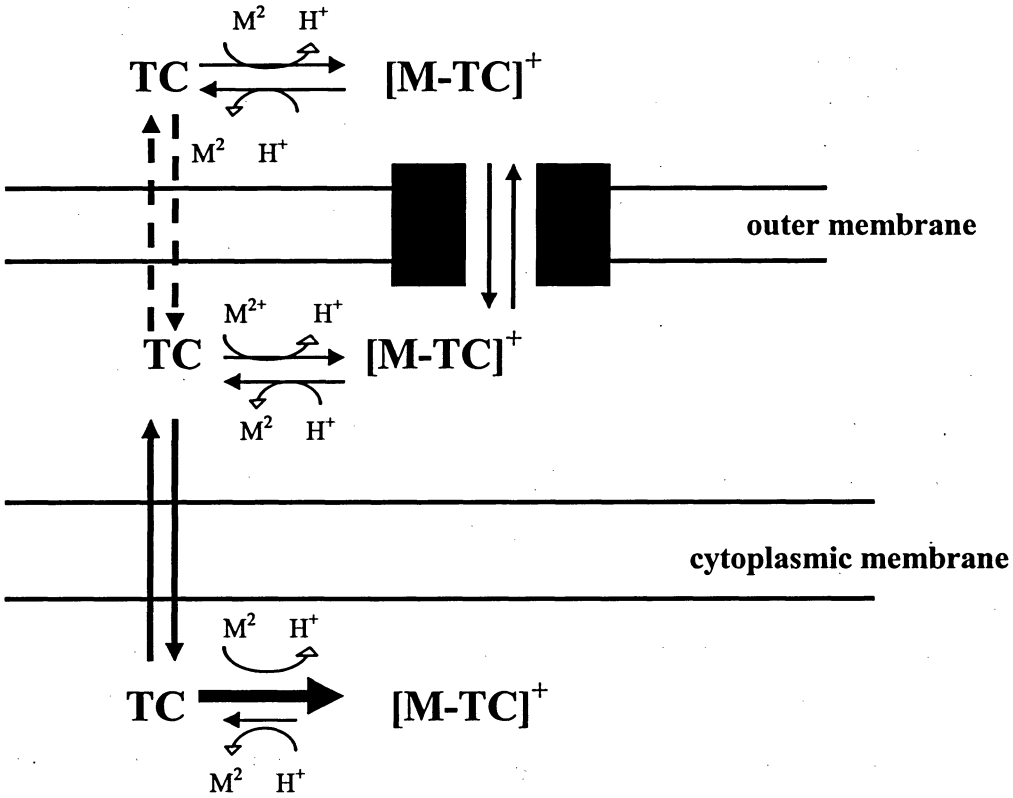


Fig. 2.6: Uptake of tetracycline by *Escherichia coli* as described in text above. Tetracycline, protons, metal cations, and the metal-tetracycline complex are shown as TC, H^+, M^{2+}, and $[M\text{-}TC]^+$ respectively (adapted from Schnappinger and Hillen, 1996)

2.7.2 Reduced Intracellular Concentration of Tetracycline and Efflux Mechanism

In view of the fact that tetracyclines target the ribosome, the antibiotic activity would depend on the presence of the drug in the cytoplasm. Hence, in order to decrease the cell's susceptibility to the antibiotic, the amount of tetracycline in the cytoplasm should be reduced. That objective can be achieved by two means: (1) the permeability of the cell envelope may be lowered, and (2) tetracycline may be pumped out of the cytoplasm in an energy-dependent fashion.

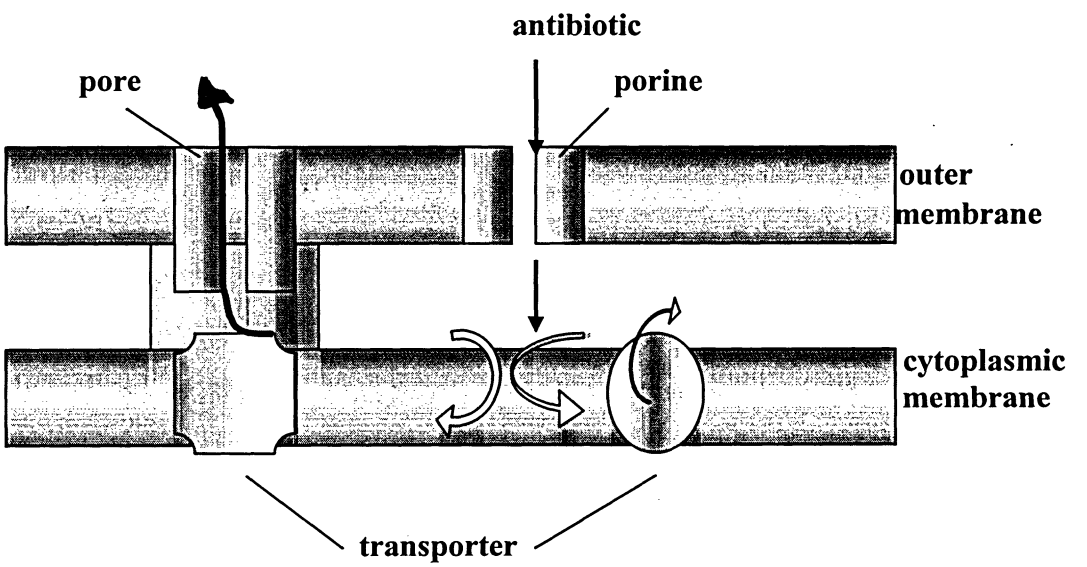


Fig. 2.7: Topology of multi-component (left) and mono-component (right) efflux pumps. Multi-component efflux pumps are specific to Gram-negative bacteria. Arrows show directions of antibiotic transport (adapted from Van Bambeke et al, 2003).

The permeability barrier effects are usually supported by additional resistance mechanisms to achieve high-level resistance; porin-deficient mutants of *E. coli* are only moderately resistant to tetracycline, and a low level, endogenous, active efflux system has also been assumed as a contributor to this resistance (Thanassi et al, 1995). Active efflux occurs in conjunction with reduced outer membrane permeability in *P. aeruginosa* (Li et al, 1994a, b) and contributes to multiple antibiotic resistance of *E. coli* (George and Levy, 1983). Yet, low permeability of the cell wall alone seems to be inadequate to create

high-level resistance phenotypes. Energy-dependent efflux of tetracycline generates in itself high level resistance in bacteria. The fact that this even occurs in gram-positive bacteria implies that this mechanism is not barrier permeability dependent (Schnappinger and Hillen, 1996) as shown in Fig 2.7.

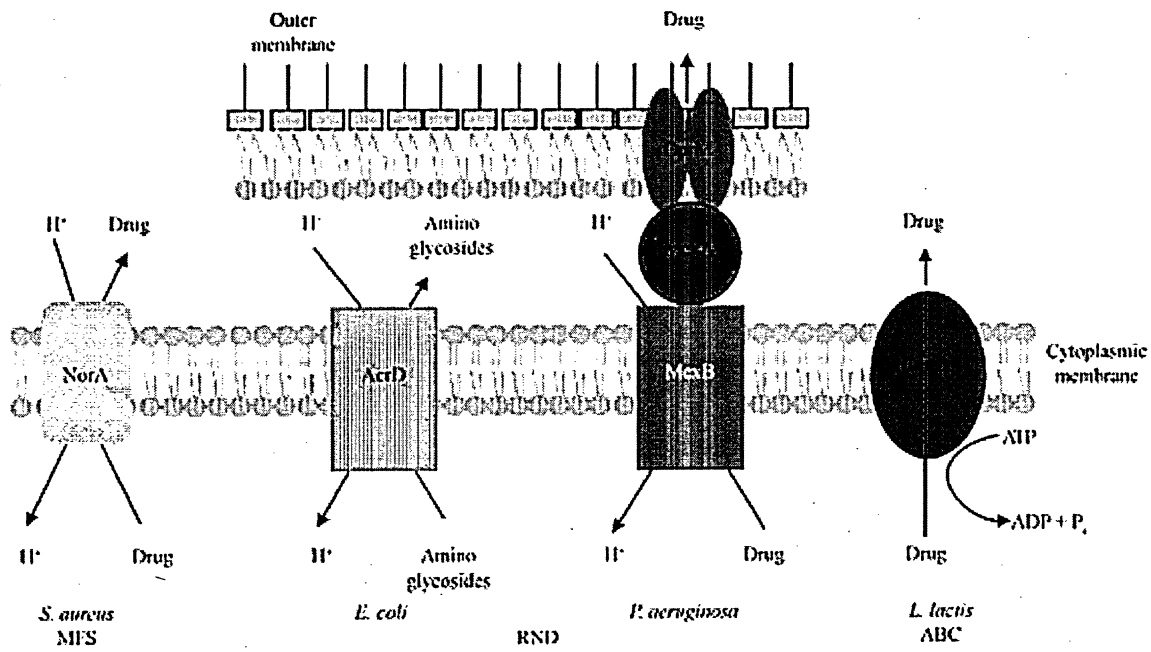


Fig 2.8: Schematic illustration of the main types of bacterial drug efflux pumps. Shown in *Staphylococcus aureus*, *Escherichia coli*, *P. aeruginosa* and *L. lactis*. Illustrated are NorA, a member of the major facilitator super-family, two members (AcrD,MexAB-OprM) of the resistance-nodulation-division family and LmrA, a member of the ATP- binding cassette family: All systems extrude drugs via an energy- dependent manner; either by using proton motif force or ATP (Schweizer, 2003)

Tetracycline-specific exporters pump their substrate into the periplasm and not across the outer membrane, as found for the multi-drug efflux pumps (Thanassi et al, 1995). The energy for active transport is provided by the pH gradient across the cytoplasmic membrane (ΔpH). Therefore, the trans-membrane electrical potential ($\Delta\psi$) is not necessary for transport (Kaneko et al, 1985), indicating that tetracycline transport is an electrically neutral anti-port of protons (Ramón-García at al, 2006) and a positively charged tetracycline complex. A model by Yamaguchi et al (1991b) explains clearly how

the same pH difference can drive tetracycline accumulation in susceptible cells and exclusion from resistant cells. That is, in susceptible cells, tetracycline is accumulated as Tc-Mg^+, which is the substrate for the tetracycline/H^+ anti-porter of resistant cells. A 1:1 stoichiometry of a monocationic metal-tetracycline/H^+ exchange has been confirmed by flow dialysis. Experiments with inverted vesicles have shown no transport in the absence of divalent cations (Yamaguchi et al, 1990a). The addition of various divalent cations stimulates tetracycline transport to different extents in the order: $Co^{2+} > Mn^{2+} > Mg^{2+}$, $Cd^{2+} > Ca^{2+}$. The respective metal-tetracycline complexes share the same affinity to the efflux protein but show different cation-specific turnover rates.

2.7.3 Tetracycline Extrusion and Efflux Mechanism in Biofilms

No bacterium is an island that is nearly all bacteria live with, and depend on other micro-organisms for energy, carbon and other nutrients. Thus, most of the bacteria in the world live in micro-ecosystems filled with hundreds of other microorganisms. Scientists have recently realized that in the natural world, more than 99% of all bacteria exist as bio-films (Prakash et al, 2003). These biofilms are marked by their heterogeneity and this heterogeneity can include gradients of nutrients, waste products and oxygen. The environmental heterogeneity that exists within a biofilm might promote the formation of a heterogeneous population of cells, such that different levels of resistance can be expressed throughout the community. In addition, cells might express a biofilm-specific resistance phenotype induced by the particular environmental factors influencing these cells (Thien and O'Toole, 2001).

The microbial community is embedded in a gel-type layer consisting mainly of extracellular polymeric substance (EPS). Just as in biofilms, the EPS produced by some sludge microorganisms plays a major role in the adhesion and formation of microbial flocs (Bitton, 2005). Antibiotics in wastewater treatment systems which tend to accumulate in the biomass (i.e. activated sludge), and the sorption of these drugs to the biomass can happen at various sites such as: EPS, cell walls, cell membranes and cell

cytoplasm, each of which has different sorption preferences, capacities and properties (Flemming, 1995).

The bacteria enclosed within the biofilm are extremely resistant to antibiotic treatments. Such resistance can be explained by several hypotheses, not necessarily limited to the following ones (Figure 2.9):

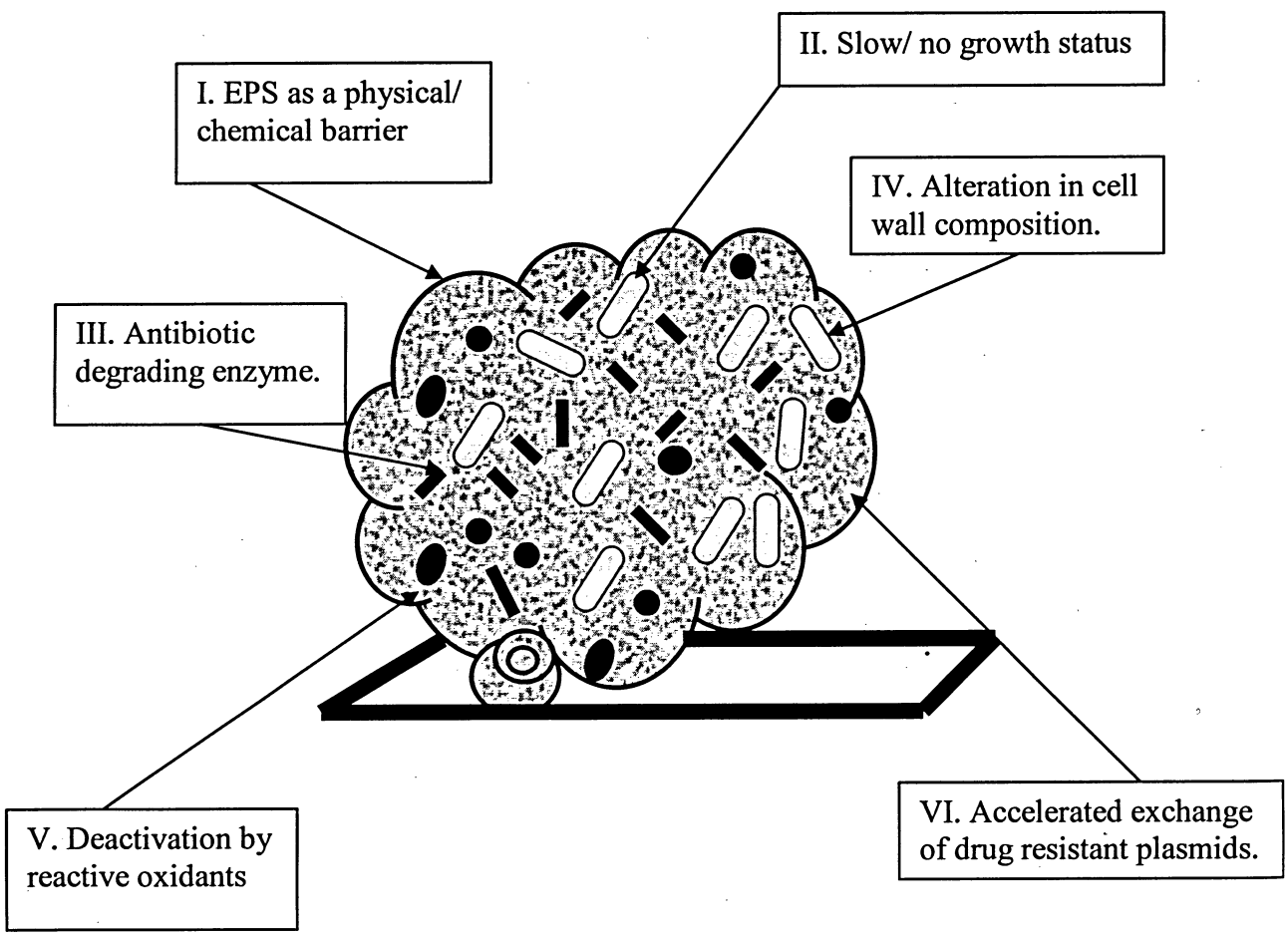


Fig.2.9: Illustration of mechanisms of antimicrobial resistance by a mature biofilm.
(adapted from Prakash et al, 2003)

First, the EPS secreted by biofilm bacteria, acts as a physical/chemical barrier, thus preventing penetration by antibodies or many antibiotics (Lewis, 2001; Costerton et al, 1995). Measurements of antibiotic penetration into biofilms in vitro have shown that some antibiotics readily permeate bacterial biofilms (Stewart, 1996). There is no generic

barrier to the diffusion of solutes at the size of antibiotics through the biofilm matrix, which is mostly water (Stewart, 1998). However, if the antibiotic is deactivated in the biofilm, penetration can be profoundly retarded (Anderl et al, 2000). Antibiotics that adsorb into the biofilm matrix could also have a retarded penetration, which might account for the slow penetration of aminoglycoside antibiotics (Kumon et al, 1994; Shigeta et al, 1997). These positively charged agents bind to negatively charged polymers in the biofilm matrix (Gordon et al, 1988; Nichols, 1988). Moreover, EPS is negatively charged and functions as an ion-exchange resin which is capable of binding a large number of the antibiotic molecules that are attempting to reach the embedded biofilm cells.

Second, embedded biofilm bacteria are generally not actively engaged in cell division, are smaller in size and less permeable to antibiotics. Virtually all antimicrobials are more effective in killing rapidly-growing cells. Further, transition from exponential to slow/no growth is generally accompanied by expression of antibiotic-resistant factors (Brown et. al., 1988; Wentland et al, 1996). That would make the cells more resistant to autolysis and could explain the mechanism of tolerance to antibiotics in slowly growing cells.

Third, antibiotic degrading enzymes such as ß-lactamase may also be immobilized in the EPS matrix, so that the incoming antibiotic molecules can be inactivated effectively. It is interesting to note that biofilm cells of the *Pseudoumas aeruginosa* have been shown to produce 32-fold more ß-lactamase than cells of the same strain grown planktonically (Potera, 1999; Thien and O'Toole, 2001).

Fourth, up to 40% of the cell-wall protein composition of bacteria in biofilms is altered from that of its planktonic brethren (Potera, 1999; O'Toole et al, 2000). The membranes of biofilm bacteria might be better equipped to pump out antibiotics before they can cause damage, or their antibiotic targets may disappear. Another alternation involves the chemical microenvironment within the biofilm. Micro-scale gradients in nutrient concentrations are a well known feature of biofilms. Concentration gradients in metabolic products reflect those of the substrates. Local accumulation of acidic waste products

might lead to pH differences >1 between the bulk fluid and the biofilm interior which could directly provoke the action of an antibiotic (Zhang and Bishop, 1996). Consequently, the depletion of a substrate or accumulation of an inhibitive waste product might cause some bacteria to enter a non-growing state, in which they are protected from killing. This alternative possibility is strengthened by direct experimental visualisation of metabolically inactive zones within continuously fed biofilms (Xu et al, 2000). Additionally, the osmotic environment within a biofilm might be altered, leading to induction of an osmotic stress response (Prigent-Combaret et al, 1999). Such a response could contribute to antibiotic resistance by changing the relative proportions of porins in a way that reduces cell envelope permeability to antibiotics.

Fifth, the antimicrobial agent is deactivated in the outer layers of the biofilm, faster than it diffuses.

Sixth, biofilms also provide an ideal niche for the exchange plasmids responsible for antibiotic resistance, virulence factors and environmental survival capabilities at accelerated rates, making it a prefect milieu for emergence of drug resistance pathogens (Hausner et al, 1999; Ghigo, 2001; Donlan, 2002). The horizontal gene transfer of resistant plasmids in wastewater treatment systems and sludge has been confirmed in many studies (Mach and Grimes, 1982; Reinthaler et al., 2003; Schwartz et al; 2003) where the conjugation rates and bacterial concentrations are high and thus the chance of contact between two suitable bacteria cells is high.

2.8 Extraction and Analysis of Tetracyclines

A number of methods have been developed for the determination of pharmaceuticals and their metabolites in the lower ng/l range using solid phase extraction (SPE), derivatization, detection and confirmation by gas chromatography /mass spectrometry (GC/MS) (Hirsch et al; 1999) and GC/MS/MS or liquid chromatography (LC)/electrospray tandem MS (LC/ES/MS/MS) (Lindsey et. al, 2001). A wide range of drugs from different medicinal classes can be determined down to the lower ng/l range.

Due to the basically elevated polarity of pharmaceuticals, either analysis by LC/ES /MS/MS or an efficient derivatization prior to measurements by GC/MS is mostly essential. A comparison between GC/ MS and LC/ES/MS/MS illustrate that only the latter allows for the analysis of the extreme polar betablockers due to an incomplete derivatization of the functional groups (Koester et al, 2003). However, when analyzing highly contaminated samples such as sewage, a suppression of the electrospray ionization is expected to occur. Thus, to assure accurate and reproducible data, either an efficient clean-up step has to be included into the sample preparation or an appropriate surrogate standard has to be spiked prior to SPE enrichment.

In a study by Hirsch et al (1998) the determination of 18 antibiotics in water down to the lower ng/l range used a multi-analytical method was described. The drugs examined belonged to different groups of antibiotics such as penicillins, tetracyclines, sulfonamides and macrolide antibiotics; analysis was performed by LC/ES /MS /MS. An analysis of the three antibiotics was conducted by Huang et al (2001) via liquid chromatography mass spectrometry (LC-MS). In addition, high performance liquid chromatography (HPLC) with fluorescence detection was used for ciprofloxacin analysis (Golet et al, 2003). The chromatography method applied for the detection and analysis of antibiotics requires the use of C_8- and C_{18}-bonded silica columns and water / acetonitrile mixtures containing ammonium acetate.

2.8.1 High Performance Liquid Chromatography (HPLC) Analysis

Tetracyclines are nonvolatile antimicrobials with high molecular weights, which make HPLC an appropriate analytical tool more for their separation and detection. Several papers dealing with the liquid chromatographic determination of tetracyclines and their degradation products have been published (Hamscher et al, 2002; Kolpin et al, 2002; Halling-Sørensen et al, 2003; Loke et al, 2003).

The tetracyclines have been analysed using separation on reversed-phase derivatized silica or polymer solid supports. Reversed-phase (RP-HPLC) has been used extensively

in combination with UV-DAD, fluorescence or mass detectors (Oka et. al, 2000; Castellari and García-Regueiro, 2003). Under these conditions mobile phases containing oxalic acid or well end-capped and high pure silica gel columns were employed to reduce tailing peaks (Castellari and García-Regueiro, 2003).

Fluorescence detection of tetracyclines is more specific and also in many cases more sensitive than UV detection (Pena et al, 1998). Normally it is used in the analysis of tetracycline residues in biological and food samples. On the other hand high-performance liquid chromatography/mass spectrometry (LC/MS) or LC/MS/MS has been used in the analysis of antibiotics because of its high sensitivity and ability to provide compound confirmation (Yang et al, 2004). Given that tetracyclines respond well to positive electrospray ionization, this would make (LC/MS) an excellent choice for separation and analysis (Lindsey et al, 2001).

2.8.2 Solid Phase Extraction (SPE)

Solid-phase extraction has become a common method of sample preparation for purifying and concentrating analytes from an aqueous matrix. SPE methods for extracting antibiotics in a water matrix using mixed-mode, cation exchange (MCX), hydrophilic-lipophilic balance (HLB), styrene-divinylbenzene (SDB), and Lichrolute EN/Lichrolute C_{18} have been developed (Fedeniuk and Shand, 1998; Hirsch et al, 1998; Lindsey et al, 2001; Miao et al, 2004; Yang et. al, 2004).

Yet since TCs have been shown to chelate metals and to bind to silanol surfaces, special techniques have been used in past studies to improve recovery. For example, as shown by Miao and coworkers (2004), EDTA has been used in the extraction of higher concentrations of TCs to improve recovery by chelating metal ions. TCs may bind residual metals on SPE cartridges, thereby irreversibly binding to the cartridge and lowering recovery. Chelation of metals was prevented by the TCs, as mentioned in the literature, was achieved study in two ways (Miao et al, 2004). The first was to wash metals off the cartridge using a solution of 0.5N HCl. The second way was to add a

strong chelator to the sample that would out-compete the TCs for metal ions. One the chelators used was Na_2EDTA because it is an excellent metal chelator that is sufficiently soluble in water. Thus, it was found when extractions were performed without rinsing the cartridge with HCL or adding Na_2EDTA to the sample tetracycline recoveries decreased by at least 50% (Lindsey et al, 2001; Yang et al, 2004).

3.0 MATERIALS AND METHODS

3.1 Experimental Design

This study involved an examination of the degradation of Chlortetracycline in wastewater treatment systems, and the distribution of CTC in three different compartments; the bulk water, in the extracellular polymeric substances (EPS) of microbial flocs and inside the cells under different environmental conditions (Figure 3.1).

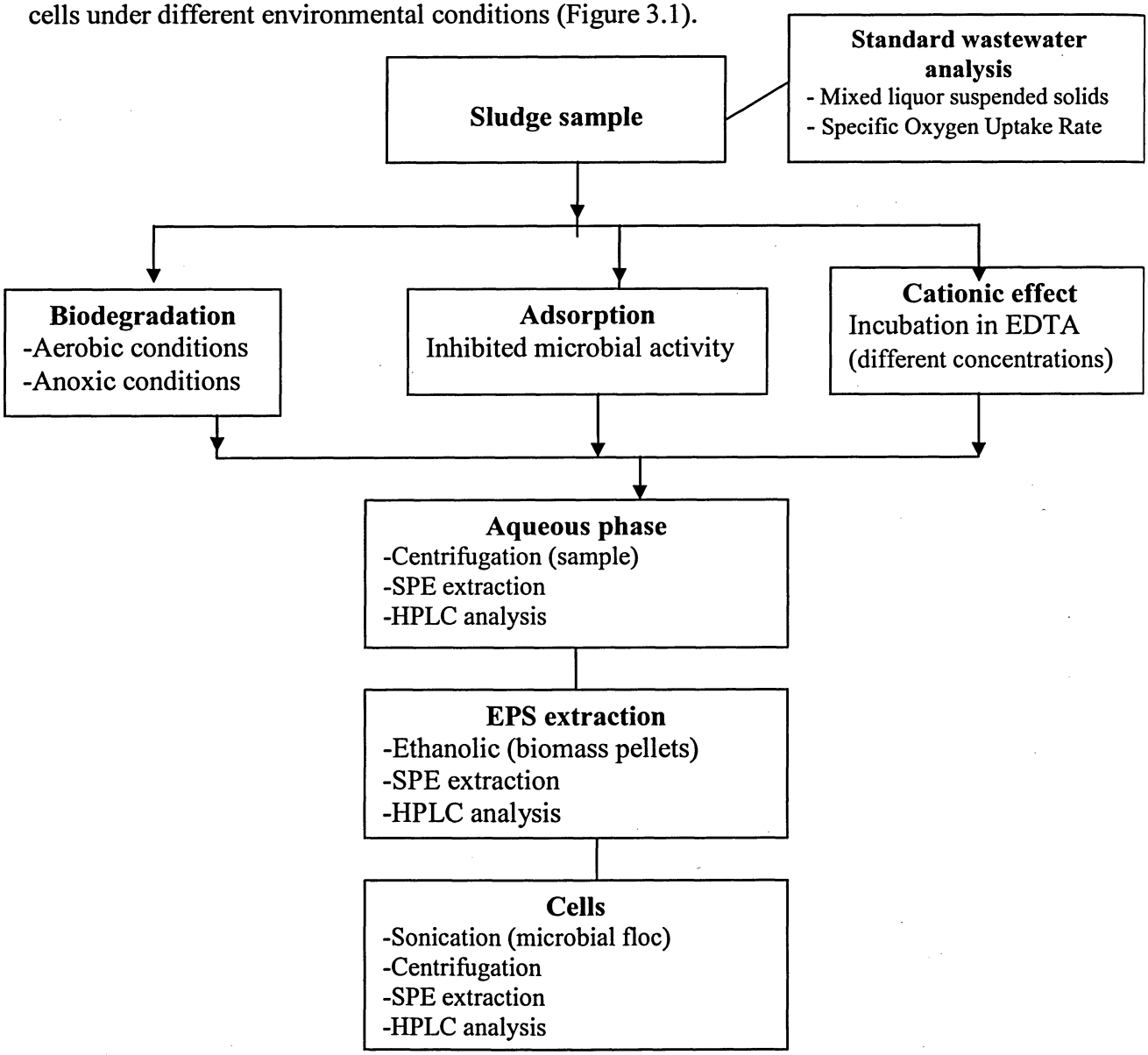


Fig 3.1: Experimental approach for the study of CTC degradation

3.2 Sample Collection

Samples were collected at the beginning of the trials at the Ashbridges Bay Treatment (ABTP) plant (formally known as Toronto Main Wastewater Treatment Plant) which is located in Toronto's east end. The samples consisted of mixed liquor which were obtained from aeration tank 2 of the activated sludge system at the Ashbridges Bay treatment plant. The samples were transferred to the lab for immediate use, or stored in the fridge (4°C) up to two weeks. Prior to use, samples were incubated in substrate and reactivated. It was noted that fresh samples and stored samples gave reproducible results.

3.3 Establishing Inhibition Concentration

Before starting the main experiments of interest for this research, inhibition concentration levels of Chlortetracycline, hydrochloride ($C_{22}H_{23}ClN_2O_8 \cdot HCl$; molecular weight 515.34) (Sigma-Aldrich CO.; Oakville - ON) needed to be established. Thus the standardized respiration inhibition test according to OECD method 209, was used to assess the effect of Chlortetracycline, hydrochloride (CTC) on aerobic bacteria present in sewage sludge. The concentrations ranged from 1.6 - 8 µg/mL. 3,5-dichlorophenol (Sigma-Aldrich CO.; Oakville - ON) were used as a positive control as recommended by the test guideline. The experiments were conducted according to the test guideline, with one modification: the final volume of the sample (250 ml) was half that mentioned in the guideline (for practicality reasons). All the chemicals used were at an analytical grade. Some of the solutions were stored at 4°C until use, and distilled water was used in the process. The biomass was from ABTP whose influent is mostly municipal. The sludge was washed three times with distilled water prior to use. Dry matter content was 3.6 g L^{-1} ± 1.02. The sludge was aerated overnight at 20± 2 °C; and in the case of using a stored sample, the pH was 6–8. The test consisted of three different vessels containing the test compound in different concentrations in addition to two controls that had only the biomass with feed. The concentration range used to test inhibitory effect was chosen according to data published by Kümmerer et al (2004).

An incubation period of 3 h at 20 ± 2 °C was the duration of the experiment, after which the respiration rate of sewage sludge in the test vessels was measured by measuring the dissolved oxygen (DO) via a DO-meter (3" Digital LCD; Cole Parmer, USA) until the oxygen became limiting (1.0 mg/L) following that the results were plotted against time, and the slope of the best fitted line was the respiration rate (or specific oxygen uptake rate- SOUR). In order to calculate the inhibitory effect of CTC at a particular concentration, the respiration rate is expressed as a percentage of the mean of the two control respiration rates:

$$\left\{ 1 - \frac{2R_s}{R_{c1} + R_{c2}} \right\} \times 100 = \text{per cent inhibition} \qquad (3.1)$$

Where:

R_s = oxygen-consumption rate at tested concentration of test substance,

R_{c1} = oxygen-consumption rate, control 1,

R_{c2} = oxygen-consumption rate, control 2.

The standard test (3 h of exposure) is valid if the respiration rates of the activity controls differ less than 15% (OECD method 209; 1984). The inhibition concentration of 50% effect (IC_{50}) for 3,5-dichlorphenol should be 5-30 µg/mL. These criteria were also applied. Hence when applying the above equation, it was found that an inhibitory effect of (44 ± 2)% was observed for the concentration between 8 – 4 µg/mL. Therefore, it was decide to use a concentration of 5 µg/mL of CTC, although that concentration is high compared to what is found in municipal influents, yet given the level of sensitivity of the analysis by HPLC-UV, a lower concentration would be difficult to detect.

3.4 Biomass Preparation

Samples used were either from those immediately picked up from ABTP or from those stored. In the latter case, they were first given 100 ml feed/L for three hours under continuous stirring and aerated conditions in order to activate the biomass and bring it

back to an aerobic stage. The feed was 1200 mg COD /L as mixture (50/50) of glucose and sodium acetate (645 mg glucose/L; 1360 mg sodium acetate/L). Then the sample was centrifuged at 3000 (HN-S centrifuge; Intl. equipment company, Mass. USA) rpm for 5 min and washed twice with distilled water and then re-suspended in a mineral medium (adapted from Kargi and Uygur, 2003) shown in Table 1, after which the sample was ready to be used.

Table 3.1: Mineral medium composition (Kargi and Uygur 2003)

Compound*	mg/L
Ammonium Chloride (NH_4Cl)	229.3
Mono-potassium Phosphate(KH_2PO_4)	78.9
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	100
Sodium Bicarbonate (Na HCO_3)	500
Sodium Chloride (NaCl)	100
Potassium Chloride (KCl)	20
Calcium Chloride ($CaCl_2 \cdot 2H_2O$)	50
Ferric Sulfate ($FeSO_4 \cdot 7H_2O$)	1.5

* Sigma- Aldrich CO., Oakville, ON.

Mixed Liquor Suspended Solids (MLSS) is composed of active microbial mass, non-active microbial mass, non biodegradable organic mass, and inorganic mass, and was measured in accordance with Standard Methods (APHA, 1980). An aliquot of well-mixed mixed liquor (5 mL) sample was filtered through a weighed standard glass-fibre filter (Whatrnan, 47mm diameter) and the residue retained on the filter was dried to a constant weight at 103-105°C for at least one hour. The increase in weight of the filter paper represented the MLSS.

The MLSS was calculated according to;

$$\text{MLSS (mg/L)} = \frac{(\text{A}-\text{B})mg \bullet 1000}{Volume_{sample}(ml)} \qquad (3.2)$$

Where; A-weight of filter + dried residue at 105°C after 1h (mg),

B-weight of filter (mg)

The Specific Oxygen Uptake Rate (SOUR), also known as the oxygen consumption or respiration rate, is defined as the milligram of oxygen consumed per gram of volatile suspended solids (VSS) per hour. This quick test has many advantages; rapid measure of influent organic load and biodegradability, indication of the presence of toxic or inhibitory wastes, degree of stability and condition of a sample, and the oxygen consumption rate of a sample of activated sludge. The SOUR test procedure to be discussed below was derived from Standard Methods (APHA, 1980-(SM 2710 B))

The sample was poured into a BOD bottle (the bottle should be completely full) and a magnetic stir bar was placed inside, then the DO probe was inserted into the BOD bottle, making sure that the probe tightly sealed the sludge from the atmosphere. After the meter reading stabilized, the initial DO reading was recorded. Then data is recorded over a 15 minute period or until the DO was less than 1.0 mg/L. The observed readings (D.O. mg/L vs. time in minutes) was then plotted, and the slope of the best fitted line was determined by using the equation

$$\text{m (oxygen consumption rate in mg/L per minute)} = \frac{y-b}{X} \qquad (3.3)$$

Where; y = y intercept
x = x intercept
m = slope
b = where line crosses y axis

To convert the slope into mg/L per hour per gram mixed liquor suspended solids, the slope is changed to a positive number, and then the uptake rate was calculated by using the following formula:

$$\text{SOUR (mg/g)/h} = \frac{m\,(mg/l)/\min}{MLSS\,(g/L)} \times \frac{60\ \min}{hour} \qquad (3.4)$$

Where:
m = positive slope

Extraction and Analysis; The sludge samples were first centrifuged at 3000 rpm for 5 min where after the supernatant was collected, and then was extracted according to the solid phase extraction method described above, after which the samples were separated in the RP-HPLC column.

A fresh stock of CTC standard solutions was run with the samples to be certain of the retention time, and so as to calculate the final concentration in the sample. The calculation was performed according to:

$$C_f\,(\text{ppm}) = \frac{peak\ area\ of\ the\ sample}{peak\ area\ of\ the\ standard} \times X\,(\mu g / mL) \qquad (3.5)$$

Where; C_f is the final concentration in the sample.

X is the intial concentration of CTC in sample.

3.5 Separation and Detection of Chlortetracycline

Samples were first extracted and cleaned using solid phase extraction (SPE), then were analyzed with reverse-phased high performance liquid chromatography (RP-HPLC) using a UV detector.

3.5.1 Extraction and Clean Up Using SPE

The SPE extraction procedure was adapted from a previously described method (Miao et al, 2004). Because CTC have been shown to chelate metals (Blanchflower et al, 1997; Varatarian et al, 1998) and to bind to silanol surfaces, special techniques have been used in past studies to improve recovery. Therefore, disodium ethylenediamine tetraacetate (EDTA, Na-form, B.D.H laboratory Chemicals Inc.; molecular weight 373.24) has been used in the extraction of CTC to improve recovery by chelating metal ions (Carson et al, 1998; Chee-Sandford et al, 2001). CTC may bind residual metals on SPE cartridges, thereby irreversibly binding to the cartridge and lowering recovery. Thus preventing

chelation of metals by CTC is accomplished in two ways. The first was to precondition the cartridge(see below). The second way was to add a strong chelator to the sample that would out-compete CTC for metal ions. EDTA was chosen because it is an excellent metal chelator that is sufficiently soluble in water.

Consequently, analytes were extracted using the 60-mg hydrophilic-lipophilic balance (HLB) cartridge from Waters (Millford, MA). Cartridges were preconditioned with 3mL of methanol (MeOH; Sigma-Aldrich CO., Oakville ON.), 3 mL of acetone (Sigma-Aldrich CO., Oakville ON.), and 3 mL of 50 mM EDTA (pH 3.0). The sludge samples were first centrifuged at 3000 rpm for 5 minutes, the supernatant was collected, acidified to pH 3.0 with 3.0 M H_3PO_4 followed by the addition of EDTA (0.5 g/L). Samples were then passed through the cartridges at approximately 10 mL/min and after passage of the samples each cartridge was eluted with three 2 mL volume of MeOH. The elutes were collected in 10 ml test- tube, after which they were concentrated with a speed vacuum centrifuge (DNA Speed Vac.; DNA 110, Savant Instrument Inc., NY) for 30 minutes at high temperature, and then reconstituted to 1.0 mL with 20% aqueous MeOH. Methanol was chosen as the universal solvent for the TCs, because of its ability to dissolve the Tetracyclines and its miscibility with aqueous and organic solvents. Methanol was also chosen because aqueous solvents tend to accelerate degradation of tetracyclines compounds (Pena et al, 1998).

3.5.2 High Performance Liquid Chromatograph-Ultra Violet Detector (HPLC-UV)

The chromatographic separation of the analytes was conducted using a liquid chromatograph of an PerkinElmer Series 200 HPLC system with a model 785A preamble absorbance detector (PerkinElmer Inc, USA) containing a 20µl injection loop and an auto-sampler. The detector was set at 264 nm wavelength, 0.05 absorbance unit full scale. The linearity of the detector response was checked using standard solutions of CTC. A C_8 Zorbax (12.5x 4.6 mm I.D., 5µm particle size) guard column and a reversed- phase C_8 Zorbax (150x 4.6mmI.D., 5µm particle size) analytical column (Chromatographic

Specialties Inc., Canada). Selectivity was checked by comparing chromatograms of blank and spiked samples obtained with the reported procedures.

The operating conditions were as follows:

injection volume	20µl
run time	10 min.
flow rate	1.0 ml/ min
mobile phase	75/25 , 0.1% Trifluoroacetic acid (TFA): Acetonitirle (Sigma-Aldrich, H PLC grade)
temperature	Ambient

3.6 Experimental Procedure

In this study, the degradation of CTC in wastewater treatment systems was investigated, in addition to the distribution of CTC in three different compartment; the bulk water, in the extracellular polymeric substances (EPS) of microbial flocs and inside the cells under different environmental conditions. All experiments were run in duplicates and analyzed in triplicate (n = 6). The MLSS to all samples ranged between 1.0- 2.0 g/L. The Feed/ Microbe (F/M) was between 1.0 – 2.5 (Reynolds and Richards, 1996; Bitton, 2005)

3.6.1 Biodegradability of Chlortetracycline

The bulk of the literature suggests that CTC is persistent in the environment and that it is not effectively removed from wastewater treatment plants (Gavalchin and Katz, 1994; Ingerslevet al, 2001; Kolpin et al, 2002; Boxall et al, 2003; Alexy et al, 2004), hence in this study, that needed to be established before pursuing the detection of CTC in the three compartments mentioned above under different environmental conditions.

First as a positive control, two samples of the wastewaters were washed and re-suspended in a mineral medium then they were run under aerobic conditions for 24 hrs where CTC

is the only carbon source, the final sample size was 400 ml, and CTC concentration was 5 μg/mL. The sample was put in an Erlenmeyer flask, with a stopper allowing an air inlet and another hole to allow air out, the sample was continuously stirred using a magnetic stirrer plate, and the flask was covered with aluminium foil to eliminate the photo-degradation possibility. Air (an oxygen supply) was introduced through a stone air diffuser. In addition a blank was run with the two samples. After 24 h had elapsed, SOUR and C_f were measured.

3.6.2 Adsorption of Chlortetracycline

Since the main removal pathway of TCs in general and CTC in particular is adsorption (Sithole and Guy, 1987a; Pouliquen and LeBris, 1996; Rabolle and Spliid, 2000), there was a need to quantify the percentage of removal via adsorption. Aliquots (150 mL) of the washed sludge were inoculated in duplicate in 400 mL Erlenmeyer flasks wrapped in aluminium foil to prevent possible photo-degradation of CTC to minimize any Chlortetracycline elimination due to biotic processes, 0.32 g (800 μg/mL) of sodium azide (NaN_3) were added into each flask. The final concentration of CTC in the samples was 5 μg/mL. As a control sample, an aliquot of 150 mL of washed sludge with 150 mL (1200 COD) feed and 100 mL distilled water in a 400 mL Erlenmeyer flask wrapped in aluminium foil was used. The samples were left to run for 24 h under aerobic conditions and continuous stirring.

On the basis of the results of the kinetic experiments presented by Kim et al (2005), 24 h proved sufficient to reach the adsorption equilibrium of tetracycline onto activated sludge. Therefore, after the 24 h period, the MLSS test, SOUR test, SPE extraction, and HPLC analysis were performed as aforementioned. The adsorbed CTC concentration in the biomass, *Cs*, was calculated using

$$Cs = \frac{X}{M} = \frac{(C_0 - C_e) \bullet V}{C_B \bullet V} \qquad (3.6)$$

Where; X is the total mass of tetracycline in the biomass
M is the total dried weight of the biomass,
C_B is the biomass concentration,
V is the solution volume,
C_0 is the initial Chlortetracycline concentration and,
C_e is the final Chlortetracycline concentration in the liquid phase after 24 h of equilibration. The final concentration in the liquid phase, C_e, was determined using HPLC

3.6.3 Co-metabolism of Chlortetracycline

Although it is not mentioned in the literature that co-metabolism biodegradation of CTC takes place, the removal of chlortetracycline in the presence of another substrate was tested and to be compared with the removal in the case where CTC was the only carbon source, and when there was no microbial activity.

Aliquots (150 mL) of the washed sludge in addition to a 150 mL (1200 COD) feed were inoculated in duplicate in 400 mL Erlenmeyer flasks wrapped in aluminium foil to prevent possible photo-degradation of CTC. The final concentration of CTC in the samples was 5 μg/mL. As a blank sample, an aliquot of 150 mL of washed sludge with 150 mL (1200 COD) feed and 100 mL distilled water in a 400 mL Erlenmeyer flask wrapped in aluminium foil was used. The samples were left to run for 24 h under aerobic conditions and continuous stirring. Consequently, after the run was completed the MLSS test, SOUR test, SPE extraction, and HPLC analysis were performed as aforesaid. The final concentration of Chlortetracycline in the aqueous phase was calculated based on equation (3.5).

3.7 Distribution of Chlortetracycline in Different Compartments

Since a portion of chlortetracycline is removed from the bulk water in wastewater treatment systems via sorption, the test was intended to closely examine how and where

the CTC might sorb. In addition the effect of different environmental conditions on that distribution of the sorbed CTC in the biomass was examined.

3.7.1 Chlortetracycline in Bulk Water

After each run, regardless of the conditions, the sample was centrifuged and the supernatant was collected and then extracted (SPE – extraction) and analyzed (HPLC-analysis) as mentioned.

3.7.2 Chlortetracycline on the EPS

Capsular biopolymers were removed mainly by ethanolic extraction, adopting the method used by Forster and Clarke (1983). The biomass remaining after the centrifugation of the samples and the collection of the supernatant was re-suspended in the original volume (20 mL) of ethyl alcohol (Aldrich Chemical Company Inc., WI) and allowed to stand in a sealed container at 23°C for 24 h. The ethanolic suspensions were then centrifuged in a Z36 HK Super Speed Centrifuge Labnet centrifuge (Anachemia Science/Mines Assay Supplies, Mississauga, ON) at 1800-g for 10 min and the clear ethanolic extract decanted. The polymeric material was obtained by removing the solvent under reduced pressure using a speed vacuum centrifuge for 45 min on high temperature after that the concentrate was re-suspended in a buffer (2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 m.M NaCl, 1 mM KCl, pH 7) then were acidified to pH 3.0 with 3.0 M H_3PO_4 followed by the addition of EDTA (0.5 g/L). Samples were then passed through the HLB cartridges at approximately 10 mL/min after passage of the samples each cartridge was eluted with three 2 mL volume of MeOH. The elutes were collected in 10 mL test- tube, after which they were concentrated with a speed vacuum centrifuge for 30 min on high temperature, and then reconstituted to 1.0 mL with 20% aqueous MeOH. Subsequently; elutes were taken to be separated by the RP-HPLC column, and the concentration of CTC in the EPS was calculated according to equation (3.5).

3.7.3 Chlortetracycline in the Cells

Following the ehtanolic extraction of the EPS, the remaining biomass (the microbial cells) was suspended in 20 mL of an extraction buffer (2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 mM NaCl, 1mM KCl, pH 7) then sonicated over a period of 2 min at an intensity of 2μm amplitude using an MSE Soniprep 150 Ultrasonic Disintegrator (Johns Scientific Inc., Toronto, Ontario) attached with a 19 mm diameter probe tip. Subsequently, the samples were centrifuged at 3000 rpm for 5 min, and the supernatant was collected and prepared for the SPE extraction, where the pH was brought to 3.0 with 3.0 M H_3PO_4 followed by the addition of EDTA (0.5 g/L). Then samples were passed through the HLB cartridges, eluted, concentrated and reconstituted to 1.0 mL and sent to HPLC separation. The concentration measured according to equation (3.5) represents the amount of CTC the accumulated inside the cells.

3.8 Chlortetracycline Removal under Aerobic and Anaerobic Conditions

There are significant findings in the literature to suggest that there are different removal behaviours under aerobic and anoxic conditions (Ingerlev et al, 2001; Thiele-Bruhn, 2003). Therefore the following experiments which were run for a period of 14 days were set to study the effect of aerobic and anaerobic conditions on the removal CTC from the aqueous phase and the nature of CTC accumulation in both the EPS and microbial cells.

3.8.1 Chlortetracycline Removal under Aerobic conditions

Microbial inoculums (200 mL) from washed sludge were added in duplicates to 200 mL of feed (1200 COD) and 100 mL CTC in 500 mL Erlenmeyer flasks wrapped in aluminium foil to prevent possible photo-degradation of CTC. The final concentration of CTC in the samples was 5 μg/mL. The flasks were continuously stirred. They were closed with rubber stoppers that allowed air inlet tube which ended in a stone air diffuser and another opening to allow air out. MLSS was measured at the beginning of the run. During the 14 days no feed was replenished, nor the loss of volume compensated.

After two weeks, the SOUR was measured to make sure there was still microbial activity, then samples were centrifuged at 3000 rpm for 5 min, the supernatant was collected for the analysis of CTC in the aqueous compartment, the remaining biomass pellets were re-suspended in ethanol and left to stand at 23°C for 24 h to be followed by the EPS extraction then analysis for CTC in the polymeric substance. Finally, what was left (of biomass) after the EPS extraction was re-suspended in the extraction buffer (pH 7), sonicated for 2.0 min at 2μm amplitude then CTC analysis was performed to quantify the amount of the antibiotic in the microbial cells.

3.8.2 Chlortetracycline Removal under Anoxic conditions

Aliquots (200 mL) of the pre-washed sludge in addition to a 200 mL (1200 COD) feed were inoculated in duplicate in 500 mL Erlenmeyer flasks wrapped in aluminium foil to prevent possible photo-degradation of CTC. The final concentration of CTC in the samples was 5 μg/mL. The samples were run for two weeks continuously stirred under anaerobic conditions where rubber stoppers were used to prevent air form coming in. At the start of the experiment the MLSS was measured. At the end of the run the SOUR was measured to check for microbial activity, and then the samples were centrifuged at 3000 rpm for 5 min to gather the supernatant in order to quantify CTC in the aqueous phase. The sludge capsules were re-suspended in ethanol and left to stand at 23 °C for 24 h after which EPS was extracted and analysed for CTC presence in the polymeric substance. Lastly, the remaining biomass from the EPS extraction was re-suspended in the extraction buffer (pH 7), dispersed via sonication for 2.0 min at 2μm amplitude followed by SPE extraction and HPLC separation to determine the amount of CTC in the microbial cells.

3.9 Influence of Divalent Cations

In order to further study the distribution of the adsorbed CTC in the both the EPS and the cells, several tests were done. A number of questions were at hand: why was the

antibiotic bypassing the EPS and accumulating in the cells when the microbial community was not active? In addition, would the distribution of CTC differ in a viable microbial community and the presence of another chelating agent, one that affects the stability of the microbial floc? Would the CTC as it effluxes out of the cells evade the EPS and amass in the water?

3.9.1 Addition of EDTA to Inactive Biomass

Three samples of 200 mL of pre-washed sludge were added into 500 mL Erlenmeyer flasks which contained 200 mL feed (1200 COD), a 100 mL CTC, and 0.4 g (800 μg/mL) of sodium azide (NaN_3). The flasks were wrapped in aluminium foil to prevent possible photo-degradation of CTC. The final concentration of CTC in the samples was 5 μg/mL. The flasks were closed with a rubber stopper allowing an air inlet via a stone air diffuser and another opening to allow air out. The samples were left to run for 24 h and the MLSS for both samples was measured at the beginning.

At the conclusion of the experiment the sample's SOUR was measured to make sure that the inhibition conditions were existent, and then 90 mL was taken from each sample and centrifuged at 3000 rpm for 5 min the supernatant was then extracted (SPE) and separated (HPLC) to quantify the CTC concentration in the water, then the remaining pellets were re-suspended in 30 ml ethanol and left to stand at ambient temperature for 24 h, in preparation for EPS extraction followed by sonication of the residual biomass. Then both the EPS and dispersed cells were extracted (SPE) and chromatographically (HPLC) analysed. Hence, CTC concentration in both compartments was measured.

The remaining volumes were centrifuged, the supernatant was discarded and the pellets were re-suspended in the mineral medium and given new substrate. Then four duplicate samples (200 mL) were incubated in EDTA under aerobic conditions for three hours in Erlenmeyer flasks wrapped in aluminium foil. The EDTA concentration was as follows: 0, 50 μg/mL, 150 μg/mL, 300 μg/mL. After the three hour period the SOUR of the samples was measured to make sure that the samples were active again. Then they were

centrifuged at 3000 rpm for 5 min where the supernatant was collected, extracted via SPE and separated using HPLC column to enumerate the concentration of CTC that was pumped out of the cells. Then the pellets were suspended in ethanol (20 mL) for 24 h to be followed by EPS extraction, finally the remaining biomass was sonicated. Both samples were later extracted using SPE and separated using HPLC column to calculate the concentration of CTC left in both the EPS and the microbial cells.

3.9.2 Testing the Import Effect to Active Biomass

To measure the effect of import, EDTA which is a strong chelating agent known for its effect on the stability of the microbial floc by breaking flocs linked by salt bridges and affecting ionic interactions through the removal of divalent cations (Liao et al 2002) was used. Aliquots (200 mL) of the pre-washed sludge in addition to a 200 mL (1200 COD) feed were inoculated in duplicate in 500 mL Erlenmeyer flasks wrapped in aluminium foil to prevent possible photo-degradation of CTC. The final concentration of CTC in the samples was 5 μg/mL. The samples were run for 24 h continuously stirred under aerobic conditions. The flasks were closed with rubber stoppers which allowed an air inlet and air outlet via two openings. At the start of the experiment the MLSS was measured. At the end of the run the SOUR was measured. The sample was then divided into two, 200 mL were taken, centrifuged at 3000 rpm for 5 minutes, to collect the supernatant which in turn would be extracted and analyzed to determine the amount of CTC in the aqueous phase. Followed by the EPS ethanolic extraction, where the EPS sample was then extracted and separated via SPE and HPLC column respectively to account for the amount of CTC in the EPS. And then the cells were sonicated, afterwards extracted and separated using SPE then HPLC column, thus quantifying the CTC present in the cells.

The remaining 300 mL were divided into two samples, and EDTA (150 μg/mL) was added to the duplicates. The samples were gently mixed in a shaker (Orbital Shaker, VWR International; Mississauga, Ontario) at a speed of 200 rpm for almost 30 minutes, the 150 μg/mL concentration of EDTA was selected based on the findings of Liao et al

(2002) where it was found that there was a critical EDTA concentration at about 100 mg/L, at which there was a significant effect on the microbial floc stability.

The samples were then centrifuged at 3000 rpm for 5 min to collect the supernatant so as to analyse and calculate the amount of CTC in the water phase, which was followed by the analysis of the EPS and cells and the enumeration of CTC in both of these compartments.

4.0 RESULTS

4.1 Establishing Inhibition Concentration

Chlortetracycline (CTC) is an antibiotic; consequently it is expected to have an inhibitory effect on the microbial community. Therefore, it is essential to establish an inhibition concentration of CTC that would satisfy two conditions. First, it exerts a minimum inhibitory effect under which the microbial community would still retain as part of its biological activity thus allowing a degree of resistance and degradation to the antibiotic. Second, it would permit an accurate level of quantification such that the concentration would be within the minimal detection limit of the analytical tool used.

Three different concentrations (8 μg/mL, 4 μg/mL, 1.6 μg/mL) were examined for their inhibitory effect Although the lowest of the three (1.6 μg/mL) was a thousand fold higher than what was detected in municipal wastewater, we didn't test a lower concentration was not tested because using a concentration lower than 1.6 μg/mL would have made it hard to distinguish it from the noise in chromatograms.

After running the test several times only three runs (Table 4.1) were accepted as they satisfies the test guidelines. When performing the experiment, the oxygen uptake rate of the two controls and each of the three test samples was measured and by using Equation (3.1), the inhibition effect was calculated accordingly (Table 4.2)

$$\left\{ 1 - \frac{2R_s}{R_{c1} + R_{c2}} \right\} \times 100 = \text{per cent inhibition} \qquad (3.1)$$

Where:

R_s = oxygen-consumption rate at tested concentration of chlortetracycline,

R_{c1} = oxygen-consumption rate, control 1,

R_{c2} = oxygen-consumption rate, control 2.

Table 4.1: Oxygen uptake rate of controls and test samples

Run No.	Oxygen uptake rate at different concentrations (µg/mL)					
	MLSS	R_{c1} [a] (mg/g)/h	R_{c2} [a] (mg/g)/h	$R_{8\ µg/mL}$ (mg/g)/h	$R_{4\ µg/mL}$ (mg/g)/h	$R_{1.6\ µg/mL}$ (mg/g)/h
1	1.14	21.55	24.64	13.62	13.88	15.13
2	2.78	20.24	22.06	12.08	12.64	15.11
3	2.81	23.49	21.35	11.96	12.51	14.95

[a] control oxygen uptake rate

From the results obtained (Table 4.2) the inhibitory effect of the three (8 µg/mL, 4 µg/mL, 1.6 µg/mL) tested concentrations of chlortetracycline over the microbial community were 42.73% (± 1.14), 40.57% (± 0.75), 31.07% (± 2.50), respectively.

Table 4.2: Inhibitory effect of CTC at different concentrations

Run No.	%Inhibition at different concentrations		
	$\%I_{8\ µg/mL}$	$\%I_{4\ µg/mL}$	$\%I_{1.6\ µg/mL}$
1	41.10	39.90	34.50
2	42.90	40.20	28.50
3	44.18	41.62	30.22
Average	**42.73**	**40.57**	**31.07**

Based on the data, a concentration of 5 µg/mL was to be used throughout the experiments. The inhibitory effect at 5 µg/mL, was estimated (from Fig. 4.1) to be 42 %, meaning that the level of activity was still around 58%.

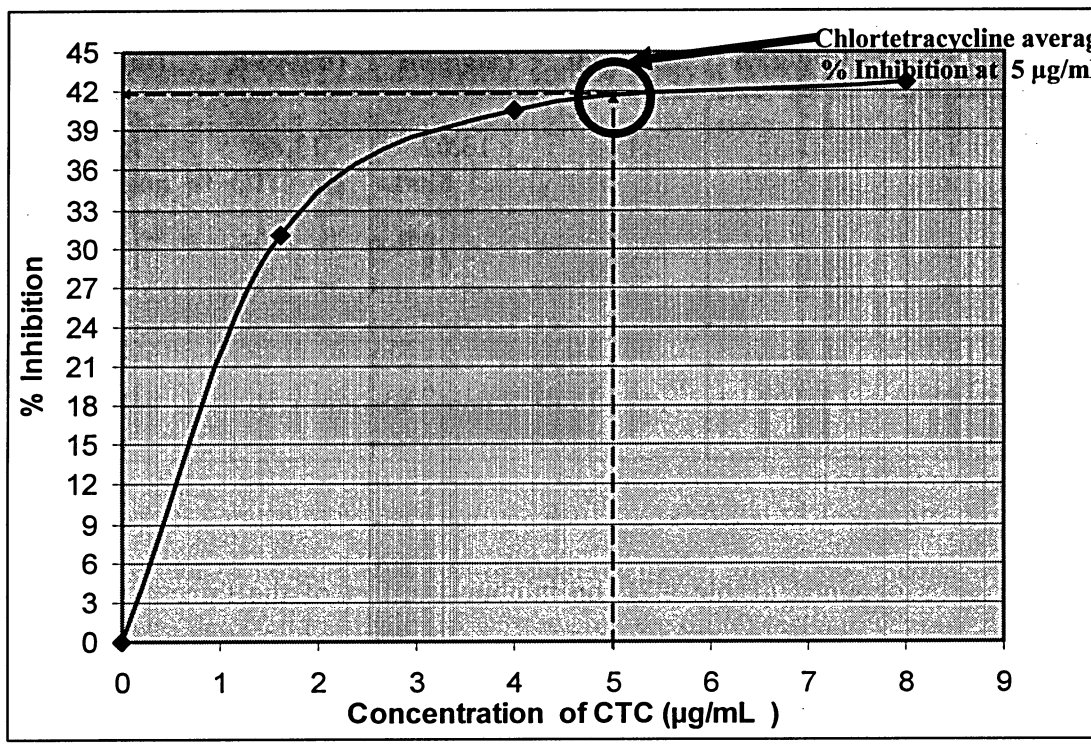


Fig. 4.1: Average % Inhibition vs. Chlortetracycline concentration (µg/mL)

4.2 Determining Chlortetracycline from the Chromatograms

Samples after being extracted and cleaned using solid phase extraction (SPE), were analyzed with reverse phased high performance liquid chromatography (RP-HPLC) where a reversed- phase C_8 (150 x 4.6mmI.D., 5µm particle size) analytical column and a UV detector (λ= 264 nm, 0.05 absorbance unit full scale) were used. Typical chromatograms of extracts obtained from standard solution, and chlortetracycline-spiked samples are shown in Figs 4.2 and 4.3 respectively. The chromatographic peaks of chlortetracycline were well resolved, and the retention time for CTC was 5.97 min (± 0.84). These results are consistent with other published results using the same

chromatographic tool (Posyniak et al, 1998). Figure 4.3 shows a representative chromatogram for the detection of chlortetracycline in the bulk water of a spiked sample.

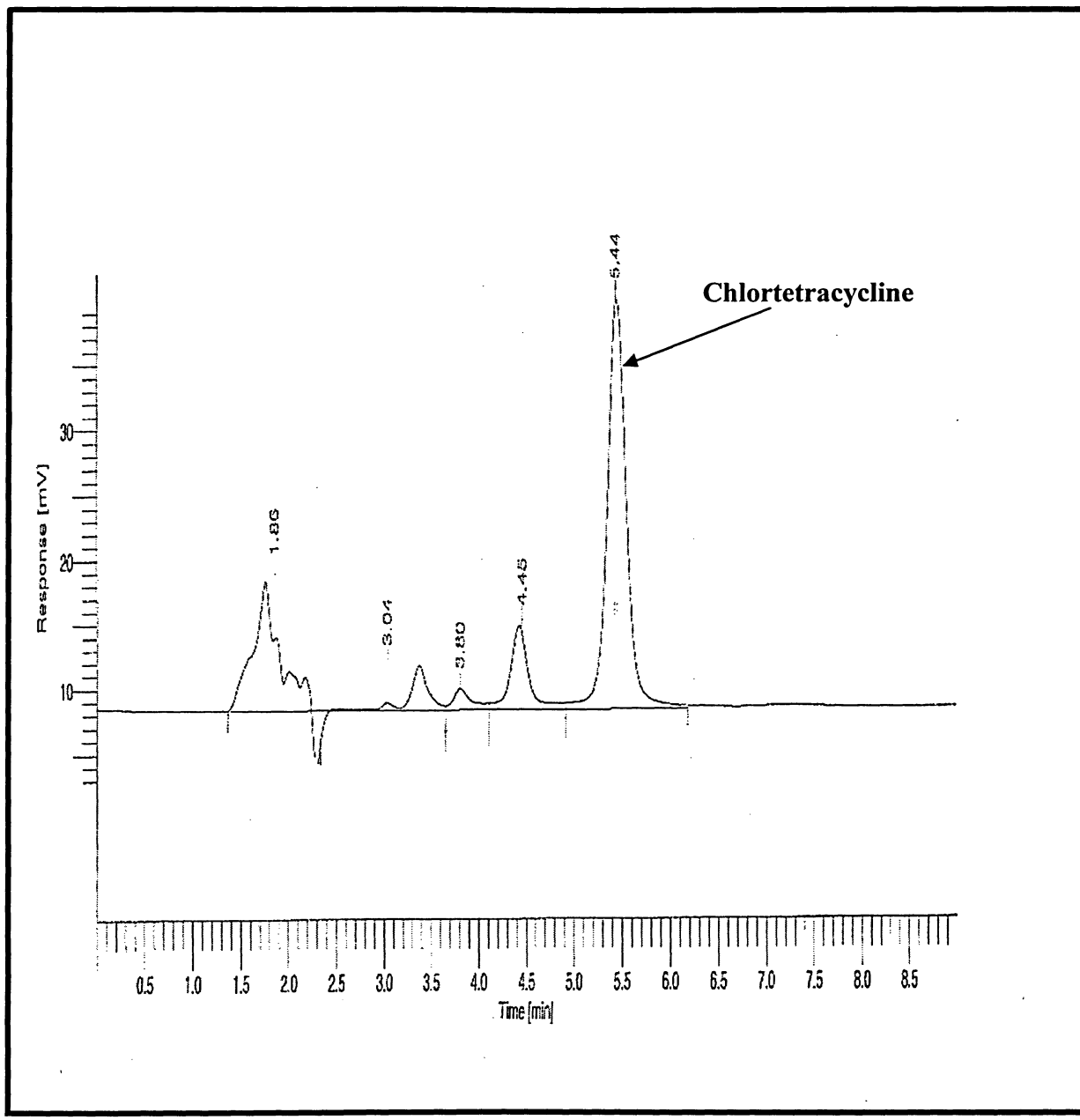


Fig. 4.2a: Chromatogram of standard CTC solution.

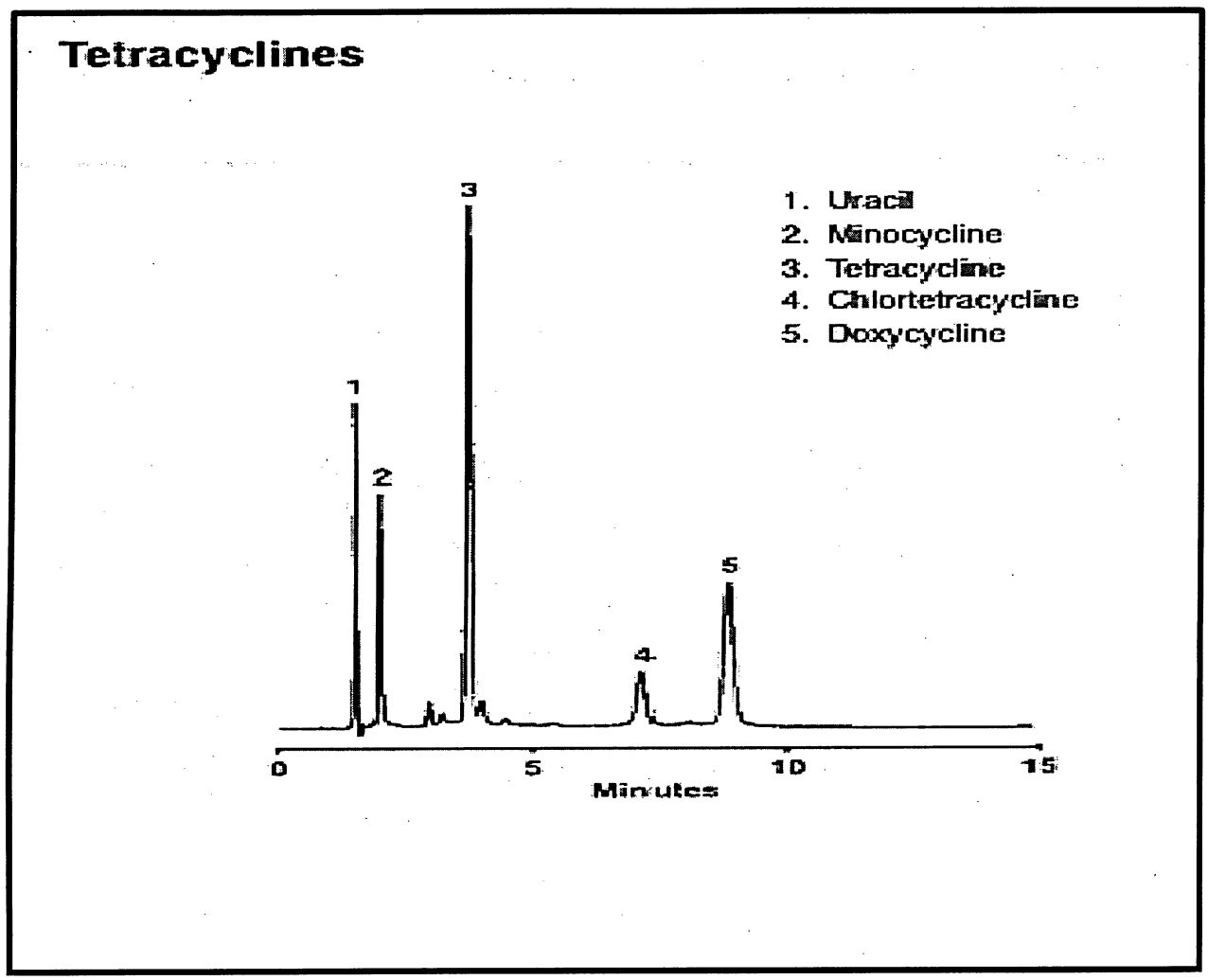
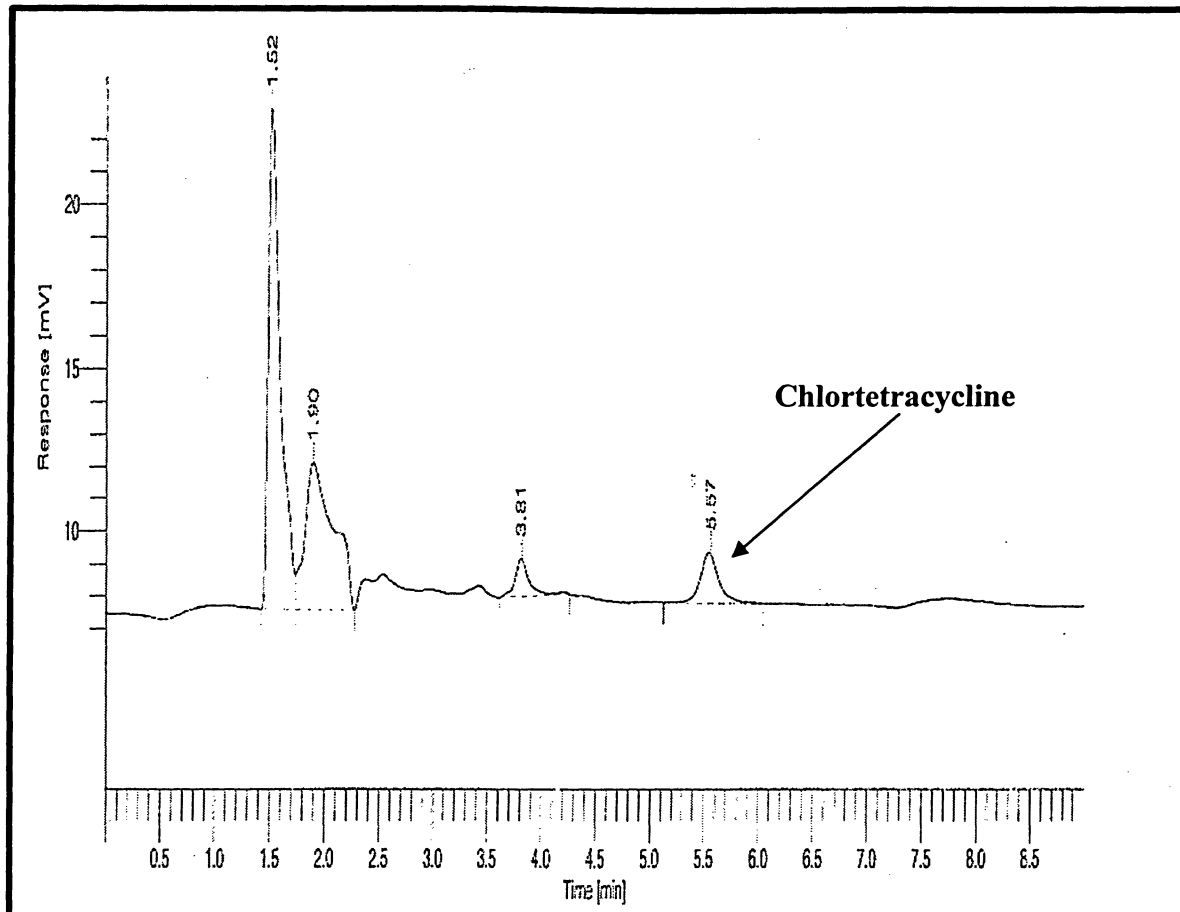


Fig. 4.2b:Chromatogram of standard TCs solution as provided by manufacturer.

Figure 4.2b is the chromatogram provided by the manufacturer showing the different retention times for tetracyclines. In it the retention time for chlortetracycline is around 7 minutes, it is also obvious that tetracyline's retention time is around 4 minutes indicating that if present in the sample it will appear before the chlortetracycline in the chromatogram.



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.515	116841.60	15340.11	51.51	51.51	BV	7.6167
2	1.897	81181.80	4544.42	35.79	35.79	VB	17.8641
3	3.810	10528.09	1163.98	4.64	4.64	BB	9.0448
4	5.568	18295.80	1484.92	8.07	8.07	BB	12.3211
		226847.20	22533.43	100.00	100.00		

Fig. 4.3: Chromatogram of CTC in the bulk water

Because the retention time tends to shift a little with each run, due to the acidic conditions in which the samples were run. With each batch of samples analysed, two standards were run, one at the beginning and the other at the end, hence compensating for

the shifts that could occur for the retention times of the samples to make sure of the accuracy of detection. The concentration in the sample was calculated based on equation (3.5), where the area under the curve for CTC as provided with the "Default Report" was divided over that provided for the standard solution and then multiplied by the concentration in the standard (5 μg/mL). In the specific example illustrated in figures 4.2a and 4.3 the concentration in the bulk water of the sample is 0.23 μg/mL.

4.3 Biodegradability of Chlortetracycline

Tetracyclines are relatively persistent antibiotics; several studies show that they are not readily biodegradable (Ingerslev et al, 2001; Alexy et al 2004). Consequently if chlortetracycline is to be introduced to a mixed microbial community as the sole carbon source under aerobic conditions, it is expected that the antibiotic would not be degraded biologically given that antibiotics are used because of their ability to kill microbes this makes these compounds hard to biodegrade. To test for the biodegradability of CTC, the antibiotic was introduced to the sludge sample as the sole substrate, under aerobic conditions. It was noted that the amount of CTC present in the aqueous phase after 24 h run was 2.34 μg/mL ± 0.34, where it initially started with 5 μg/mL. The amount quantified in the bulk water suggests a removal of 46.7% of the initial concentration. The SOUR of the samples was 3.68 (mg O_2/ g MLSS)/h, which indicates low microbial activity. This indicates that the removal from the bulk water could be attributed to something else other than biodegradation. This experiment served as a positive control.

4.4 Adsorption of Chlortetracycline

The literature suggests that the main removal pathway of the drug is through sorption. And as noted when CTC was spiked to the sludge sample, almost 50% removal from the bulk water was recorded, although there was minimal activity. Subsequently, to test the removal of CTC through an abiotic mechanism, 5 μg/mL of the antibiotic were added to the sludge sample under aerobic conditions, in the presence of sodium azide (800 μg/mL)

as a respiration inhibitor that de-energizes the bacterial cytoplasmic membrane potential (i.e., proton motive force [PMF]) and is known to cause increases in cellular accumulation of various substances, including certain antibiotics (i.e., tetracyclines, fluoroquinolones, and some b-lactams) (Levy, 1992; Nikaido, 1996). The increased accumulation occurs because PMF-dependent multidrug efflux pumps become inactivated in de-energized cells (Nikaido; 1996). Thus, these amphipathic antibiotics are no longer subject to active efflux out of the cells, resulting in the higher cellular antibiotic levels (Nikaido; 1996). After 24 h the final concentration of CTC in the bulk water was 2.09 μg/mL ± 0.80 indicating a removal of 41.7%, an amount very close to the removal recorded when there was no inhibitor present, and only the antibiotic was present as a sole carbon source. Whereas the amount of CTC present in the EPS was 0.41 μg/mL ± 0.03 which is 8.2% of the initial concentration. The amount of the antibiotic that might have been present inside the cells was not investigated under these conditions. Yet the theoretical adsorbed amount could be calculated by applying Equation (3.6),

$$Cs = \frac{(C_0 - C_e) \bullet V}{C_B \bullet V}$$

Where; C_B is the biomass concentration,
V is the solution volume,
C_0 is the initial Chlortetracycline concentration and,
C_e is the final Chlortetracycline concentration in the liquid phase after 24 h of equilibration. The final concentration in the liquid phase, C_e, was determined using HPLC

Given that CTC concentration measured in the bulk water (2.09 μg/mL) after 24 h is the equilibrium concentration present in the bulk water, the mixed liquor suspended solids is 1.68 g/L. This yields a theoretical adsorbed $(Cs)_{theoretical}$ value of chlortetracycline of 1.74 μg/mL. In light of what has already adsorbed to the EPS, the amount which would have accumulated theoretically inside the microbial cells would be around 1.33 μg/mL; this is equivalent to 26.5% of the initial concentration. The above mentioned Experiment served as our negative control, and shows that adsorption does play a major role in the removal of the antibiotic from the bulk water.

Since all biodegradation experiments were performed in the presence of another carbon source, the removal of CTC was also measured under suppressed microbial activity using

sodium azide (800 μg/mL), but in the presence of a biodegradable substrate (glucose/acetate), where the F/M ratio was 1.01. The distribution of the antibiotic in the bulk water, the EPS, and in the microbial cells was: 0.45 μg/mL (± 0.29), 0.36 μg/mL (± 0.014), and 3.96 μg/mL (± 0.09) respectively, which accounts for 95.2% (4.80 μg/mL) of the initial concentration, given that only the concentration of parent compound was accounted for and none of its transformation products. The amount present in the bulk water indicates a removal of 91.1%; which is much higher than the removal when the CTC was present in the sample as the sole carbon source, thus indicating that the transport of the antibiotic maybe associated with uncoupled metabolism. In both cases the amounts that accumulated in the EPS were very close. In the case where CTC was the only carbon source, 0.41 μg/mL was found in the EPS while in the presence of another substrate there was 0.39 μg/mL, once again indicating that the main difference is in the amounts that reside in the cells and those remaining in the bulk water. The results could be related to the effect of sodium azide has on microbes in the presence of two types of carbon a substrate (glucose/ acetate) and a toxic material in our case CTC. It is known that sodium azide is a compound known to eliminate bacterial growth by inhibiting cytochrome oxidase and ATPase, and it therefore inhibits respiration and uncouples the cell membrane (Harold; 1972). It facilitates diffusion of protons across the plasma membrane, which is otherwise largely impermeable to them (Harold; 1972). It is thus a reasonable working hypothesis that the inhibition of active transport by sodium azide may be due to its ability to collapse gradients of pH and of electrical potential across the membrane.

4.5 Co-metabolism of Chlortetracycline

Co-metabolism entails the metabolic transformation of a substance while a second substance serves as primary energy or carbon source. Because in wastewater treatment systems, CTC would be present in addition to more biodegradable chemicals, it was important to investigate the uptake of the antibiotic the presence of a biodegradable substrate in order to test for the possibility of co-metabolism as another pathway of degradation. After the spiked sludge samples (n = 6) with 5 μg/mL CTC were run for 24

h under aerobic condition where the F/M was 1.15, the final concentration of the chlortetracycline was measured in the bulk water, in the EPS and inside the microbial cells. When calculating the theoretical adsorbed CTC using Eq. (3.6), where C_e is 2.00 µg/mL and mixed liquor suspended solids is 1.19 g/ L MLSS the value of $(Cs)_{theoretical}$ is 2.51 µg/mL. The results in table 4.3 show that only 1.21µg/mL were accounted for, which is 48.3% of the theoretical value expected to be sorbed to the biomass, but that is due mainly to the fact that we were only accounting for the parent compound and none of its isomers or *epi*-isomers.

Table 4.3: Distribution of CTC in different compartments, Co-metabolism experiment*

Compartment	Concentration (µg/mL)	SD (±)	Percentage of the initial concentration (%)
Bulk water	2.00	0.64	40.1
EPS	0.42	0.21	8.3
Cells	0.80	0.33	15.9
Total	**3.22**		**64.32**

* All concentration measurement were performed after 24 h run using HPLC-UV

The percent of CTC present in the EPS compared to that in the water is 20.7%, while that in the cells compared to the CTC concentration in the bulk water is 39.7%. Yet the amount in the bulk water is double the amount sorbed to the biomass, and a mass balance of the parent compound as measured by the HPLC-UV accounts for only 64.3% which means 35% is still not accounted for. That amount could be partially the presence of the other isomers, but also it is possible that the antibiotic was co-metabolised.

4.6 Differences in Distribution of Chlortetracycline in Active and Inhibited Biomass samples

The behaviour of CTC removal when spiked to an active biomass or inactive floc (see Figure 4.4) is different under both conditions and. Results show that the there is statistically significant difference between the distribution of CTC in the three compartments; bulk water, EPS, and microbial cells, when acted upon by active and inactive biomass. The main difference lay in the portions accumulated in the microbial cells and that left in the bulk water. The amount present in the EPS in both cases seems to be the lowest of the three compartments though it's higher in the case of inactive biomass compared to that of an active microbial community but not really very significant (p = 0.2) .

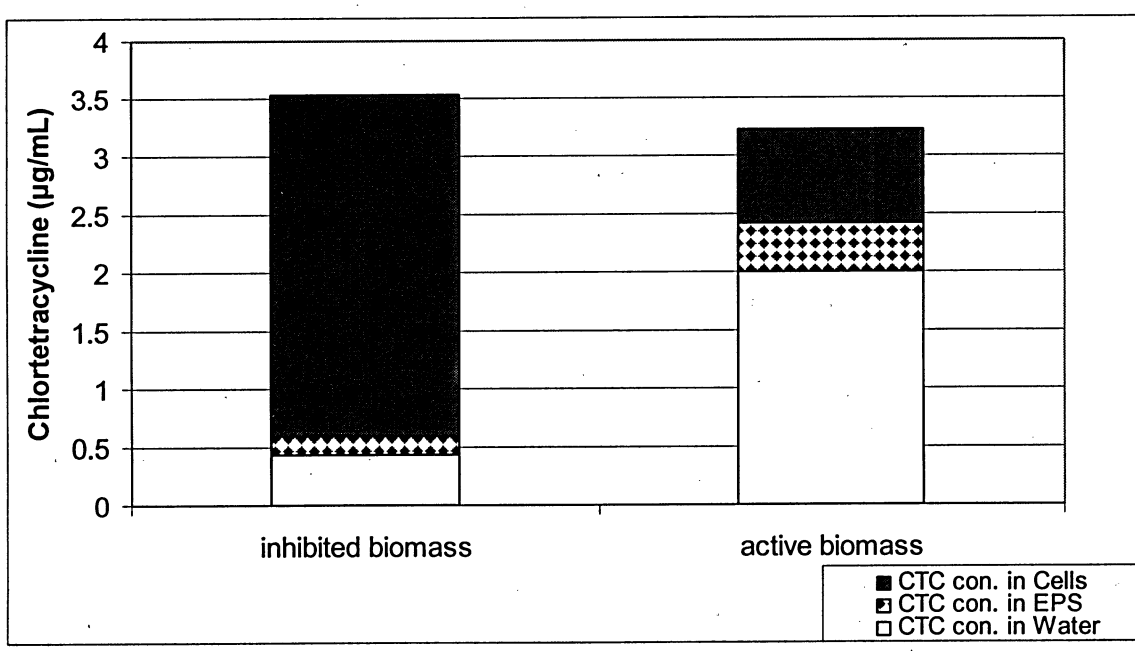


Fig. 4.4: CTC concentration in three compartments in active and inhibited biomass samples after 24 h

The removal of the antibiotic from the bulk water and its accumulation in the microbial cells as shown in figure (4.5) reflects two opposite behaviours. The difference is statistically significant when performing a one tail student t-test (p = 0.0007, 0.03 respectively) and can be explained by the fact that initially a passive uptake of CTC by

binding to cell components such as phospholipids or proteins (Argast and Beck; 1984) takes place leading to its accumulation in the cell. Yet, in the case of an active microbial floc the accumulation of the antibiotic would decreases in the cells since it would be pumped out of via an energy dependent mechanism. While in the case of inactive biomass the mechanism of the energy-dependent transport of chlortetracycline from the cells, as in the case when metabolic energy was suppressed by the use of sodium azide, there is no driving force to efflux the antibiotic out. The removal of the antibiotic from the bulk water depends inversely upon activity i.e. the more active the microbial community the less the removal from the bulk water. The findings also suggest that the antibiotic undergoes only primary biodegradation where the parent compound is transformed to other products such as tetracycline indicating that a de-chlorination reaction might be taking place.

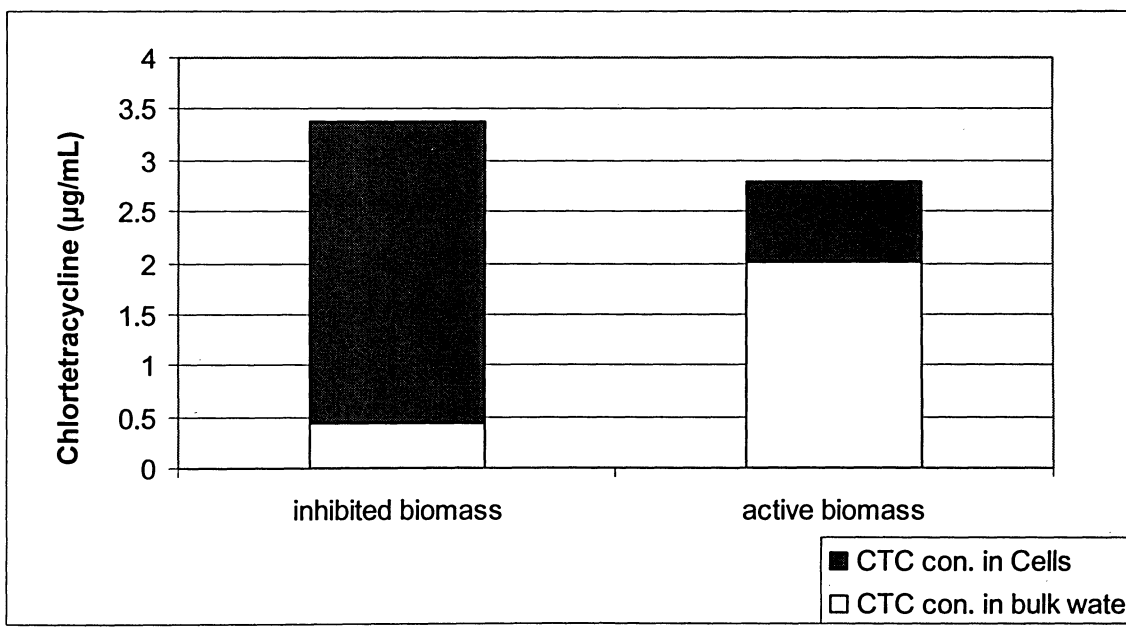


Fig. 4.5: CTC concentration in bulk water vs. the microbial cells in active and inhibited biomass samples after 24h

The comparison between the concentrations of CTC in the EPS verses what amassed in the microbial cells (Fig. 4.6) in the case of an active floc and a metabolically restricted microbial community indicate that what is present in the EPS is almost half of that in the

cells when there is an active microbial floc, while that ratio is much lower in the case of an inactive microbial floc. The amount of CTC in the EPS in the later case reaches no more than 0.05 of what accumulated in the cells.

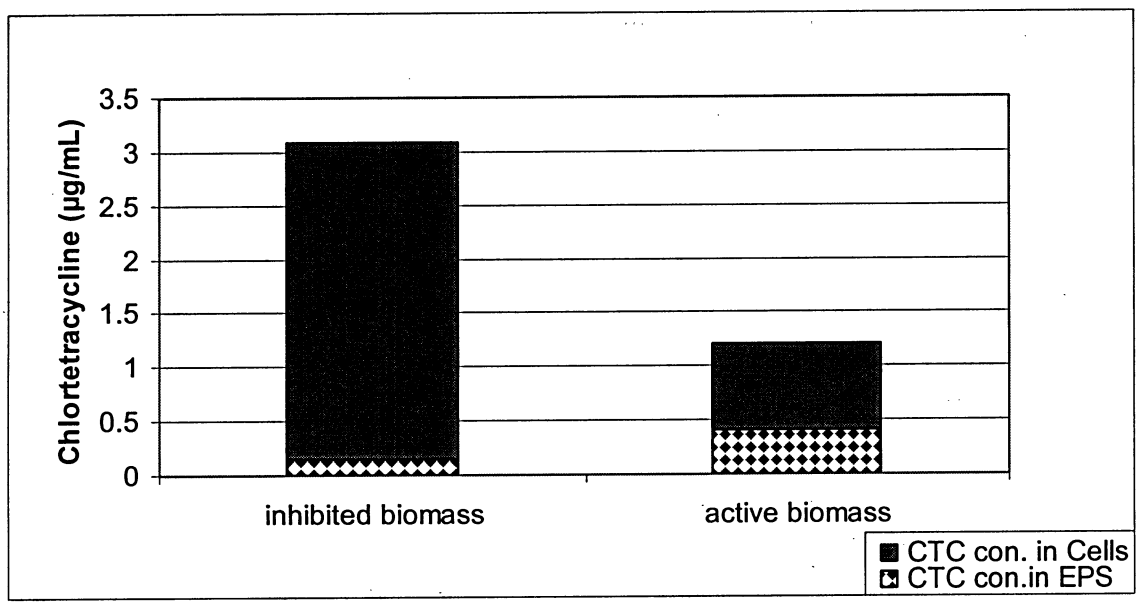


Fig. 4.6: CTC concentration in the EPS vs. the microbial cells in active and inhibited biomass samples after 24 h

4.7 Chlortetracycline Removal under Aerobic and Anaerobic Conditions

The removal of chlortetracycline was measured under different conditions over a period of two weeks. All experiments were run in the dark to eliminate the possibility of photo-degradation. A summary of the results is shown in figure (4.7).

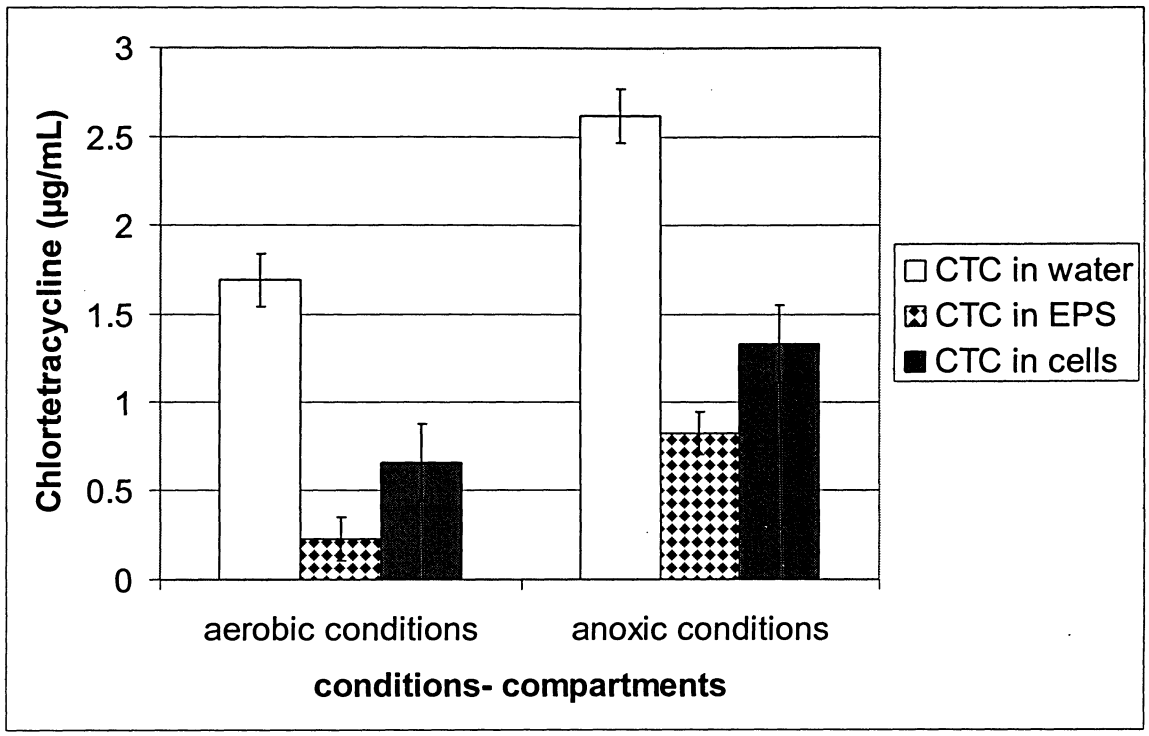


Figure 4.7: CTC concentration under aerobic and anoxic conditions after 14 days

4.7.1 Chlortetracycline Removal under Aerobic conditions

The samples were run in duplicates and analyzed in triplicates. The MLSS of the samples was 0.91 g/ L MLSS (SD ± 0.07) the specific oxygen uptake rate was 3.6 mg O_2/ L (SD ± 0.29), the F/M ratio was 1.325 (SD ± 0.105). And a pH range of 7.65 - 8.3. According to the results in Table 4.4 only 50.8% were accounted for of the initial concentration of the parent compound (see Fig. 4.8 below) any transformations were not accounted for, even though the likelihood of some degree of biodegradation is still possible.

Table 4.4: Distribution of CTC in three compartments under aerobic conditions*

Compartment	Concentration (μg/mL)	SD (±)	Percentage of the initial concentration (%)
Bulk water	1.69	0.15	33.8
EPS	0.23	0.13	4.6
Cells	0.62	0.23	12.4

* All concentration measurement were performed after 14 days using HPLC-UV

However, the percent of what was present in the cells compared to that of present in the bulk water is 36.7%, which was close to that when the sample was run over 24 h only. Whereas that present in the EPS compared to what was left in the bulk water is 13.6% which is two thirds of the ratio under aerobic conditions when the sample was run over a short period of time. In the latter case the reaction was run for 14 days there was no replenishment of the substrate, thus microbial community is not as viable as that when the sample was run over a short period of time, hence the EPS as indicated in the literature must be different in structure (Liao et al; 2002) and viability from that run over a period of 24 hours.

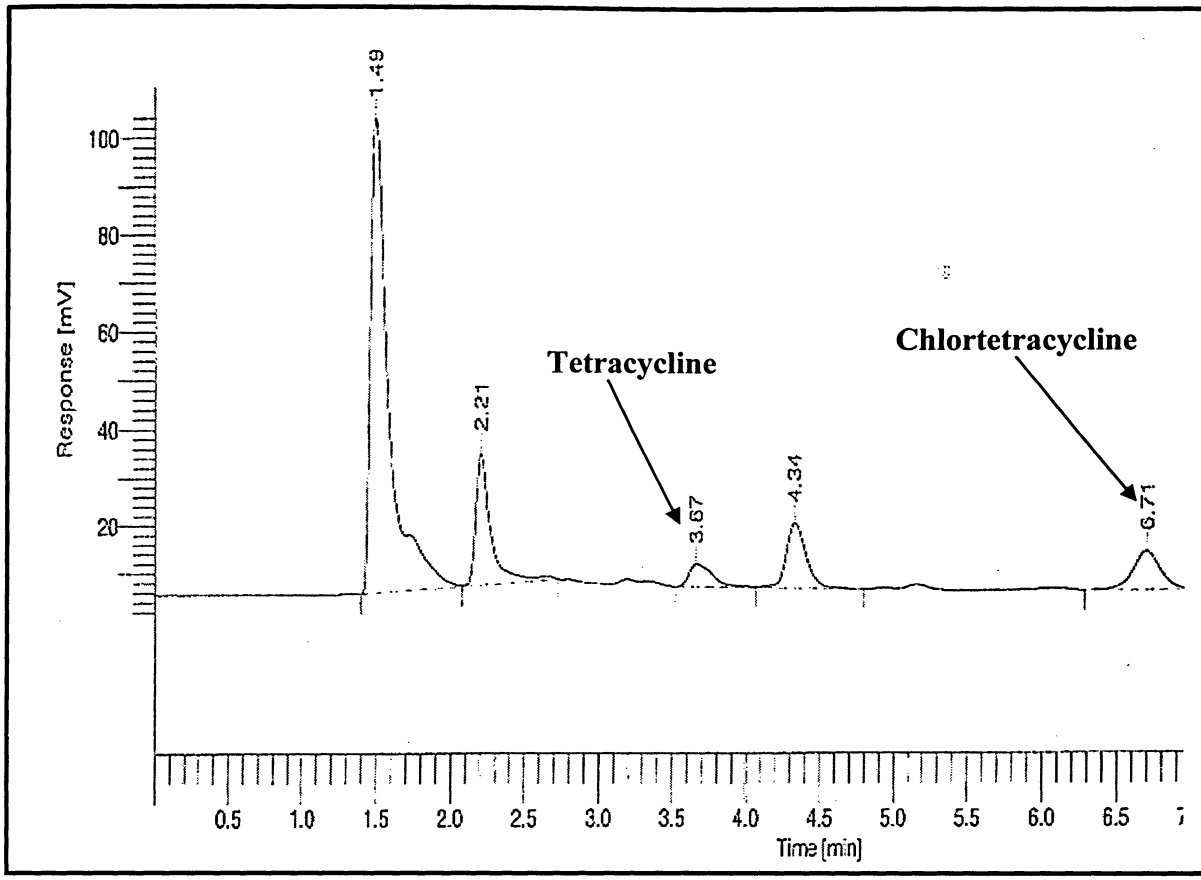


Fig. 4.8: Chromatogram of CTC in the bulk water under aerobic conditions (A chromatogram of a bulk water sample after 14 days)

4.7.2 Chlortetracycline Removal under Anoxic conditions

Samples that were left in closed Erlenmeyer flasks wrapped in aluminium foil for two weeks to investigate the removal and distribution of chlortetracycline under anoxic conditions. The mixed liquor suspended solids of the samples was 0.94 g / L MLSS, the F/M ratio was 1.28, and the pH range was 7.5 – 8.0. The distribution of the antibiotic was determined (Table 4.5) but accounting only for the parent compound and not any of its transformations (see Fig. 4.9 and Fig 4.10) we were able to account for 95.5% of the parent compound.

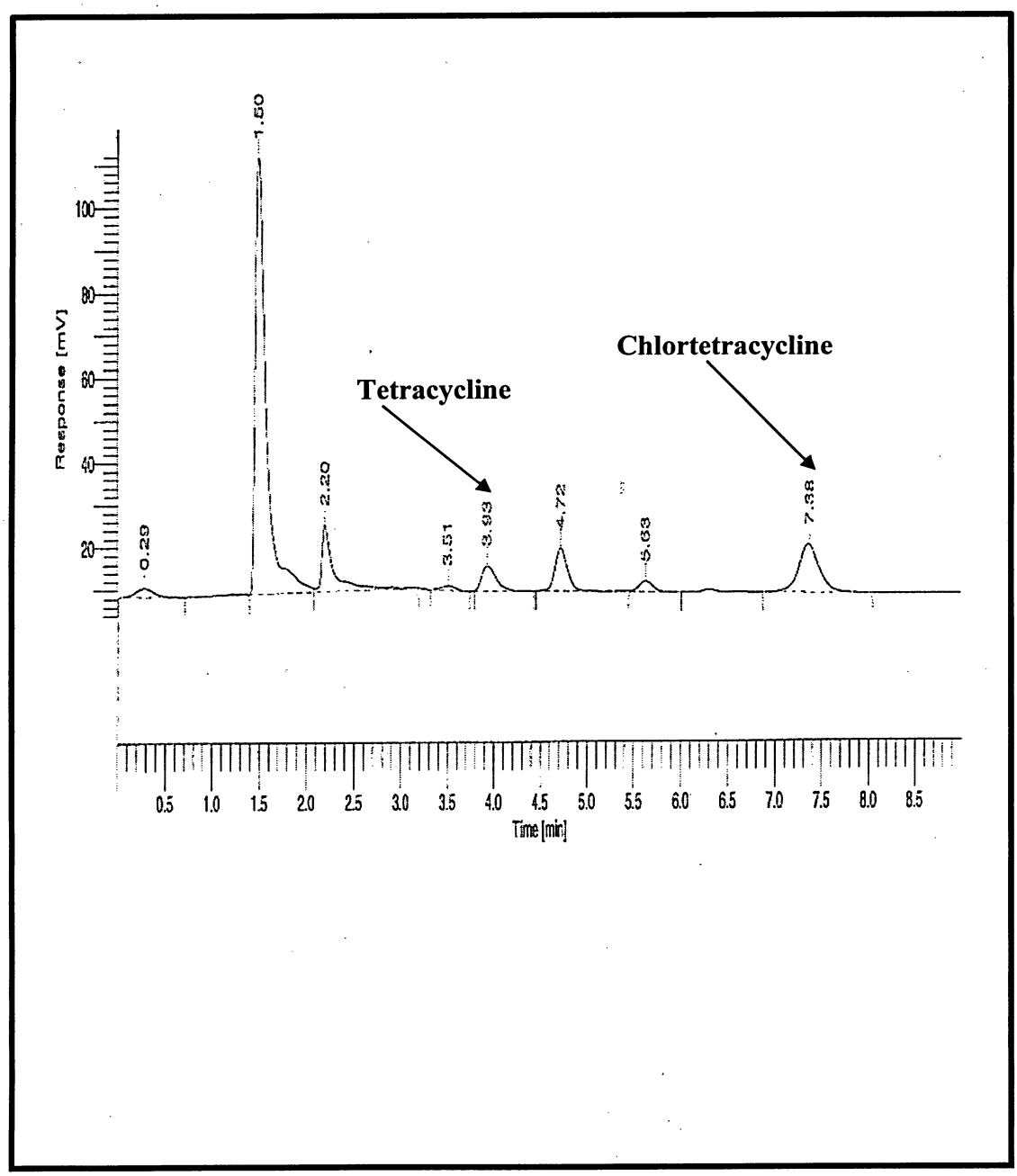


Fig. 4.9: Chromatogram of CTC in the bulk water under anoxic conditions (A chromatogram of a bulk water sample after 14 days)

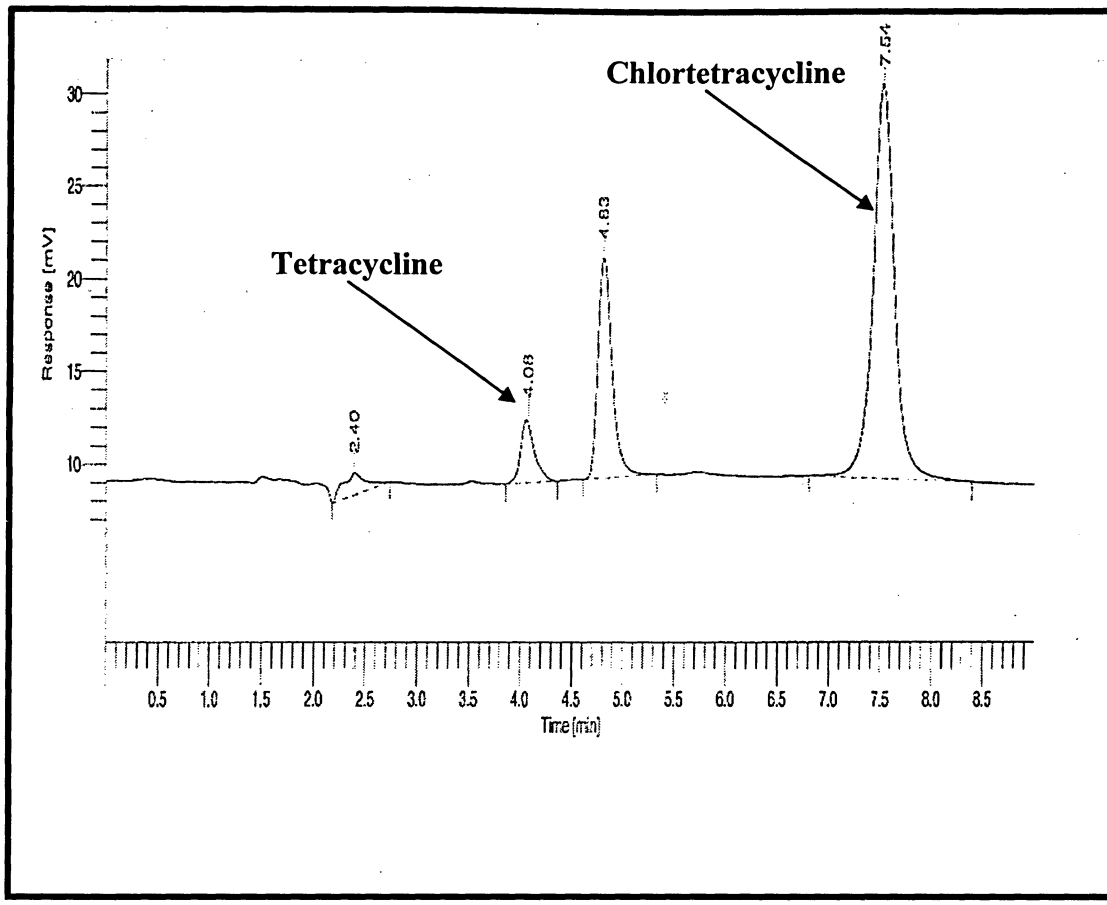


Fig. 4.10: Chromatogram of standard CTC solution.

The percent of what was on the EPS compared to that left in the water was 31.7% which is close to but lower than the percent (35.3%) between two compartments when microbial activity was restricted. While the ratio of what accumulated in the cells compared to that left in the water was 1:2, in comparison the same ratio under restricted microbial activity was 9:1 which gives a very different value, although in both cases there was minimal microbial activity or none. Despite the fact that in each of the two cases the limiting factor is different, in the former case the microbial community was deprived from oxygen hence there was minimal growth and less drive for the antibiotic to accumulate in the cells, while in the latter the bacterial cytoplasmic membrane potential was de-energized thus deactivating the efflux pumps leading to high accumulation in the cells.

Table 4.5: Distribution of CTC in three compartments under anoxic conditions*

Compartment	Concentration (µg/mL)	SD (±)	Percentage of the initial concentration (%)
Bulk water	2.62	0.44	52.4
EPS	0.83	0.2	16.6
Cells	1.33	0.51	26.6
Total	**4.78**		**95.6**

* All concentration measurement were performed after 14 days using HPLC-UV

4.7.3 Distribution under Aerobic and Anoxic Conditions, a Comparison.

The distribution of CTC in the bulk water, the EPS and the microbial cells was different, as shown in Tables 4.4 and 4.5. It was clear that the accumulation in the cells and the EPS was much higher under anoxic conditions compared to that under aerobic conditions. Whereas the amount present in the bulk water under aerobic conditions tended to be much higher than under anoxic conditions.

Comparing the three compartments to each other under the different conditions was statistically significant. The concentration of the antibiotic in the bulk water as shown in Figure (4.11) was lower under aerobic conditions compared to that under anoxic conditions. Applying the student t-test shows that the difference was statistically significant (p = 0.004). Suggesting that under aerobic conditions the amount of CTC adsorbed was much less than that under anoxic conditions, which could be highly associated with the microbial community activity and its resistance to the antibiotic.

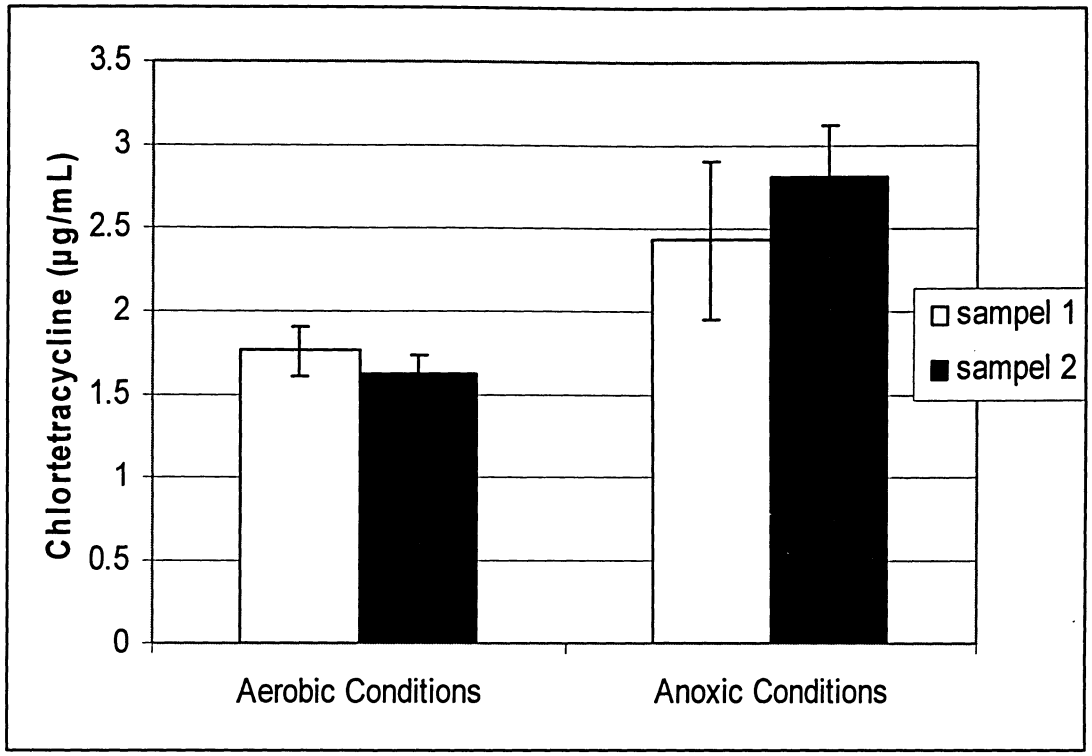


Fig. 4.11: CTC concentration in bulk water under aerobic and anoxic conditions after 14 days

The values of what accumulated in the EPS under aerobic in contrast to those anoxic conditions (Fig.4.12) show that the later was four folds higher, this significant difference between the two conditions was displayed in the results of the student t-test ($p = 0.003$). The higher accretion on the EPS may be due to several reasons; either the distribution of the surface charge of the EPS under anoxic conditions was different. Or the whole structure and architecture of the EPS went under changes and shifts under anoxic as apposed to aerobic conditions, hence providing more sorption sites which could explain the higher accumulation of CTC in the EPS under anoxic conditions. Clearly the EPS matrix's role as a physical barrier to CTC penetration was dependent to a large extent upon the binding capacity of the matrix, and the growth rate of the microbial community relative to the diffusion rate, in addition to the effect of the environmental parameters (i.e. temperature, pH) on the behaviour of the antibiotic.

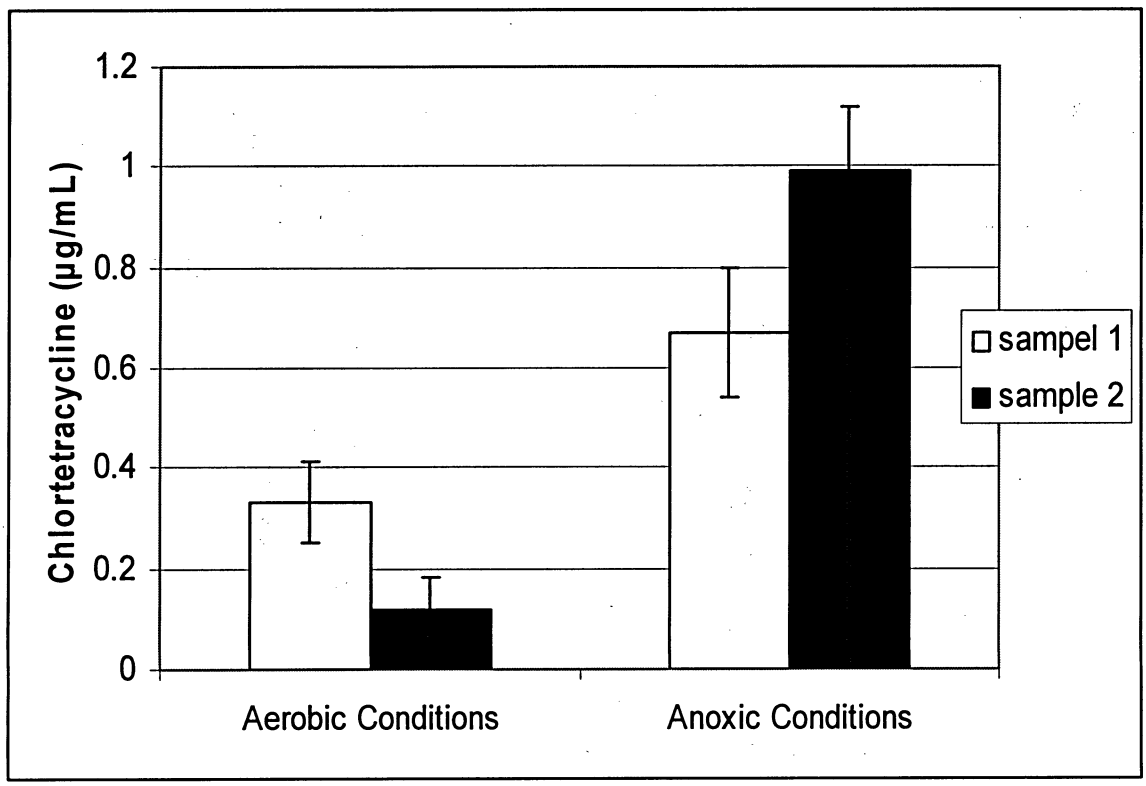


Fig. 4.12: CTC concentration in the EPS under aerobic and anoxic conditions after 14 days

The amount of CTC amassed in the microbial cells is twice as high under anoxic condition (fig.4.13) compared to that under aerobic conditions. A significant difference (p = 0.03) that can be attributed to the fact that CTC efflux out of the microbial cells is energy dependent. Therefore, in the case of anoxic condition where respiration is minimal the efflux mechanism is deactivated which results in a build up inside the cells.

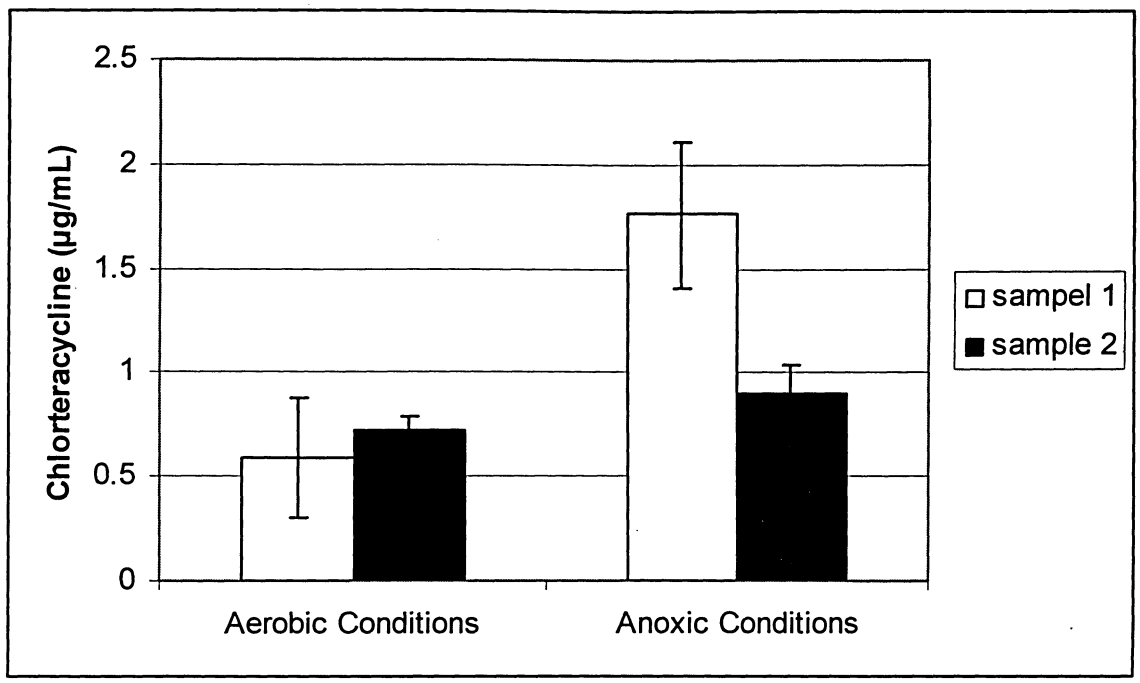


Figure 4.13: CTC concentration in the microbial cells under aerobic and anoxic conditions after 14 days

It is clear from the data that removal of CTC via sorption is higher in the absence of microbial activity. In addition, it was obvious that the resistant mechanism was mainly dependent on the efflux out of the cells and not on deactivating the antibiotic, because the parent compound was still being detected in addition to some of its *epi-isomers*, mainly what was noted was transformation of the parent compound which could be attributed to the instability of the drug given the temperature and the pH of the medium not the microbial activity.

4.8 The Divalent Cation Effect

By examining the distribution of the antibiotic in the bulk water, the EPS, and the microbial cells, it was clear that the portion with the least accumulation of the three was the EPS, which raises the question on how does the antibiotic manages to bypass the EPS and accumulate in the microbial cells, or in other cases as it was pumped out of the cells it just goes to the bulk water with only a small portion being trapped on the polymeric

substance. Another observation was that the portion of CTC in the EPS under anoxic conditions was almost four folds higher than that under aerobic conditions. These different sorption distributions suggest the need to further investigate the factors affecting the adsorption of CTC. Given that cationic interactions play a major role in the sorption mechanism, it was important to examine the effect of introducing a strong chelating agent such as EDTA on the sorption, and distribution of CTC.

4.8.1 Addition of EDTA to Inactive Biomass

Samples that ran for 24 h under aerobic conditions and inhibited respiration, were washed then reactivated by the addition of substrate and incubated with different concentrations of EDTA: 50 µg/mL, 150 µg/mL, 300 µg/mL, in addition to a control, the samples were run for three hours then the CTC was measured in the bulk water, the EPS and the microbial cells as shown in Figure (4.14). Only the parent compound was accounted for. The mixed liquor suspended solids was 0.74 g/ L MLSS, the SOUR was 7.98 mg O_2/ L, and the pH 7.48.

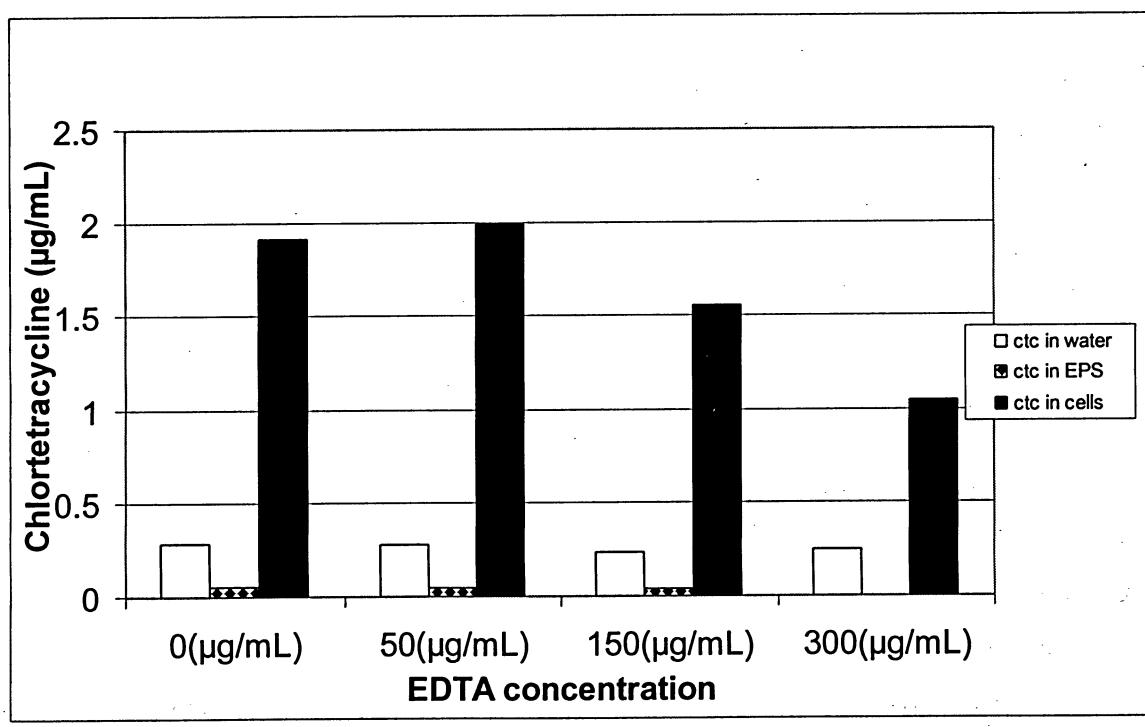


Figure 4.14: CTC concentration in three compartments after incubating in different EDTA concentrations after suspension in a new substrate for 3 h

The samples when re-suspended in the substrate (glucose/ acetate) the only chlortetracycline present in the system was the amount adsorbed to the biomass; in the EPS and the microbial cells, after the three hour reactivation the control shows that around 0.29 μg/mL was pumped out of the cells which is 12.7% of the initial amount that was sorbed to the biomass. It was clear with regards to the distribution in the bulk water, the EPS, and the microbial cells that the there was no significant difference (p = 0.49) between the control and the samples incubated in 50 μg/mL EDTA. Whereas when the concentration of the EDTA increases the amount present in the EPS starts to decrease, the decrease in the antibiotic concentration in the bulk water and the cells was significant as the chelating agent's concentration increased (p = 0.00011). The decrease in the CTC concentration in the EPS was expected since in the presence of another competing chelating agent that binds to the divalent cations present in the EPS would lower the amount of CTC present in the EPS. It was also expected in consistence with overall mass balance that as the amount in the cells and EPS decrease the amount in the bulk water should increase, yet that was not what was apparent in the results provided in Table 4.6.

Table 4.6: CTC distribution in three compartments after incubation in different EDTA concentrations*

EDTA Concentration (μg/mL)	CTC con. in bulk water (μg/mL)	CTC con. in EPS (μg/mL)	CTC con. in microbial cells (μg/mL)	Total concentration (μg/mL)
0	0.29 ± 0.07	0.06 ± 0.002	1.91± 0.19	2.26
50	0.28 ± 0.050	0.05 ± 0.007	1.99 ± 0.09	2.32
150	0.29 ± 0.06	0.04 ± 0.006	1.56 ± 0.030	1.88
300	0.25 ± 0.1	Nd**	1.05 ± 0.09	1.30

* All concentration measurement were performed after suspending the samples in a new substrate for 3 h using HPLC-UV
**Not detected

4.8.2 The Import Effect

EDTA is known to remove some lipopolysaccharide from the outer membranes of cells (McMurry et al; 1983), making them more permeable to lipophilic substances. In addition it is a strong chelating agent known for its effect on the stability of the microbial floc by breaking flocs linked by salt bridges and affecting ionic interactions through the removal of divalent cation. Thus EDTA would be a suitable substance to be used to test the import effect. Samples under aerobic conditions and active microbial community were tested for the effect of the presence of EDTA on the distribution of CTC; the concentration used was 150 µg/mL over two different time periods; 30 min and 24 h. In the case of the 30 min period the 150 µg/mL EDTA was added to the samples containing 5 µg/mL CTC after they were run under aerobic conditions for 24 hours. The samples were gently mixed using a shaker. The mixed liquor suspended solids for the samples was 0.76 g/L MLSS and the F/M ratio was 1.58. While as in case of the 24 h time the EDTA was added at the beginning of the experiments then the samples were run under aerobic conditions. The mixed liquor suspended solids for the samples was 0.84 g/L MLSS, and the F/M ratio was 1.42. Results shown in Table 4.7

Table 4.7: Distribution of CTC in presence of EDTA*

Compartment	CTC Concentration (µg/mL) Control (no EDTA)	CTC Concentration (µg/mL) Incubation in EDTA for ≈ 30min	CTC Concentration (µg/mL) Incubation in EDTA for 24 hours
Bulk water	2.10(± 0.042)	1.77 (± 0.091)	2.84 (± 0.075)
EPS	0.06 (±0.003)	Nd**	0.13 (± 0.035)
Cells	2.20 (±0.052)	1.84 (± 0.120)	2.15 (±0.090)

*All concentration measurement were performed after the conclusion of the expriments using HPLC-UV
**Not detected

When EDTA was added for a short period of time under gentle shaking there was an immediate loss of chlortetracycline from the EPS, also a decrease in what was there in the cells and in the bulk water was observed. Over a longer time period it was apparent that there was not any significant difference between the control and the sample that was incubated under aerobic conditions in 150 μg/mL EDTA. Results show that there was no import effect for EDTA given the ratio (almost 1:1) of what was in the cells to what was in the bulk water was consistent between the three samples; the control, when incubating for 24 h and when incubating for 30 min.

5.0 DISCUSSION

5.1 Distribution under Aerobic and Anoxic Conditions

When examining the distribution of CTC in the bulk water, the EPS and the microbial cells there is an apparent and significant difference between the two conditions as shown in tables 4.4 and 4.5. It is clear that the accumulation in the cells and the EPS is much higher under anoxic conditions compared to that under aerobic conditions. Whereas the amount present in the bulk water under aerobic conditions tends to be much higher than under anoxic conditions.

The concentration of the antibiotic in the bulk water was higher under aerobic conditions compared to that under anoxic conditions. Suggesting that under aerobic conditions the amount of CTC adsorbed is much less than that under anoxic conditions, which could be highly associated with the microbial community activity and its resistance to the antibiotic. Since under anoxic conditions there is no metabolic energy to facilitate the transport of CTC outside the cells, i.e. the resistance is not functioning. While under aerobic conditions the microbial community is active and able to efflux the antibiotic out of the cells resulting in a decreased amount in the cells and an increased concentration in the bulk water.

In alkaline conditions, which were the pH range of all experiments (7.48-8.70), Chlortetracycline would be negatively charged which should prevent it from penetrating the negatively charged EPS due to the electrical repulsion forces between the two. But since it seems the repulsion is not as strong to counter diffusion; it is expected that the higher concentration should be in the EPS rather than the cells, since the extracellular polymeric substance forms some kind of a physical/ chemical barrier, in addition the presence of divalent cations in the EPS that should bind the negatively charged antibiotic and deter its diffusion to the cells, thus leading to a higher concentration in the EPS than that in the cells. But what the results indicate, that CTC diffuses through the EPS and

penetrates the outer membranes of the microbial cells and resides inside, which is in accordance with the theory behind the use of antibiotics. Infections are caused by microbes and from direct observation of a wide variety of natural habitats it has been established that the majority of microbes persist attached to surfaces within a structured biofilm ecosystem and not as free-floating organisms (Costerton et al, 1995) consequently bacteria causing infections are usually bacterial biofilms somewhere in the body, and for antibiotics to cure and kill the bacteria it should be able to penetrate the biofilm and reach the bacterial cells in order to kill or inhibit their effect. Therefore chlortetracycline is most probably trapped in the EPS as it is transported out of the microbial cell; for it leaves the inner cell as a positively charged complex (Fig. 2.6), which could lead to its binding to the surface of the negatively charged polymeric substance.

Comparing the values of what accumulated in the EPS under aerobic and anoxic conditions shows that the later is four folds higher, the higher accretion in the EPS may be due to several reasons; either the distribution of the surface charge of the EPS under anoxic conditions was different, although the overall charge of the EPS is negative, the charge is distributed as both positive and negative patches all over the EPS (Wolfaardt; 2006) therefore it could be speculated that under anoxic conditions the charge distribution could become in a sense stronger than aerobic conditions thus trapping more of the positively charged complex being pumped out of the cells.

Another possible explanation could be attributed to a change and shifts in the whole structure and architecture of the EPS under anoxic as apposed to aerobic conditions, hence providing more sorption sites which could explain the higher accumulation of CTC in the EPS under anoxic conditions. Several studies support the notion that the EPS does in fact undergo some shifts in structure and composition under different environmental conditions (Morgan et al, 1990; Nielsen et al, 1996; Finlayson et al, 1998). Yet up to this point there is no clear information on the dynamics of diffusion and sorption of antibiotics to the EPS, but clearly the EPS matrix's role as a physical barrier to CTC penetration is dependent to large extent upon the binding capacity of the matrix, and the

growth rate of the microbial community relative to the diffusion rate, in addition to the effect of the environmental parameters (i.e. temperature, pH) on the behaviour of the antibiotic.

The amount of CTC amassed in the microbial cells is twice as high under anoxic condition compared to that under aerobic conditions. A significant difference ($p = 0.03$) that can be attributed to the fact that CTC efflux out of the microbial cells is energy dependent. Therefore in the case of anoxic condition where respiration is minimal the efflux mechanism is deactivated which results in a build up inside the cells. So the accumulation in the cells will continue because the cells are viable, but since there is no energy production then the opposite direction by which the cells exorcize the antibiotic is deactivated. In addition, both changes in growth rate and nutrient limitation are often accompanied by changes in cell envelope components such as fatty acids and phospholipids (Gilbert and Brown, 1978), metal cations (Boggis et al, 1979; Kenward et al, 1979), envelope proteins (Brown and Williams, 1985) and extracellular enzymes and polysaccharides (Sutherland, 1982; Ombaka et al, 1983). Also an increase in the lipopolysaccharide content as a result of changes in growth rate would result in a decrease in the uptake of compounds correlating with a decrease in susceptibility to the drug (Caldwell and Lawrence, 1986) all these factors, in turn, influence the susceptibility of the cells to antimicrobial agents. The fact that accrual in the cells under aerobic conditions is less supports the convention that microbial communities are resistant to tetracyclines in general and CTC in specific. Despite the fact that the samples that were used show there was no CTC present in the background at least within the detection limits of the HPLC-column used for separation and analysis, nonetheless the microbial community seems quite resistant, especially that the concentration used exerts a 42% inhibitory effect over the floc.

It is clear from the data that removal of CTC via sorption is high in the absence of metabolic activity. In addition it is obvious that the resistant mechanism, is mainly dependent on the efflux out of the cells and not on deactivating the antibiotic, because the parent compound was still detected in all three compartment, and no degradation of the

antibiotic was detected, mainly what was noted was a transformation of the parent compound which could be attributed to the instability of the drug given the temperature and the pH of the medium not the microbial activity.

5.2 Chlortetracycline Removal in Viable and Inactivated Biomass Samples

The behaviour of CTC removal when spiked to an active biomass or inactive floc (see Figure 4.4) resembles to a large extent the behaviour under aerobic conditions and anoxic conditions. Results show that the there is statistically significant difference between the distribution of CTC in the three compartments; bulk water, EPS, and microbial cells, when acted upon by active and inactive biomass. The main difference lay in the portions accumulated in the microbial cells and that left in the bulk water. The amount present in the EPS in both cases seems to be the lowest of the three compartments and there doesn't seem to be any significant difference ($p = 0.2$) between the amount sorbed in the presence of an inactive biomass compared to that of an active microbial community.

The removal of the antibiotic from the bulk water and it's accumulation in the microbial cells as shown in Figure (4.5) reflects two opposite behaviours. The difference is statistically significant ($p = 0.0007$, 0.03 respectively) and is explained by the fact that initially a passive uptake of CTC by binding to cell components such as phospholipids or proteins (Argast and Beck, 1984) takes place leading to its accumulation in the cell. Yet in the case of an active microbial floc, the accumulation of the antibiotic would decreases in the cells since it would be pumped out of via an energy dependent mechanism. While in the case of inactive biomass the mechanism of the energy-dependent transport of chlortetracycline from the cells, as in the case when metabolic energy was suppressed by the use of a respiration inhibitor, it is disabled in the absence of ATPase which constitutes the driving force to efflux the antibiotic out. sodium azide is known to eliminate bacterial growth by inhibiting cytochrome oxidase and ATPase, and it therefore inhibits respiration and uncouples the cell membrane (Harold, 1972). In addition it facilitates diffusion of protons across the plasma membrane, which is otherwise largely impermeable to them (Harold, 1972). Therefore it is a reasonable hypothesis that the inhibition of active

transport by sodium azide may be due to its ability to collapse gradients of pH and of electrical potential across the membrane, this effect of sodium azide over the microbial cells is the one leading to the accumulation of the antibiotic and hindering the efflux out of the cells. Since the energy for active transport of chlortetracycline is provided by the pH gradient across the cytoplasmic membrane (ΔpH) (Kaneko et al, 1985), indicating that chlortetracycline transport is an electrically neutral anti-port of protons (Ramón-García at al, 2006) and a positively charged tetracycline complex.

This apparent difference between the distribution of CTC in the microbial cells and the bulk water is in consistence with what was described in the work of McMurry and coworkers (1980). In their experiment radioactive tetracycline entered the inverted vesicles in the presence of an energy source, which actually represented what would normally be pumped out of whole cells. The results obtained demonstrated very clearly that in the presence of an energy source tetracycline accumulated in the everted membrane vesicles significantly above the external concentration. While as when an energy inhibitor was added, the accumulated tetracycline came streaming out of the vesicles. This finding showed that the drug was not accumulating in the everted membrane vesicles because of precipitation, but was clearly being pumped into the vesicle; in the absence of energy, this accumulation did not occur.

In addition, the manner by which CTC accumulates in the cells and bulk water in the presence/ absence of metabolic activity indicate two main things: that there is substantial resistance among sludge bacteria to chlortetracycline and that the removal of the antibiotic from the bulk water depends inversely upon activity i.e. the more active the microbial community the less the removal from the bulk water. The findings also suggest that the antibiotic undergoes only primary degradation where the parent compound is transformed to other products such as tetracycline indicating that a de-chlorination reaction might be taking place.

With respect to the third compartment; a comparison between the concentrations of CTC in the EPS verses what amassed in the microbial cells in the case of an active floc and a

metabolically restricted microbial community shows that what is present in the EPS is almost half of that in the cells when there is an active microbial floc, while that ratio is much lower in the case of an inactive microbial floc. The amount of CTC in the EPS in the later case reaches no more than 0.05 of what accumulated in the cells. That difference in portions between the two conditions could be attributed to the fact that in the former case there is an active efflux out of the cells, where chlortetracycline is transported as a positively charged complex, thus providing an opportunity for it to be trapped in the EPS while as in the latter case most probably what is on the EPS is an amount that was trapped due to the diffusion equilibrium between either side of the microbial cell walls which lost their permeability in the presence of sodium azide. Yet most of the CTC would be immobilized in the cells in the absence of a counter transport driving force because tetracyclines tend to accumulates in the compartment having a higher pH and a higher magnesium concentration (Yamaguchi et al, 1991b).

5.3 Effect of Prolonged Aerobic Batch Conditions on Chlortetracycline Removal

Experiments under aerobic conditions were run under batch conditions over a short time span (24 h) and a prolonged period (14 days). The former is the minimum time required to reach equilibrium between adsorption / desorption as demonstrated by Kim and coworkers (2005) while the latter resembles an extended solid retention time (Bitton, 2005). Whilst the concentration of CTC in both the bulk water and the microbial cells is reasonably close in value, the amount present in the EPS is significantly different (p = 0.067). The amount that accumulated in the EPS under 24 hours (8.3%) is double that when the sample was run for 14 days (4.6%). One main reason could be attributed to the reduced surface charge, for as the solid retention time increases the floc surface is less negatively charged which explains why under shorter time frame the amount trapped in the EPS was double that under the extended time (Liao et al, 2001). In addition the results obtained for all three compartments under a longer and shorter time confirm previous findings by Kim et al (2005) where it was found that the reduction of solid retention time while maintaining constant hydraulic retention time decreased tetracycline removal efficiency from the bulk water in activated sludge processes.

Another factor to be considered with respect to the removal and accumulation of CTC in the cells (i.e. cell resistance) is the maturity of the floc and its EPS. In a study by Anwar et al (1992) of the kinetics of biofilm formation, it was found that the population of biofilm cells of *P. aeruginosa* that adhered to a solid surface increased exponentially from days 1 to 5 and remained constant after day 5. When the biofilm bacteria were removed on day 2 (young biofilms) and exposed to two antimicrobial agents results indicated that the young biofilm cells were susceptible to these agents (Anwar et al 1989 a, b; Anwar and Costerton, 1990). On the other hand when the cells were allowed to continue the colonization of the surface until day 7 (old biofilms) and were then exposed to the two antimicrobial agents, the results indicated that old biofilm cells were very resistant to killing by these agents. In that light, the lower accumulation in the both the EPS and the microbial cells when the samples were run for an extended time period could be attributed to a higher resistance which is in agreement with those finding (Anwar et al, 1992).

5.4 Accumulation of Chlortetracycline in a Respiration Inhibited Biomass

To investigate the adsorption of CTC to biomass in the absence of metabolic activity, sodium azide was added (800 μg/mL) under two different conditions. In one instance chlortetracycline was the only carbon source, while in the other it was present in addition to another substrate (glucose/ acetate). The behaviour of the antibiotic sorption under the two conditions was notably different, in particular the amount left in the bulk water was significantly different (p = 0.002). The amount left in the bulk water when the antibiotic was the only carbon source (2.083 μg/mL) is almost five times higher (0.447 μg/mL) than that when there another substrate was present. Theses result demonstrate that uptake of chlortetracycline in the presence of sodium azide could be associated with uncoupled metabolism. In addition when CTC is present in the system as the sole carbon source (since it's not a biodegradable compound) cells tend to lyse, because as Joliffe et al (1981) found; sodium azide alone, in the absence of a substrate, caused cell lysis due to the high concentration of Na^+ during the inhibition of respiration. Therefore the antibiotic

which would attack living cells has no driving force to accumulate in the bacterial cells thus quite a considerable portion of it will remain in the water (almost 50%). Whereas when another carbon source such as glucose/ acetate is present the lysis doesn't take place and viability is maintained (Joliffe et al, 1981; Svarachorn et al, 1989), thus there would be an uptake of the drug but because there is no metabolic energy produced the efflux pumps are impaired hence the antibiotic will remain inside the cells.

5.5 Distribution in the Presence of EDTA

Given that solids and biomass are the main sinks of Tetracyclines in the different environmental compartments, since TCs have a high sorption tendency to solid matter. Cationic interactions play a major role in the sorption mechanism of tetracyclines; because, generally speaking, cation exchange is thermodynamically more favourable than hydrophobic partitioning-type processes (Horvath et al, 1976); therefore, cation exchange may dominate even when only a small fraction of the aqueous-phase species exists as a cation (Fabrega et al, 1998). From the results obtained in this research when samples were incubated in different concentration of EDTA (50 μg/mL, 150 μg/mL, 300 μg/mL) there was a negative correlation (-0.98) between the amount present in the EPS and the concentration of the EDTA, and there was a significant difference (p= 0.0001) between what was pumped out to the bulk water and what was trapped on the EPS. But there wasn't any significant difference (p = 0.49) between the control sample and that incubated in 50 μg/mL EDTA These results indicate that the stability of the EPS was disrupted in the presence of EDTA above the 50 μg/mL concentration, and further imply that ionic interactions through salt bridging were disrupted (Liao, 2002) thus the decrease in the CTC concentration in the EPS was noted. The declination in CTC concentration in the EPS as the EDTA concentration is increased is expected since in the presence of another competing chelating agent that binds to the divalent cations present in the EPS would lower the amount of CTC present in the polymeric matrix. It is expected that as the amount in the cells and EPS decrease the amount in the bulk water should increase, yet that is not what is apparent in the results provided in Table 4.6 which could be

attributed to the fact that the amount that are detected are considerably low, and loss in accountability could be due to the error margin.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Investigating the accumulation and distribution of the chlortetracycline in activated sludge batch experiments within three compartments; the bulk water, the extracellular polymeric substance and the microbial cells under different environmental conditions was the aim of this study. This is the first time, to the knowledge of the author; there is an attempt to quantify the accumulation distribution of chlortetracycline within the biomass in activated sludge process. Previous work only indicated either kinetics of sorption or investigated the differences in the sorption capacities of different matters, such as soils and humic acids.

In our study, it was found that extracellular polymeric substance (EPS) may contribute to the antimicrobial resistance properties of biofilms by impeding the mass transport of antibiotics through the microbial flocs, probably by binding directly to these agents. The importance of these findings, relay in the role of the EPS which is multifaceted. When associated with organisms, EPS can play roles in attachment, formation of biofilms, aid to locomotion, protection against physico-chemical change in environment, adsorption of nutrients, etc (Decho, 1990; Costerton et al, 1978; Hoiczyk, 2000; Murray, 1995). When EPS is released to the surrounding environment and becomes remote from the organisms, it plays critical roles in the biogeochemical cycle (Wotton, 2004; Bhaskar and Bhosle, 2005). For example; mucus form of EPS can serve as food sources for animals (Wotton, 2004). The unique properties of EPS make them not only a source for organic carbon pool, but also play an integral role in the entire biogeochemical process. EPS are already known for their ability to bind and remove heavy metals and nucleotides from natural water and waste waters (Dhami et al, 1998; Wilhelmi and Duncan, 1995), and the findings in our research show that it also plays a role in the removal of antibiotics from the bulk water in wastewater treatment processes. That removal via the EPS which varied between 5-17% depending on the environmental conditions which the microbial

community went under is in fact significant for many reasons. First, EPS as mentioned above could become detached from the microbial community thus mobilizing the antibiotic; in addition the EPS is present in the aqueous phase which facilitates its introduction to the food chain. Second, although the amount present in the EPS is minimal it is around the sub-therapeutic concentrations which would result in induced resistance, and given that plasmid transfer has be detected between bacteria in sediment, soil, water, and wastewater (Machand Grimes, 1982; Top et al, 1994), the presence of an antibiotic within minimal levels enhances the acquisition of resistance which is main concern with regards to the low removal efficiency witnessed in biological waste water treatment processes.

Under the different conditions the microbial community was subjected to, accumulation of the antibiotic within the biomass varied between as low as 27% of the initial concentration to up to 43%. The latter being under anoxic conditions which again puts forward the concern that under these conditions, where microbial flocs are unable to efflux the antibiotic out of the cells, that would lead to the alteration of the capacity of activated sludge in the sewage treatment plants to degrade other organic xenobiotics or to nitrify ammonia due to their bacterial potency This may result in a shift in the microbial sludge population enabling unaffected species to create dominants and potentially alter the activity of the sludge, i.e., reduce the nitrification capacity (Halling-Sørensen, 2001). On the other hand if the activated sludge is used as soil conditioner, antibacterial agents and residues sorbed to the sludge may also affect the soil bacterial community. These indigenous communities of bacterial and fungal populations are very complex and they have the important task of cycling nutrients. Proper cycling of nutrients is critical for quality soils and essential for maintaining sustainable use of agricultural lands. Consequently, when sludge containing Tetracyclines is applied to soil that could have an effect on microbial community structure, not only through its direct bacteriostatic effect, but also indirectly by influencing microbial interactions among different populations. (Rysz and Alvarez, 2004)

Accumulation of contaminants by microbial biomass; especially cell aggregates and micro-organisms growing attached to surfaces in biofilms or microbial flocs, play an important role in the functioning of biological waste-water treatment systems. Differentiation between sorption to live and inactivated biomass, and quantifying the sorption distribution between the cells and the EPs was determined by the study at hand. It was found that the active biomass only retains 24% of the initial concentration, while the inactive community amassed almost 90% of the initial concentration. Thus it is crucial to maintain an active healthy microbial community in wastewater treatment systems especially those dealing with pharmaceutical influents to reduce the amount removed via sorption to avoid the introduction of the antibiotic to soils and plants.

The addition of EDTA which is a strong chelator affected the amount of the antibiotic retained on the EPS, there was a high negative correlation (-0.98) between the EDTA concentration and the amount accumulated on the EPS. Yet it did not show any strong import effect.

6.2 Recommendations

- The findings suggest that Technologies that maximizes the removal of solids such as membrane bioreactor technologies could be more effective in antibiotics removal from waste water since sorption tends to be the main pathway of removal. Yet it also indicates that further treatment, physical or chemical, to the biofilms is required because the antibiotic may still retain its activity.

- Future work could be invested in modeling the manner by which the antibiotic is distributed in the three compartments, in specific the EPS and the cells, and looking into the different driving forces affecting the distribution; such as the surface charge and the presence of cations.

- Also, the determination of CTC metabolites and their fate in wastewater processes needs to be further investigated.

7.0 References

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APPENDIX A: PARAMETERS OF CHLORTETRACYCLINE REMOVAL UNDER AEROBIC AND ANOXIC CONDITIONS EXPERIMENT

Table A.1: Concentration of Chlortetracycline (μg/mL) in water

sample	Chlortetracycline Concentration under aerobic conditions (μg/mL)	Chlortetracycline Concentration under anaerobic conditions (μg/mL)
Sample 1	1.7	2.81
	1.96	2.7
	1.62	1.77
Sample 2	1.77	2.38
	1.56	3.07
	1.53	2.98

Student t-test: paired, one tailed distribution

$p = 0.004$

Table A.2: Concentration of Chlortetracycline (μg/mL) in extracellular polymeric substance

sample	Chlortetracycline Concentration under aerobic conditions (μg/mL)	Chlortetracycline Concentration under anaerobic conditions (μg/mL)
Sample 1	0.42	0.51
	0.23	0.67
	0.35	0.82
Sample 2	0.07	0.81
	0.2	1.04
	0.1	1.11

Student t-test: paired, one tailed distribution

$p = 0.003$

Table A.3: Concentration of Chlortetracycline (µg/mL) in microbial cells

sample	Chlortetracycline Concentration under aerobic conditions (µg/mL)	Chlortetracycline Concentration under anaerobic conditions (µg/mL)
Sample 1	0.62	2.21
	0.93	1.37
	0.23	1.71
Sample 2	0.71	1.07
	0.67	0.73
	0.81	0.90

Student t-test: paired, one tailed distribution
$p = 0.03$

Aerobic conditions

MLSS (mg/L) ; sample (1) = 0.98 mg/L
sample (2) = 0.84 mg/L

SOUR (mg/g)/h; sample (1) = 3.31 (mg/g)h
sample (2) = 3.89(mg/g)h

F/M (g BOD/ g MLSS); sample (1) = 1.22
sample (2) = 1.43

Anoxic conditions

MLSS (mg/L) ; sample (1) = 0.94 mg/L
sample (2) = 0.94 mg/L

F/M (g BOD/ g MLSS); sample (1) = 1.28
sample (2) = 1.28

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 1:35:01 PM
Operator	: manager	Sample Name	: deg.,PS43
Sample Number	: 137	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/22
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
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Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
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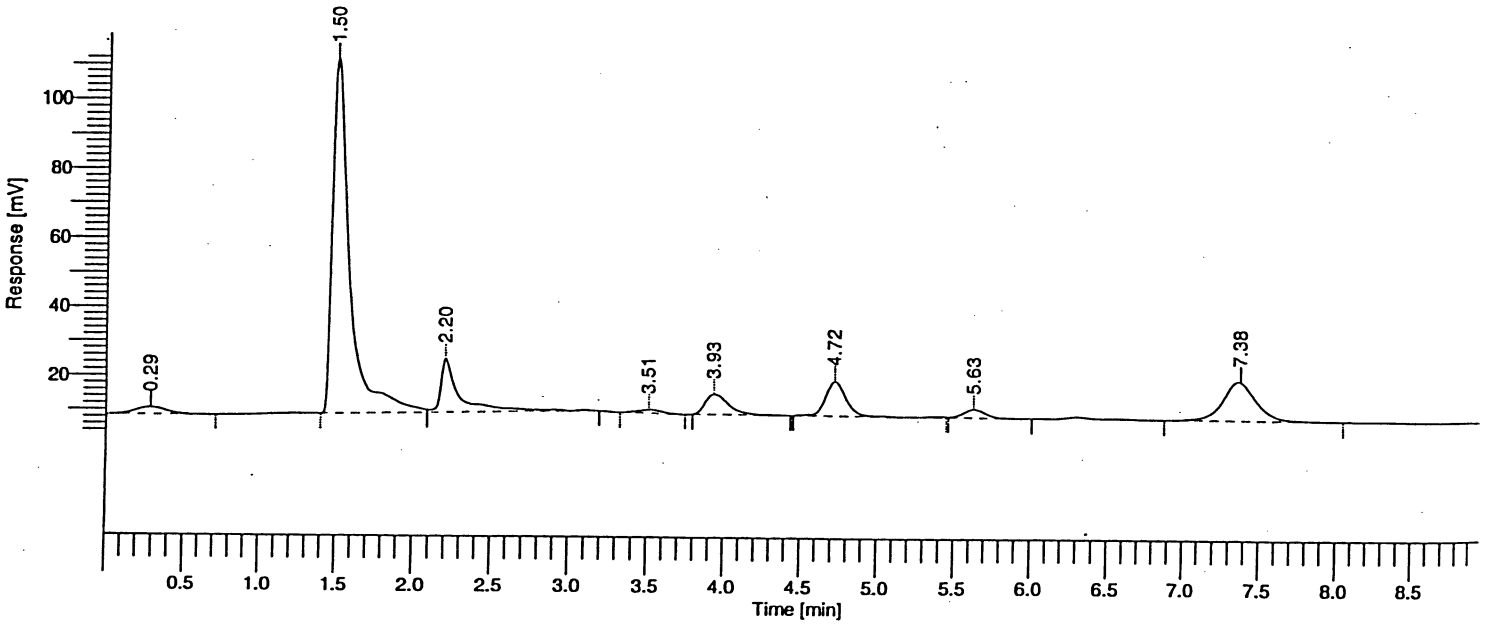

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2	1.499	817754.79	102907.33	60.21	60.21	BV	7.9465
3	2.204	138298.81	15654.30	10.18	10.18	VB	8.8346
4	3.511	11388.40	1018.99	0.84	0.84	BB	11.1762
5	3.934	64244.20	5984.33	4.73	4.73	BB	10.7354
6	4.723	98590.24	10130.43	7.26	7.26	BV	9.7321
7	5.630	26219.82	2525.92	1.93	1.93	VB	10.3803
8	7.377	173294.40	11125.37	12.76	12.76	BB	15.5765
		1358132.46	151427.14	100.00	100.00		

Missing Component Report

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Sample Number	: 126	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/11
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
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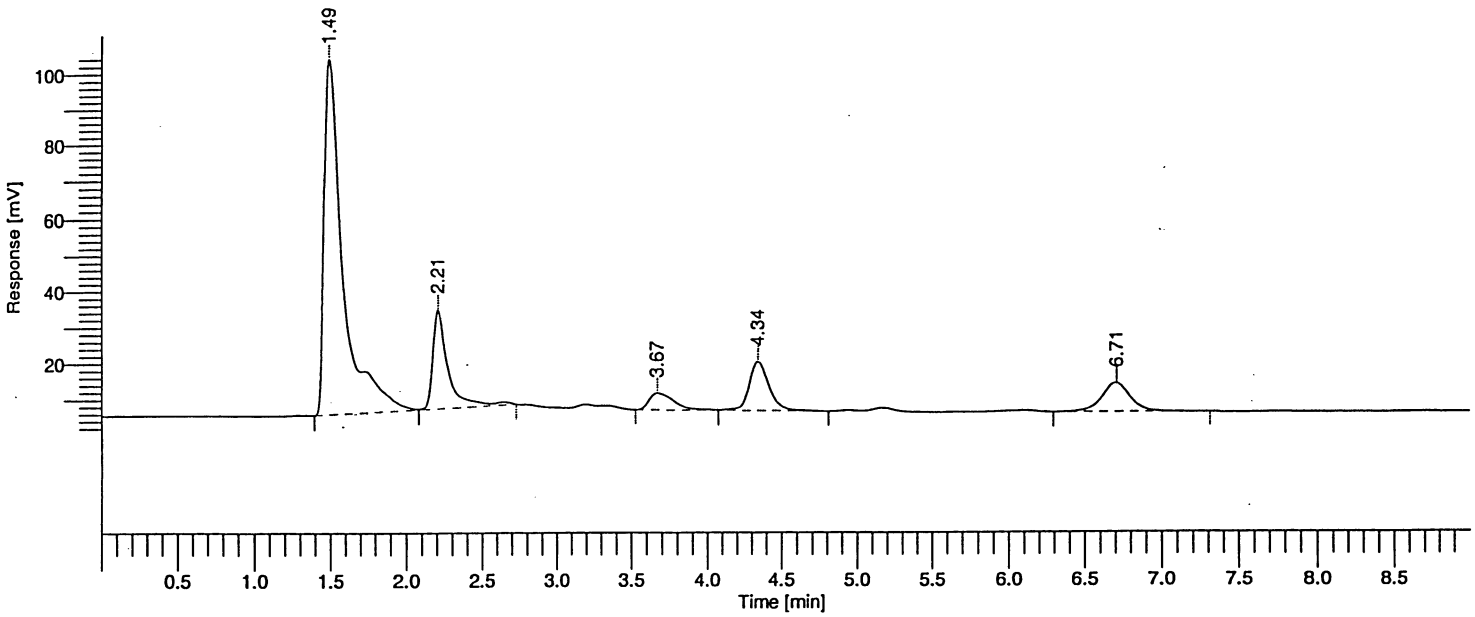

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2	2.209	193029.02	27526.15	14.56	14.56	VB	7.0126
3	3.667	53252.29	4701.21	4.02	4.02	BV	11.3274
4	4.340	126875.71	13651.71	9.57	9.57	VB	9.2938
5	6.710	104870.00	7967.24	7.91	7.91	BB	13.1626
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Instrument Name	: LC	Channel	: A
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Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
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Sample Amount	: 1.0000	Dilution Factor	: 1.00
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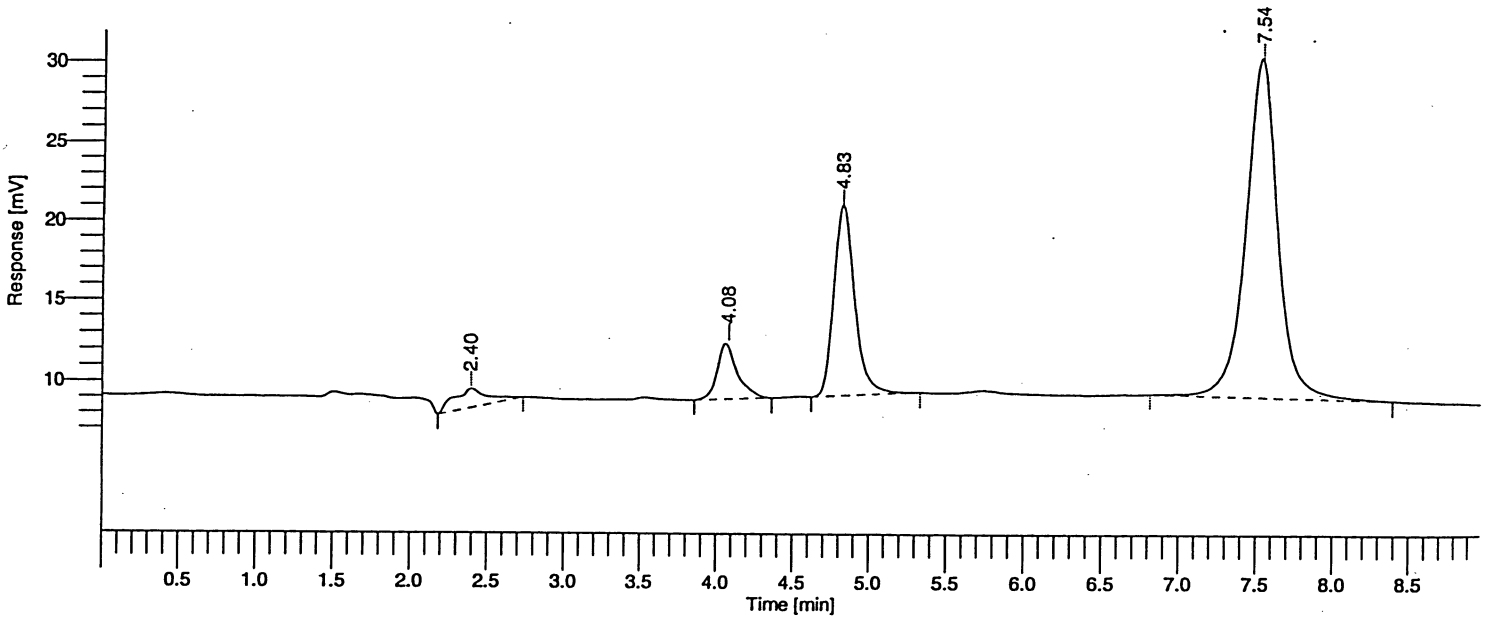

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2	4.083	32592.80	3195.24	6.89	6.89	BB	10.2004
3	4.825	113591.20	11918.08	24.01	24.01	BB	9.5310
4	7.538	308275.20	21078.44	65.16	65.16	BB	14.6251
		473103.20	37414.58	100.00	100.00		

Missing Component Report

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Instrument Serial #	: None	A/D mV Range	: 1000
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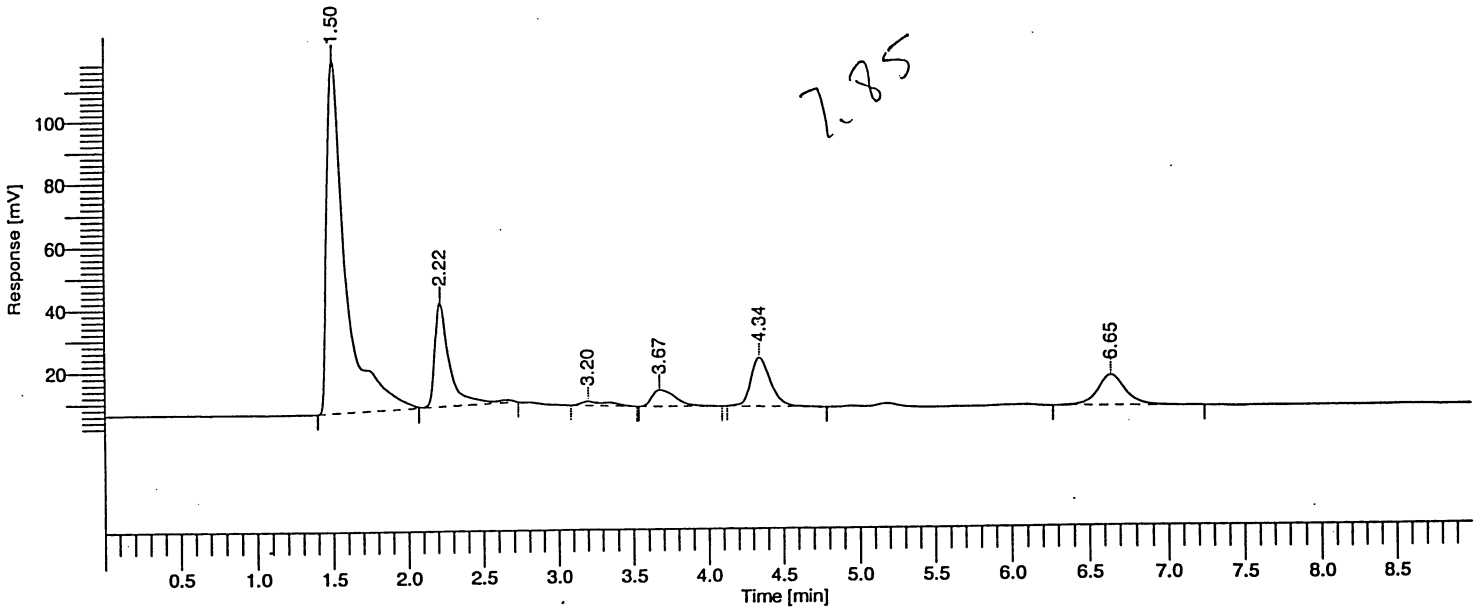

DEFAULT REPORT

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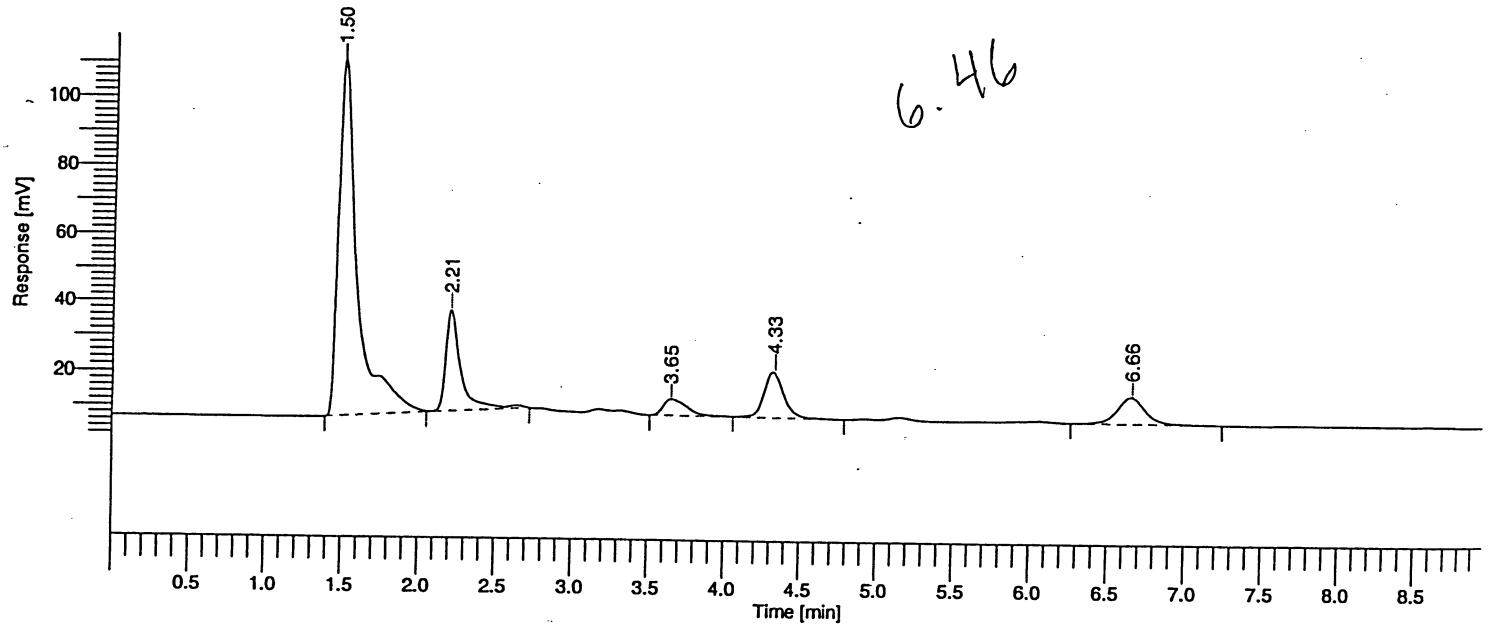
2	2.215	230861.10	33415.52	14.85	14.85	VB	6.9088
3	3.199	18809.00	1320.33	1.21	1.21	BB	14.2457
4	3.671	58417.60	5302.07	3.76	3.76	BB	11.0179
5	4.340	140186.00	15439.06	9.02	9.02	BB	9.0800
6	6.646	121044.60	9634.90	7.79	7.79	BB	12.5631
		1554364.20	177928.59	100.00	100.00		

Missing Component Report
Component Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 12:05:00 PM
Operator	: manager	Sample Name	: deg.,PS13
Sample Number	: 128	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/13
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 1:03:29 PM	Cycle	: 134

Raw Data File : \\Poweredge\E drive\TC\dennis\data124.raw
Result File : \\Poweredge\E drive\TC\dennis\data124.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data124.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.498	885836.91	104052.85	64.94	64.94	BV	8.5133
2	2.212	200588.09	29378.22	14.70	14.70	VB	6.8278
3	3.653	53576.24	4784.32	3.93	3.93	BV	11.1983
4	4.334	124543.16	12825.67	9.13	9.13	VB	9.7105
5	6.662	99591.20	7689.25	7.30	7.30	BB	12.9520
		1364135.60	158730.31	100.00	100.00		

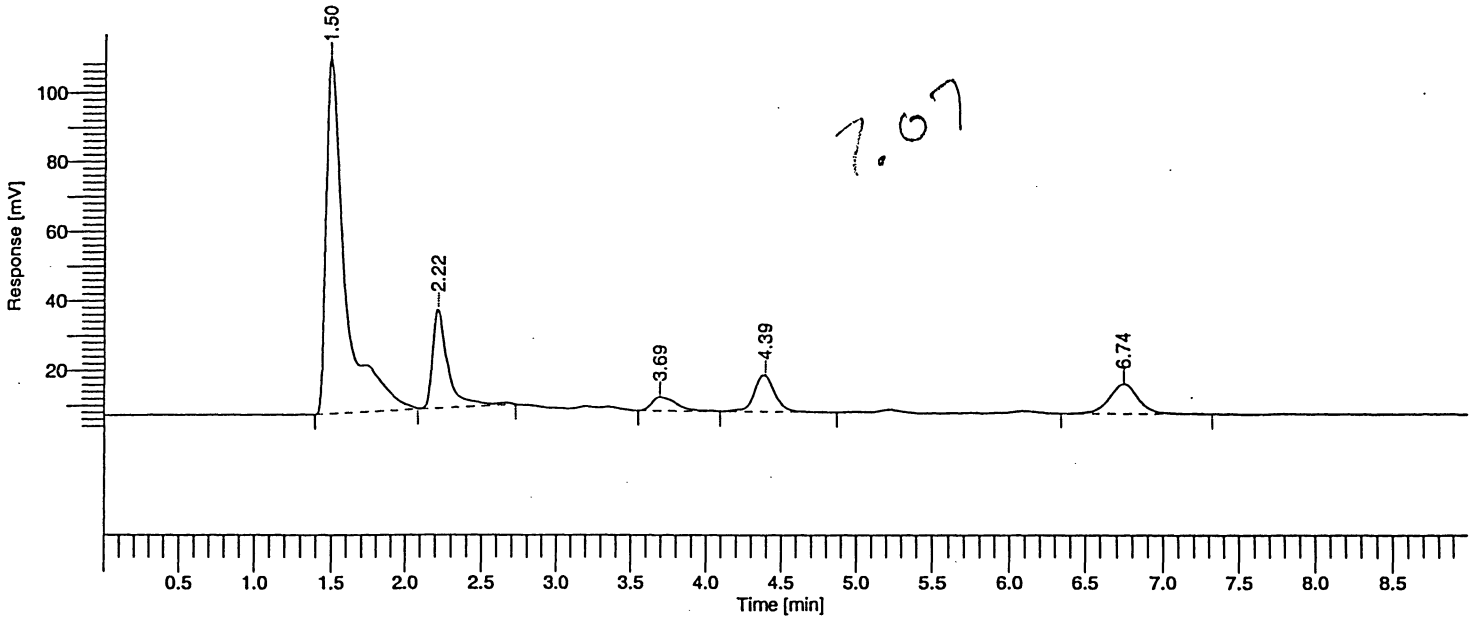
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 12:15:02 PM
Operator	: manager	Sample Name	: deg.,PS21
Sample Number	: 129	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/14
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 1:13:30 PM	Cycle	: 135

Raw Data File : \\Poweredge\E drive\TC\dennis\data125.raw
Result File : \\Poweredge\E drive\TC\dennis\data125.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data125.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.496	897923.30	102270.17	66.67	66.67	BV	8.7799
2	2.219	193855.10	28708.73	14.39	14.39	VB	6.7525
3	3.692	45112.52	3958.33	3.35	3.35	BV	11.3969
4	4.389	100846.88	10263.17	7.49	7.49	VB	9.8261
5	6.744	108979.60	8495.62	8.09	8.09	BB	12.8277
		1346717.40	153696.02	100.00	100.00		

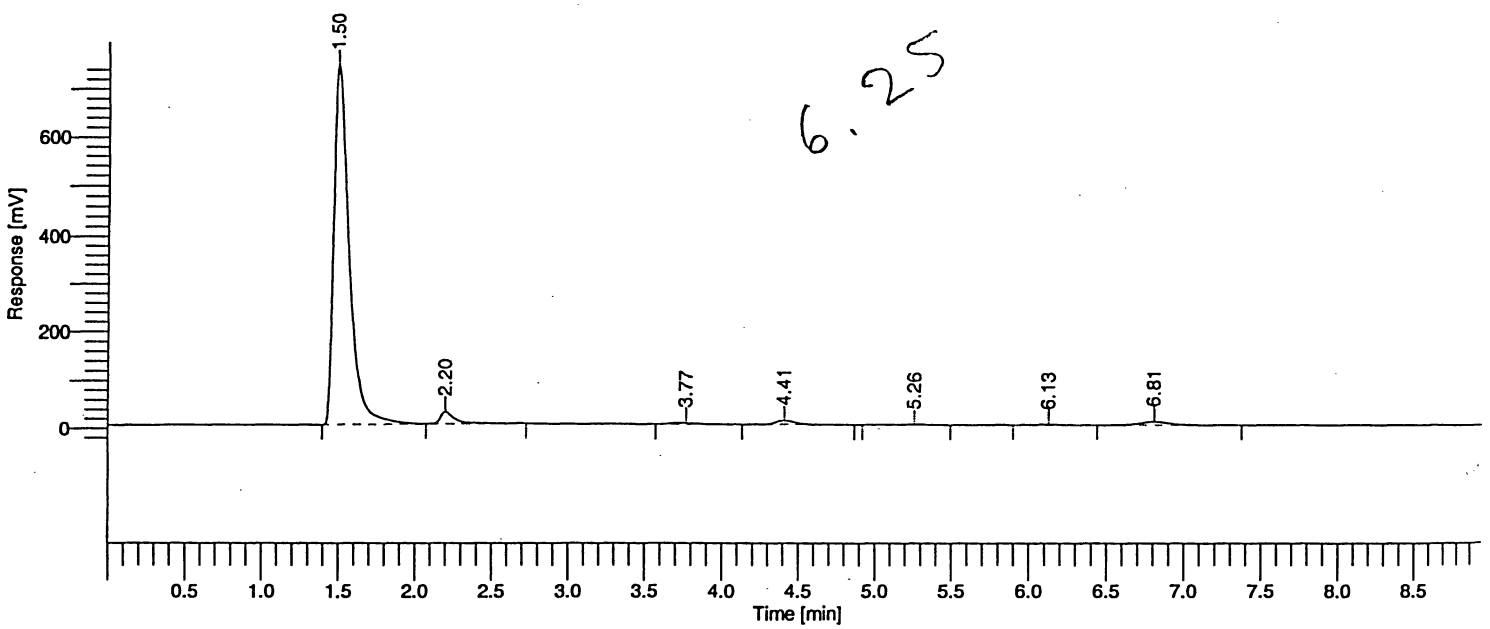
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 12:25:03 PM
Operator	: manager	Sample Name	: deg.,PS22
Sample Number	: 130	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/15
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 1:23:30 PM	Cycle	: 136

Raw Data File : \\Poweredge\E drive\TC\dennis\data126.raw
Result File : \\Poweredge\E drive\TC\dennis\data126.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data126.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.499	5174734.19	742662.27	92.35	92.35	BV	6.9678
2	2.199	185513.41	26468.99	3.31	3.31	VB	7.0087
3	3.767	39461.97	2976.85	0.70	0.70	BV	13.2563
4	4.410	84637.23	8810.13	1.51	1.51	VB	9.6068
5	5.260	11349.60	977.40	0.20	0.20	BB	11.6120
6	6.128	11623.96	1170.44	0.21	0.21	BV	9.9313
7	6.810	96268.44	7390.18	1.72	1.72	VB	13.0265
		5603588.80	790456.27	100.00	100.00		

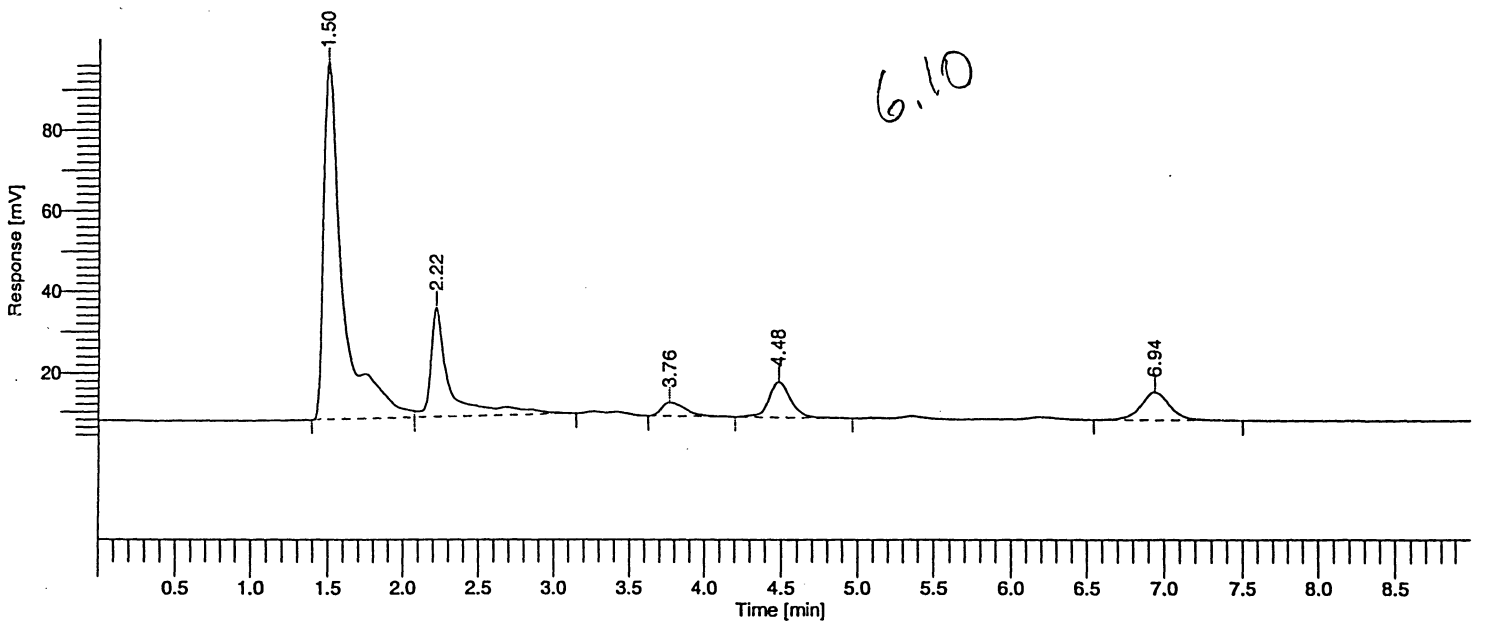
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 12:35:05 PM
Operator	: manager	Sample Name	: deg.,PS23
Sample Number	: 131	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/16
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 1:33:30 PM	Cycle	: 137

Raw Data File : \\Poweredge\E drive\TC\dennis\data127.raw
Result File : \\Poweredge\E drive\TC\dennis\data127.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data127.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.498	786969.09	88773.53	62.68	62.68	BV	8.8649
2	2.216	246064.11	27437.45	19.60	19.60	VB	8.9682
3	3.760	40177.07	3547.45	3.20	3.20	BV	11.3256
4	4.479	88198.53	9136.86	7.03	7.03	VB	9.6530
5	6.938	94045.60	7183.13	7.49	7.49	BB	13.0926
		1255454.40	136078.41	100.00	100.00		

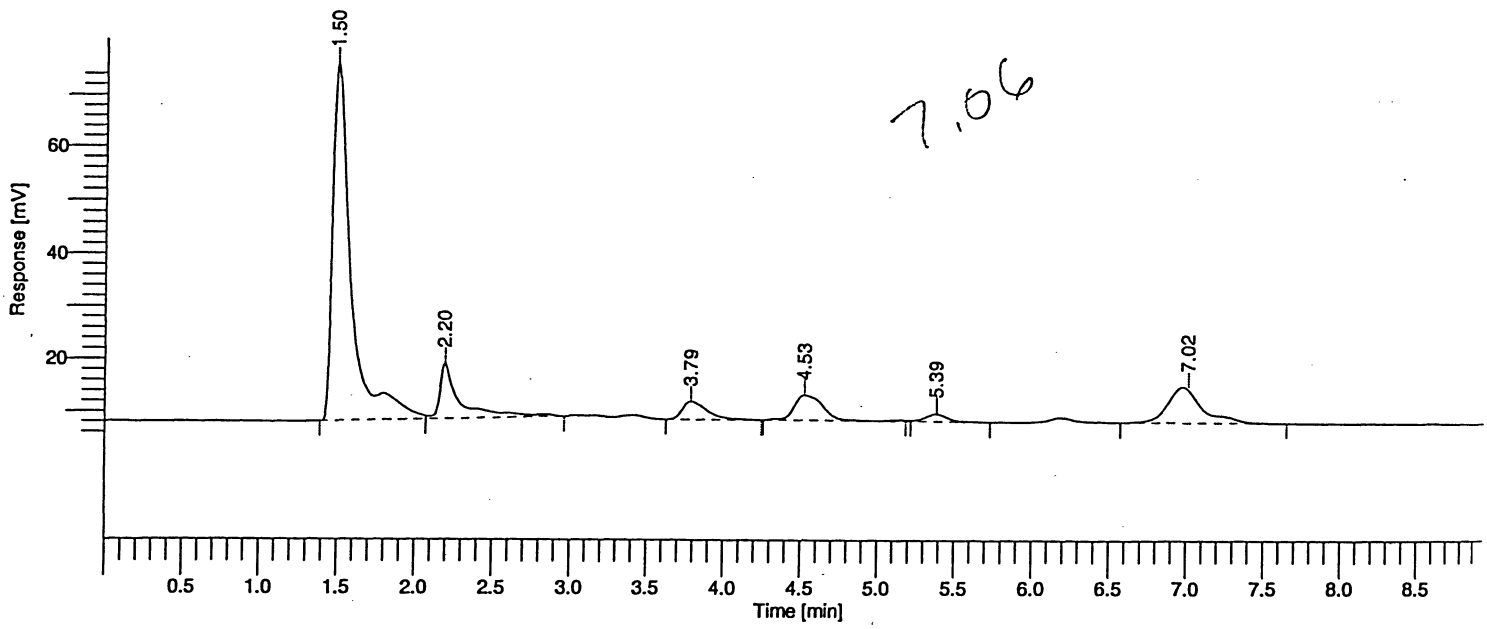
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 12:44:59 PM
Operator	: manager	Sample Name	: deg.,PS31
Sample Number	: 132	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/17
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 1:43:30 PM	Cycle	: 138

Raw Data File : \\Poweredge\E drive\TC\dennis\data128.raw
Result File : \\Poweredge\E drive\TC\dennis\data128.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data128.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.499	559625.93	67835.01	63.36	63.36	BV	8.2498
2	2.196	96609.07	10592.26	10.94	10.94	VB	9.1207
3	3.792	38877.60	3498.99	4.40	4.40	BB	11.1111
4	4.528	64711.84	4839.59	7.33	7.33	BV	13.3714
5	5.387	14585.64	1438.39	1.65	1.65	VB	10.1403
6	7.016	108862.60	6311.27	12.32	12.32	BB	17.2489
		883272.68	94515.50	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 12:55:01 PM
Operator	: manager	Sample Name	: deg.,PS32
Sample Number	: 133	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/18
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 1:53:30 PM	Cycle	: 139

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Result File : \\Poweredge\E drive\TC\dennis\data129.rst
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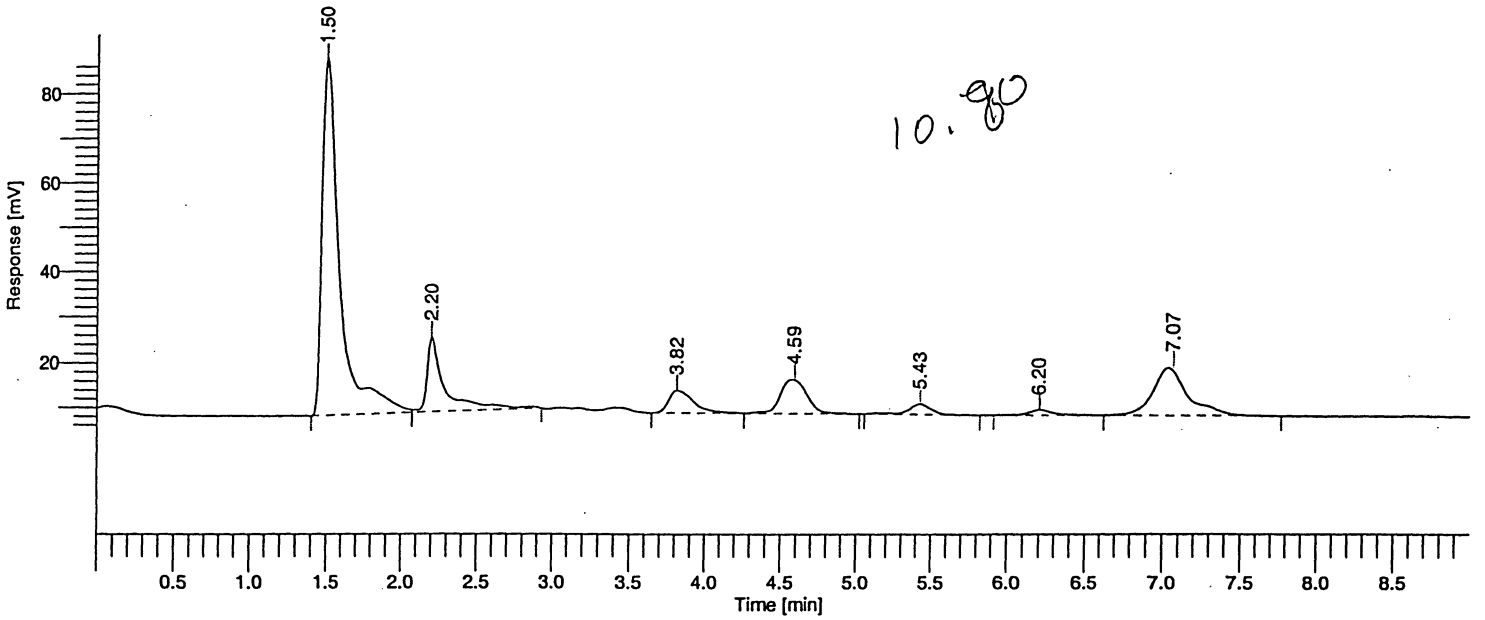
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.499	651542.20	79557.54	56.54	56.54	BV	8.1896
2	2.202	138196.20	16516.14	11.99	11.99	VB	8.3673
3	3.815	60091.62	5198.59	5.21	5.21	BV	11.5592
4	4.589	96429.78	7641.01	8.37	8.37	VB	12.6200
5	5.425	25856.40	2370.84	2.24	2.24	BB	10.9060
6	6.204	13831.51	1227.37	1.20	1.20	BV	11.2692
7	7.075	166399.29	9971.91	14.44	14.44	VB	16.6868
		1152347.00	122483.38	100.00	100.00		

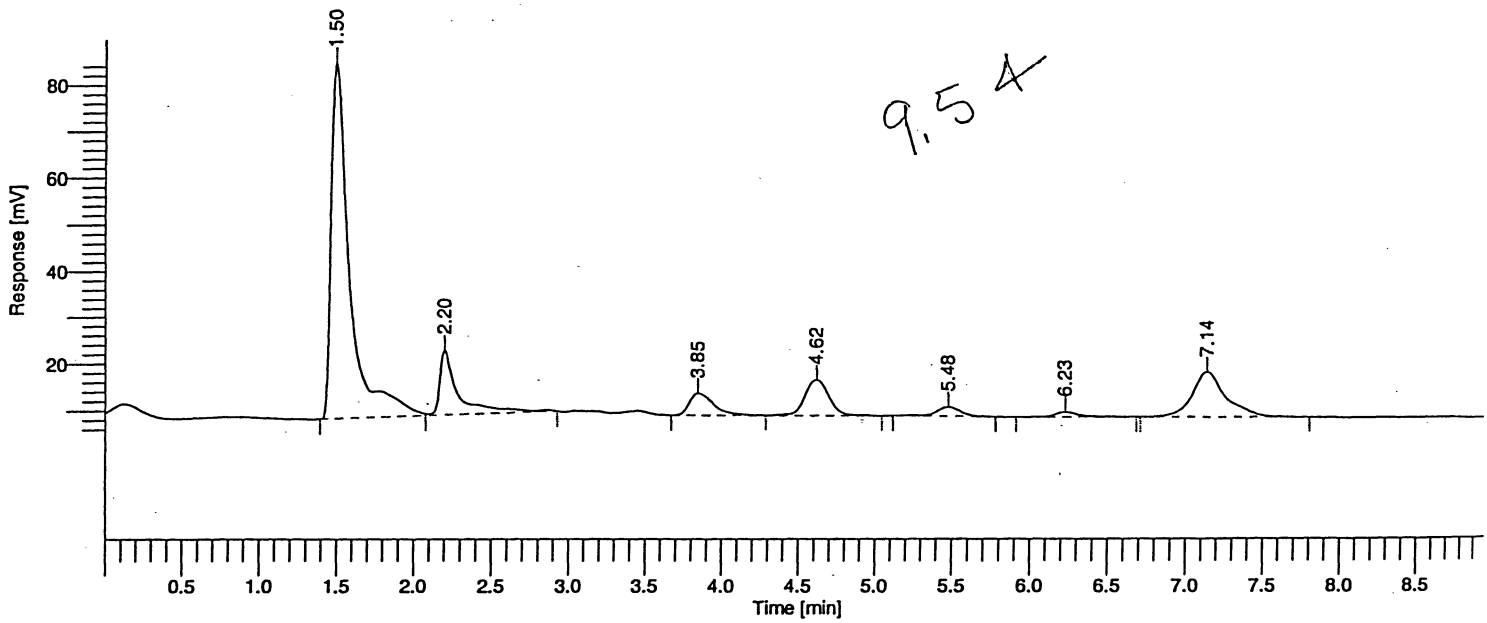
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 1:05:03 PM
Operator	: manager	Sample Name	: deg.,PS33
Sample Number	: 134	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/19
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 2:03:31 PM	Cycle	: 140

Raw Data File : \\Poweredge\E drive\TC\dennis\data130.raw
Result File : \\Poweredge\E drive\TC\dennis\data130.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data130.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.498	635848.16	76567.57	59.49	59.49	BV	8.3044
2	2.201	114536.84	13873.25	10.72	10.72	VB	8.2559
3	3.846	53396.31	4676.41	5.00	5.00	BV	11.4182
4	4.620	85170.09	7653.72	7.97	7.97	VB	11.1279
5	5.481	21179.60	2006.82	1.98	1.98	BB	10.5538
6	6.231	11692.40	1068.69	1.09	1.09	BB	10.9409
7	7.144	146972.20	9711.43	13.75	13.75	BB	15.1339
		1068795.60	115557.89	100.00	100.00		

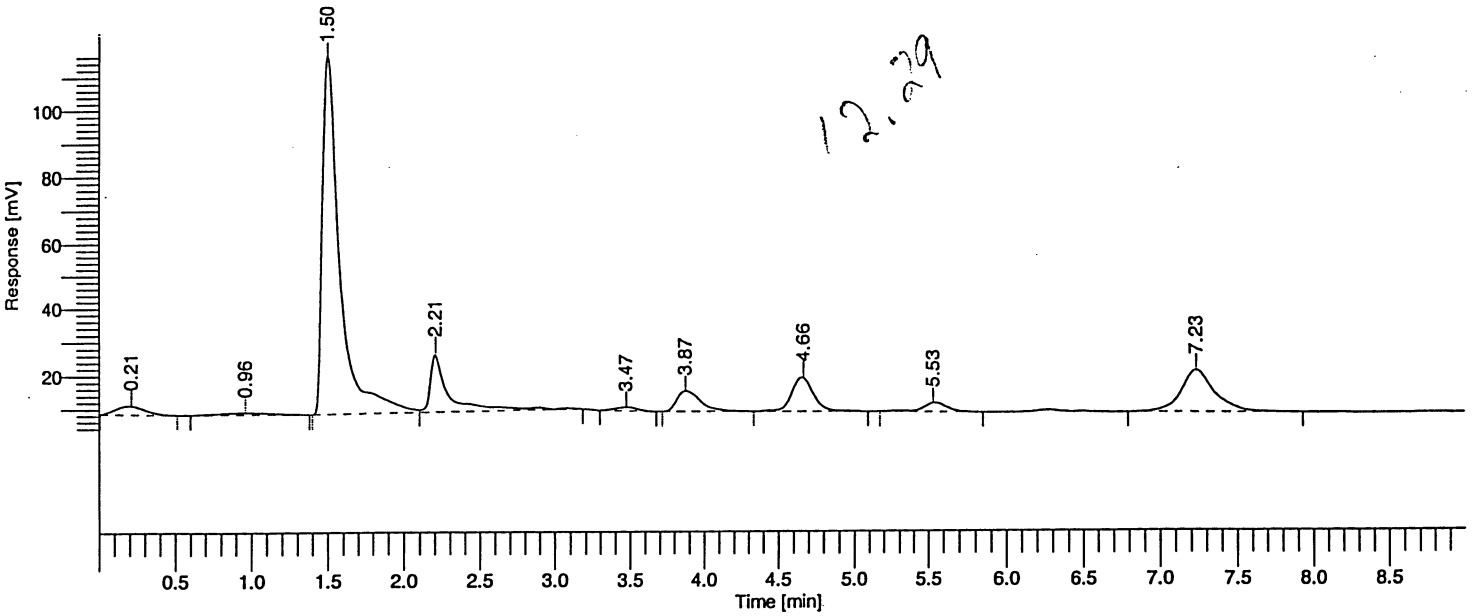
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 1:15:05 PM
Operator	: manager	Sample Name	: deg.,PS41
Sample Number	: 135	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/20
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 2:13:31 PM	Cycle	: 141

Raw Data File : \\Poweredge\E drive\TC\dennis\data131.raw
Result File : \\Poweredge\E drive\TC\dennis\data131.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data131.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.211	36507.40	2701.42	2.48	2.48	BB	13.5141
2	0.962	16299.60	666.05	1.11	1.11	BB	24.4722
3	1.499	858444.52	107779.06	58.30	58.30	BV	7.9649
4	2.206	151503.48	17544.27	10.29	10.29	VB	8.6355
5	3.473	11836.40	1169.81	0.80	0.80	BB	10.1182
6	3.872	70969.41	6244.98	4.82	4.82	BV	11.3642
7	4.663	106992.39	10169.95	7.27	7.27	VB	10.5204
8	5.530	30332.00	2741.56	2.06	2.06	BB	11.0638
9	7.235	189480.00	12567.13	12.87	12.87	BB	15.0774
		1472365.20	161584.22	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 1:25:06 PM
Operator	: manager	Sample Name	: deg.,PS42
Sample Number	: 136	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/21
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 2:23:33 PM	Cycle	: 142

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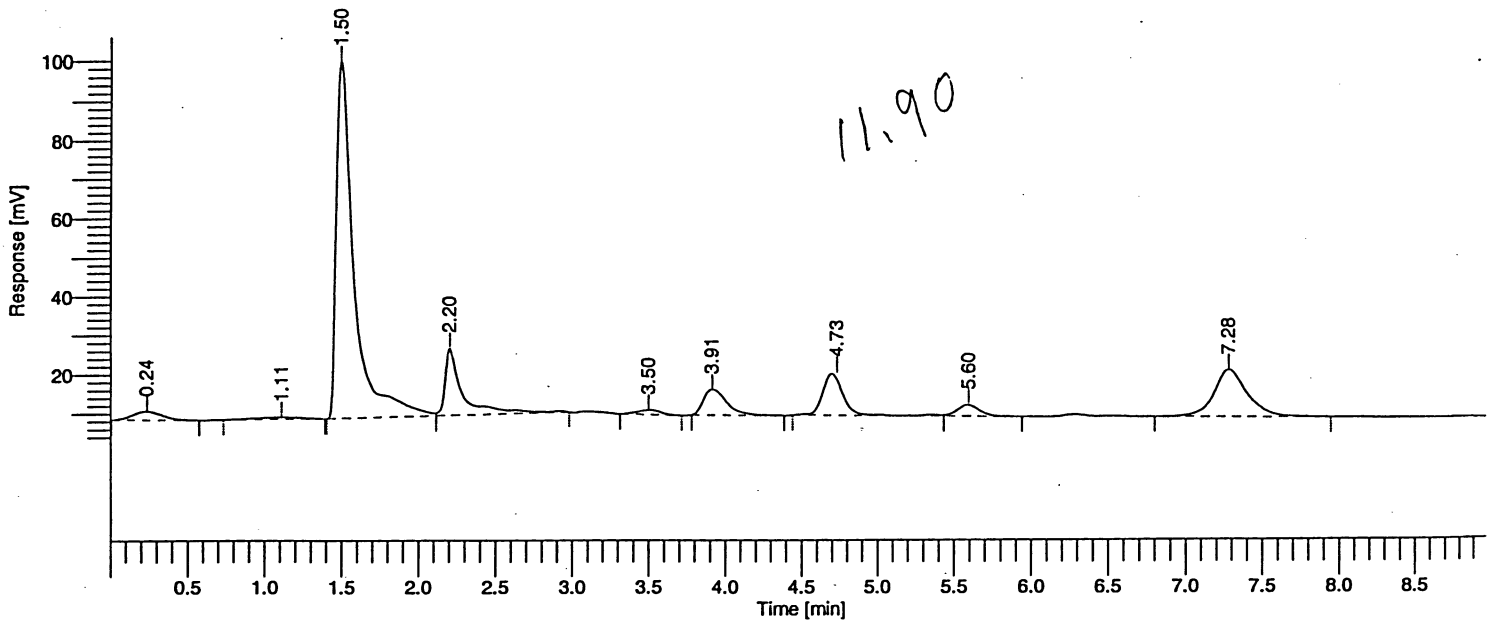
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.238	30128.00	2257.78	2.29	2.29	BB	13.3441
2	1.111	11012.40	497.85	0.84	0.84	BB	22.1200
3	1.500	736143.26	91359.84	55.95	55.95	BV	8.0576
4	2.204	137697.54	17208.87	10.47	10.47	VB	8.0015
5	3.500	12503.80	1158.17	0.95	0.95	BB	10.7961
6	3.914	70559.60	6660.25	5.36	5.36	BB	10.5941
7	4.732	104706.09	9160.77	7.96	7.96	BV	11.4298
8	5.597	29358.71	2901.80	2.23	2.23	VB	10.1174
9	7.284	183495.20	12129.62	13.95	13.95	BB	15.1279
		1315604.60	143334.94	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 1:45:02 PM
Operator	: manager	Sample Name	: deg.,P11,EPS
Sample Number	: 138	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/23
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 2:43:33 PM	Cycle	: 144

Raw Data File : \\Poweredge\E drive\TC\dennis\data134.raw
Result File : \\Poweredge\E drive\TC\dennis\data134.rst
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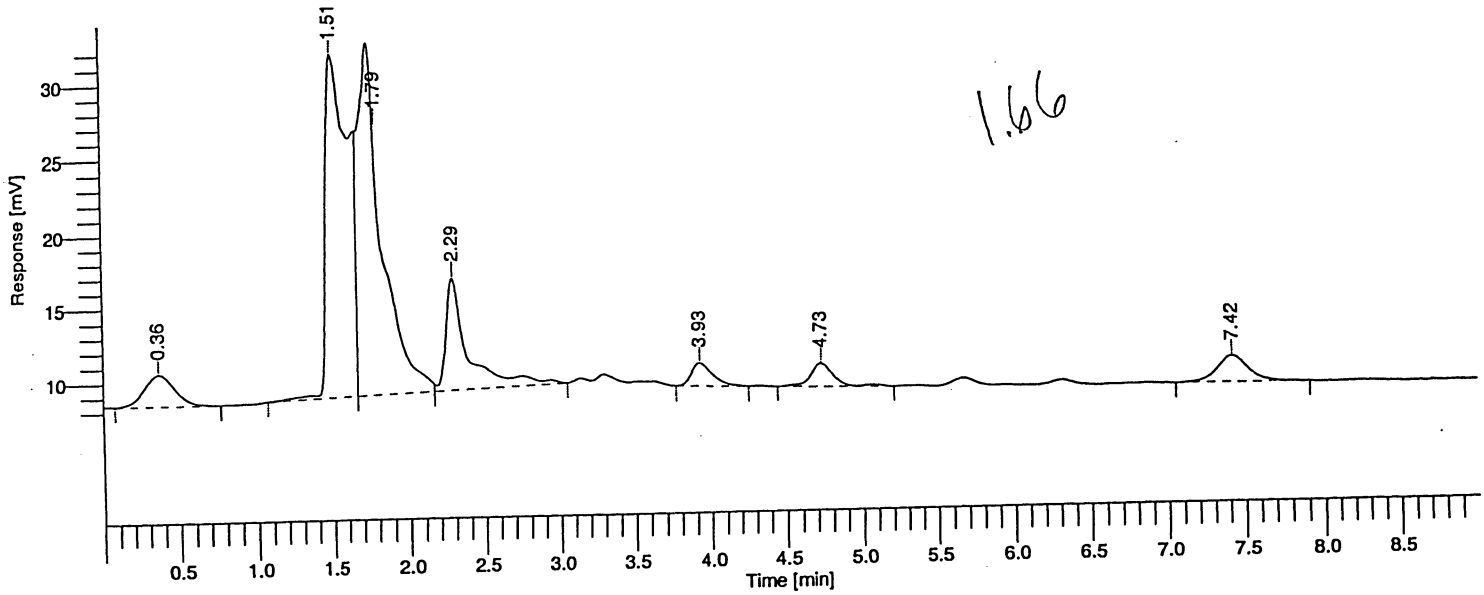
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.362	29913.40	2137.32	4.51	4.51	BB	13.9957
2	1.505	245620.73	23005.52	37.07	37.07	BV	10.6766
3	1.793	251692.04	15795.31	37.99	37.99	VV	15.9346
4	2.292	77592.83	7621.90	11.71	11.71	VB	10.1803
5	3.927	15364.80	1566.22	2.32	2.32	BB	9.8101
6	4.727	16798.00	1571.25	2.54	2.54	BB	10.6909
7	7.421	25606.40	1796.31	3.86	3.86	BB	14.2550
		662588.20	53493.84	100.00	100.00		

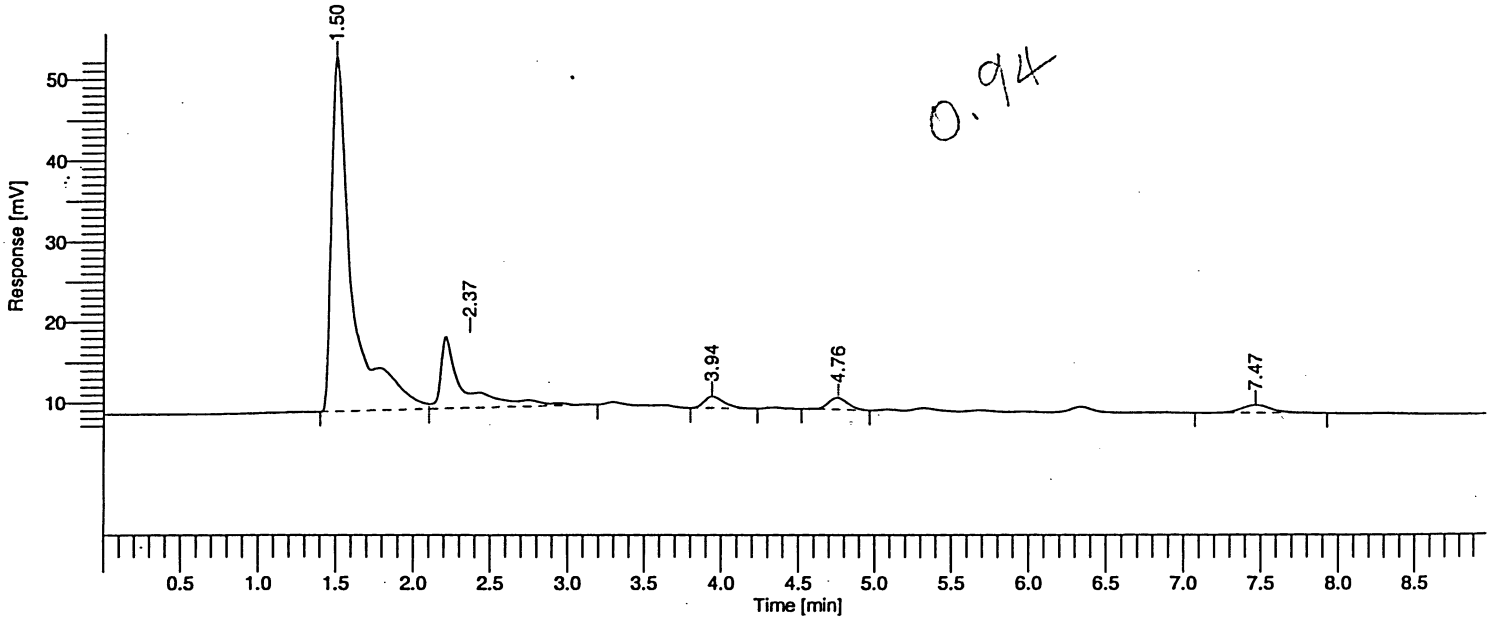
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 1:55:04 PM
Operator	: manager	Sample Name	: deg.,P12,EPS
Sample Number	: 139	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/24
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 2:53:34 PM	Cycle	: 145

Raw Data File : \\Poweredge\E drive\TC\dennis\data135.raw
Result File : \\Poweredge\E drive\TC\dennis\data135.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data135.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.502	404512.26	43819.77	74.95	74.95	BV	9.2313
2	2.367	94146.94	1779.31	17.44	17.44	VB	52.9119
3	3.941	13122.00	1472.01	2.43	2.43	BB	8.9143
4	4.762	13491.60	1491.18	2.50	2.50	BB	9.0476
5	7.467	14430.60	1032.43	2.67	2.67	BB	13.9774
		539703.40	49594.70	100.00	100.00		

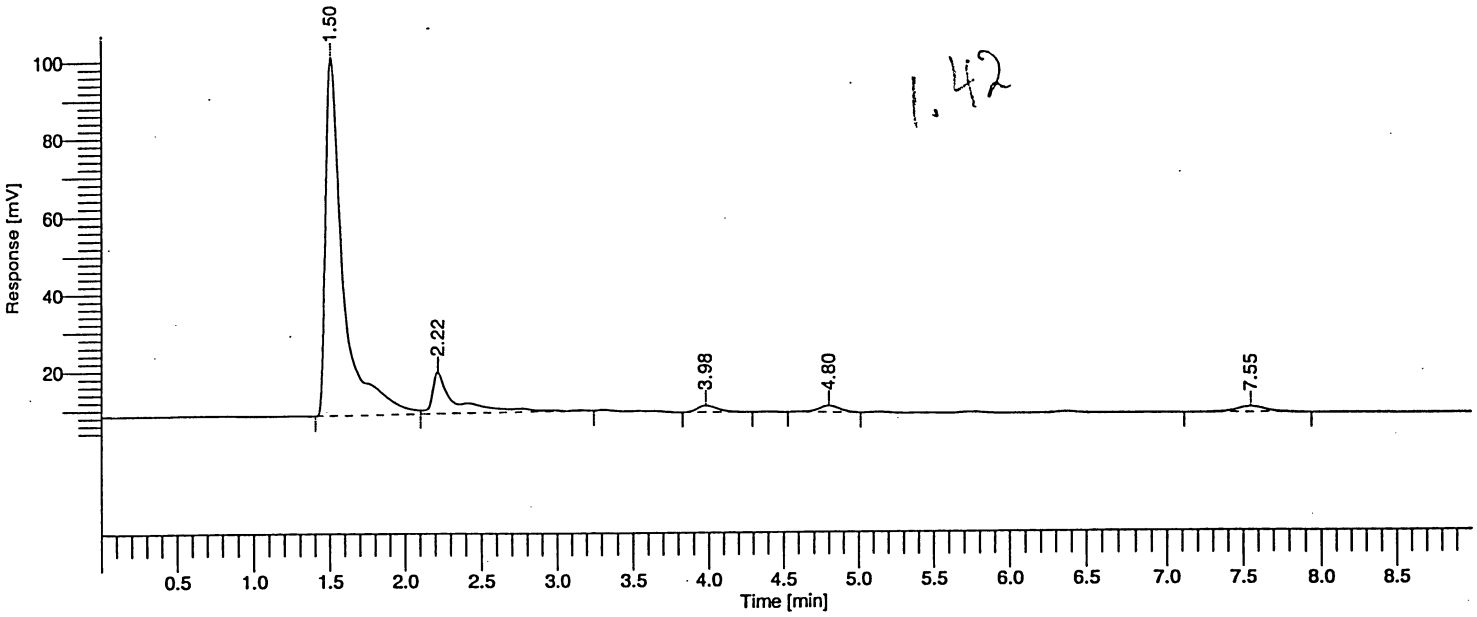
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 2:05:06 PM
Operator	: manager	Sample Name	: deg.,P13,EPS
Sample Number	: 140	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/25
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 3:03:34 PM	Cycle	: 146

Raw Data File : \\Poweredge\E drive\TC\dennis\data136.raw
Result File : \\Poweredge\E drive\TC\dennis\data136.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data136.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.502	763511.49	92231.26	81.68	81.68	BV	8.2782
2	2.215	116796.11	10732.80	12.49	12.49	VB	10.8822
3	3.978	15876.60	1711.24	1.70	1.70	BB	9.2779
4	4.800	16749.20	1678.37	1.79	1.79	BB	9.9794
5	7.552	21823.00	1530.43	2.33	2.33	BB	14.2594
		934756.40	107884.09	100.00	100.00		

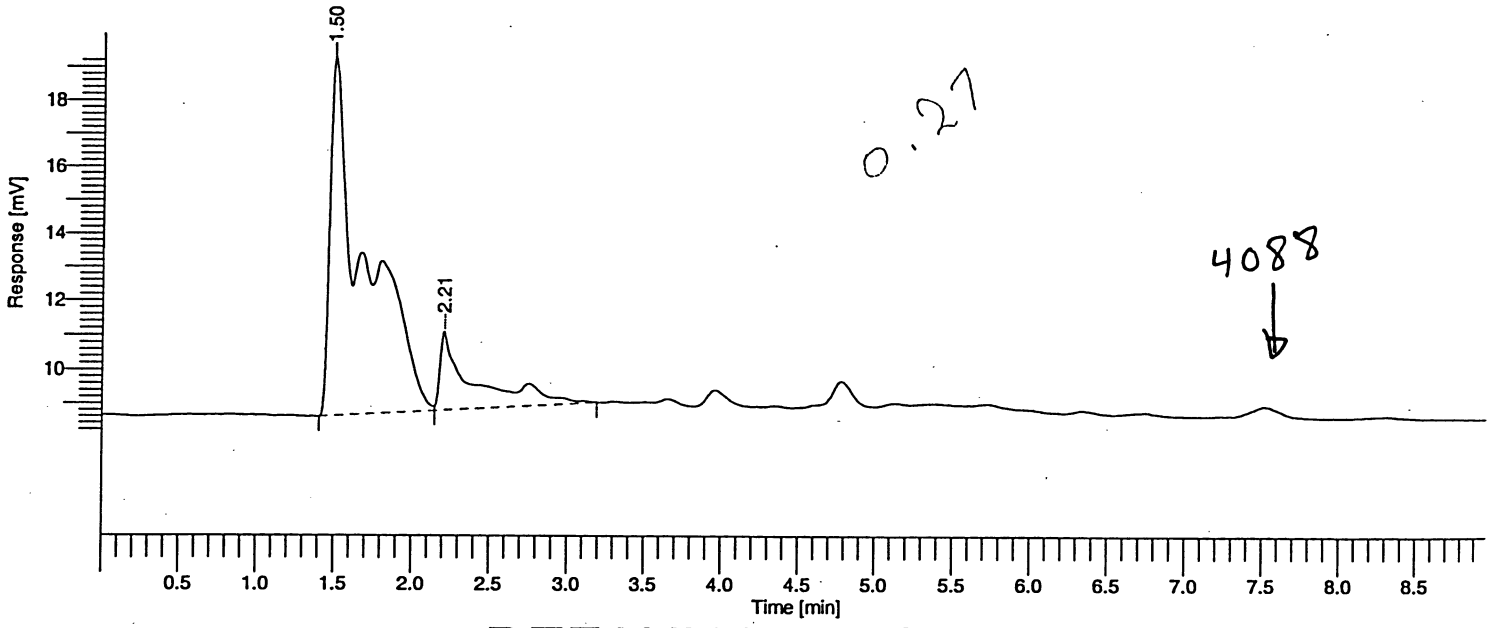
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 2:15:07 PM
Operator	: manager	Sample Name	: deg.,P21,EPS
Sample Number	: 141	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/26
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 3:13:34 PM	Cycle	: 147

Raw Data File : \\Poweredge\E drive\TC\dennis\data137.raw
Result File : \\Poweredge\E drive\TC\dennis\data137.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data137.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.500	164479.92	10684.40	82.36	82.36	BV	15.3944
2	2.210	35229.28	2333.08	17.64	17.64	VB	15.0999
		199709.20	13017.48	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 2:25:01 PM
Operator	: manager	Sample Name	: deg.,P22,EPS
Sample Number	: 142	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/27
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 3:23:34 PM	Cycle	: 148

Raw Data File : \\Poweredge\E drive\TC\dennis\data138.raw
Result File : \\Poweredge\E drive\TC\dennis\data138.rst
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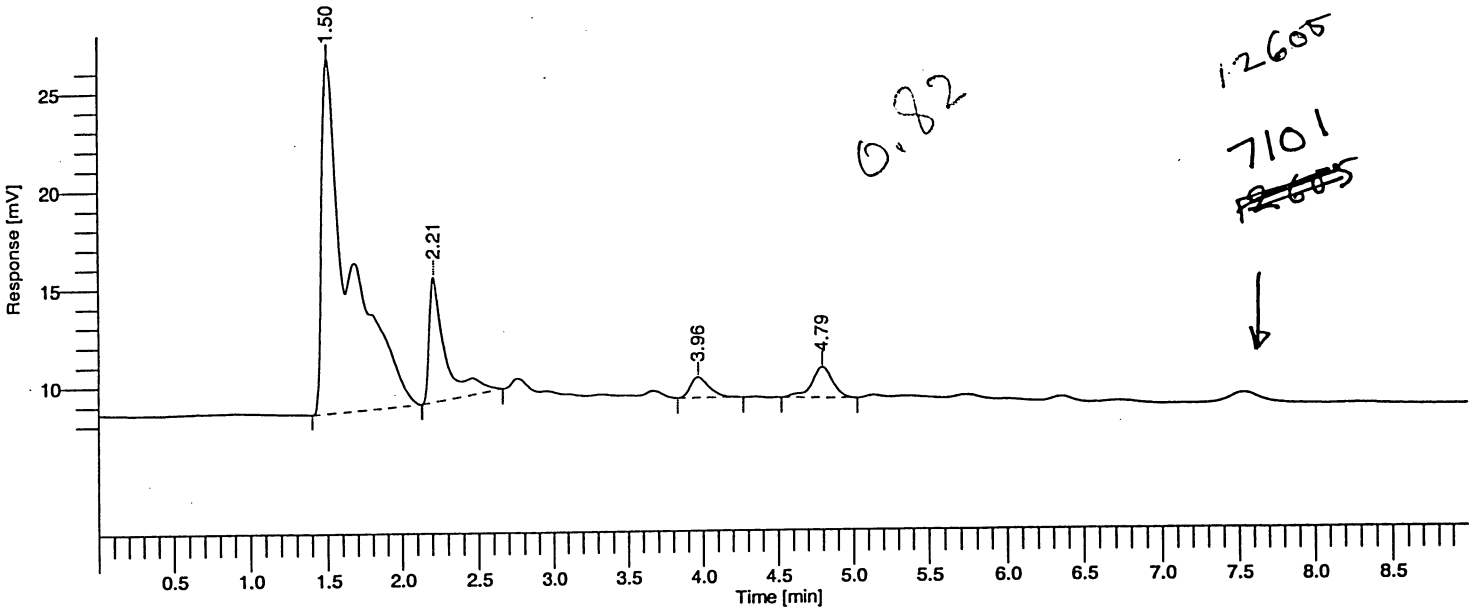
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.500	230809.60	18089.09	76.61	76.61	BB	12.7596
2	2.211	46175.60	6446.85	15.33	15.33	BB	7.1625
3	3.964	9214.80	1050.94	3.06	3.06	BB	8.7682
4	4.792	15086.40	1562.09	5.01	5.01	BB	9.6578
		301286.40	27148.96	100.00	100.00		

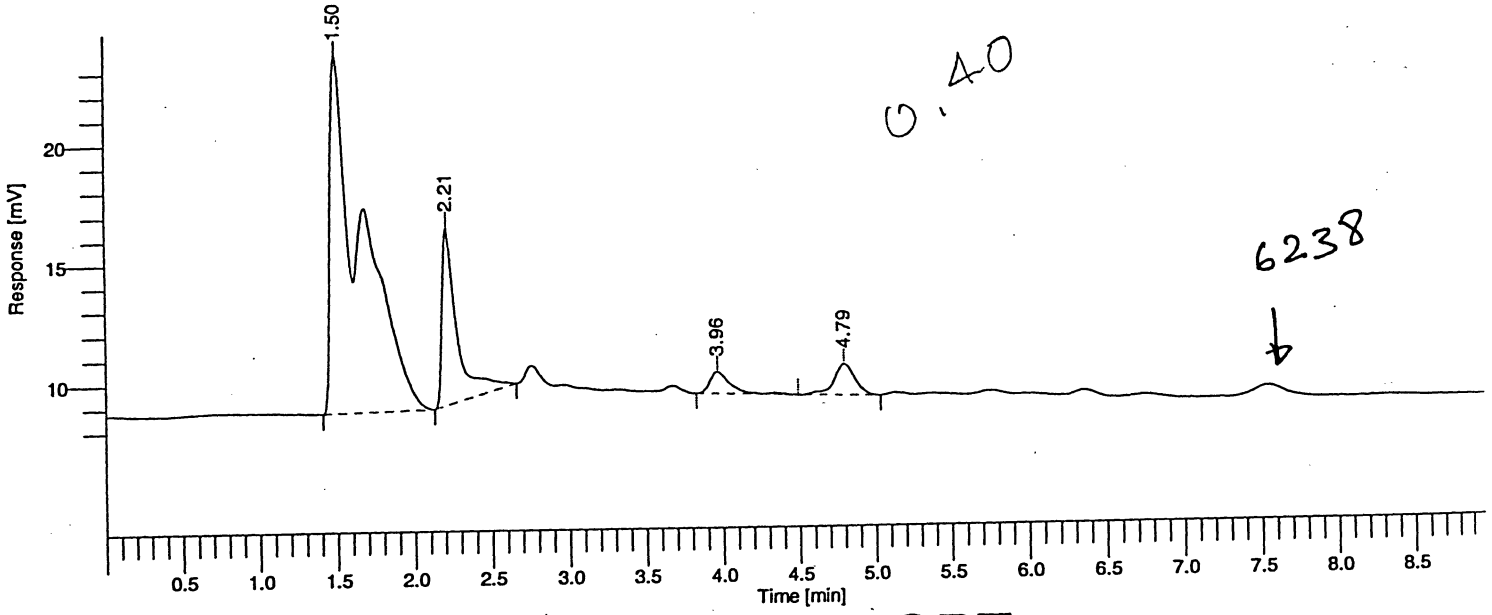
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 2:35:03 PM
Operator	: manager	Sample Name	: deg.,P23,EPS
Sample Number	: 143	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/28
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 3:33:34 PM	Cycle	: 149

Raw Data File : \\Poweredge\E drive\TC\dennis\data139.raw
Result File : \\Poweredge\E drive\TC\dennis\data139.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data139.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.500	212902.60	14969.48	74.86	74.86	BB	14.2224
2	2.213	50405.60	7436.86	17.72	17.72	BB	6.7778
3	3.963	8595.00	916.99	3.02	3.02	BV	9.3730
4	4.795	12490.33	1294.84	4.39	4.39	VB	9.6462
		284393.53	24618.18	100.00	100.00		

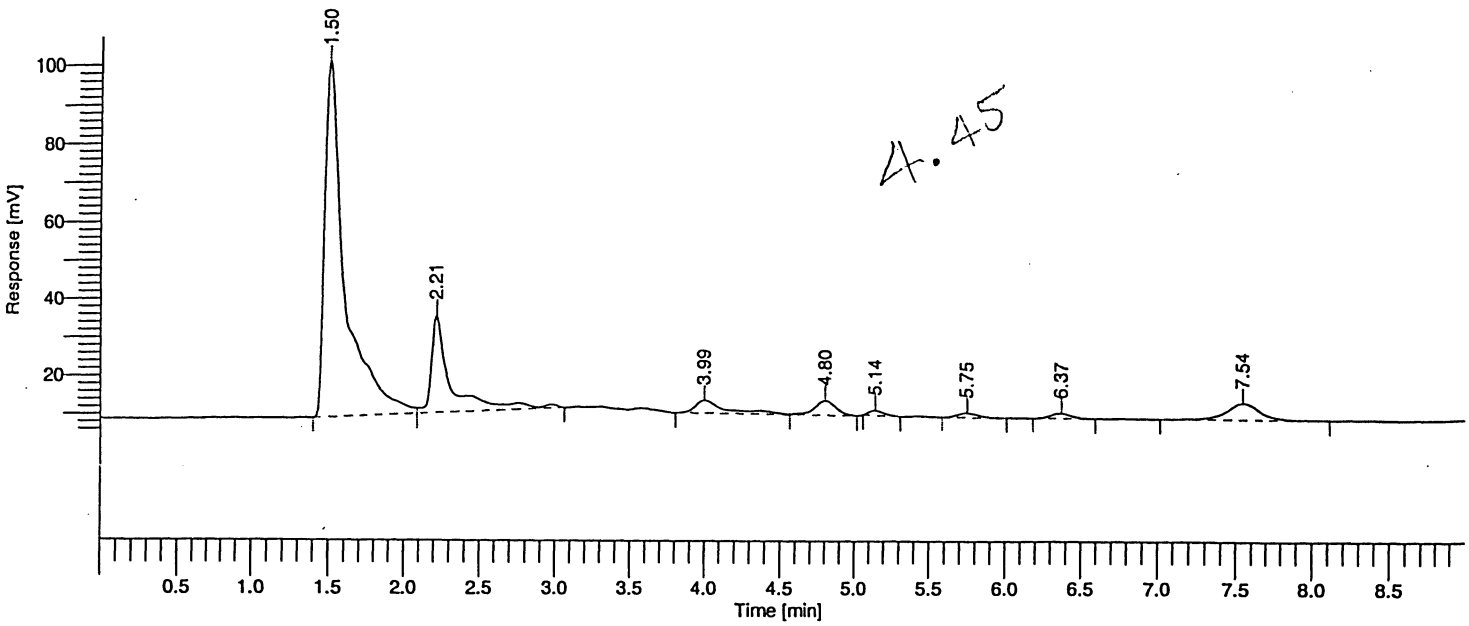
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 2:45:05 PM
Operator	: manager	Sample Name	: deg.,P31,EPS
Sample Number	: 144	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/29
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 3:43:35 PM	Cycle	: 150

Raw Data File : \\Poweredge\E drive\TC\dennis\data140.raw
Result File : \\Poweredge\E drive\TC\dennis\data140.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data140.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.505	843865.28	91807.40	66.89	66.89	BV	9.1917
2	2.215	228599.92	25346.24	18.12	18.12	VB	9.0191
3	3.995	49539.24	3389.99	3.93	3.93	BV	14.6134
4	4.799	38335.36	3802.53	3.04	3.04	VB	10.0815
5	5.138	9177.60	1316.16	0.73	0.73	BB	6.9730
6	5.745	11390.80	1196.97	0.90	0.90	BB	9.5164
7	6.369	12197.00	1349.17	0.97	0.97	BB	9.0403
8	7.545	68531.60	4418.35	5.43	5.43	BB	15.5107
		1261636.80	132626.81	100.00	100.00		

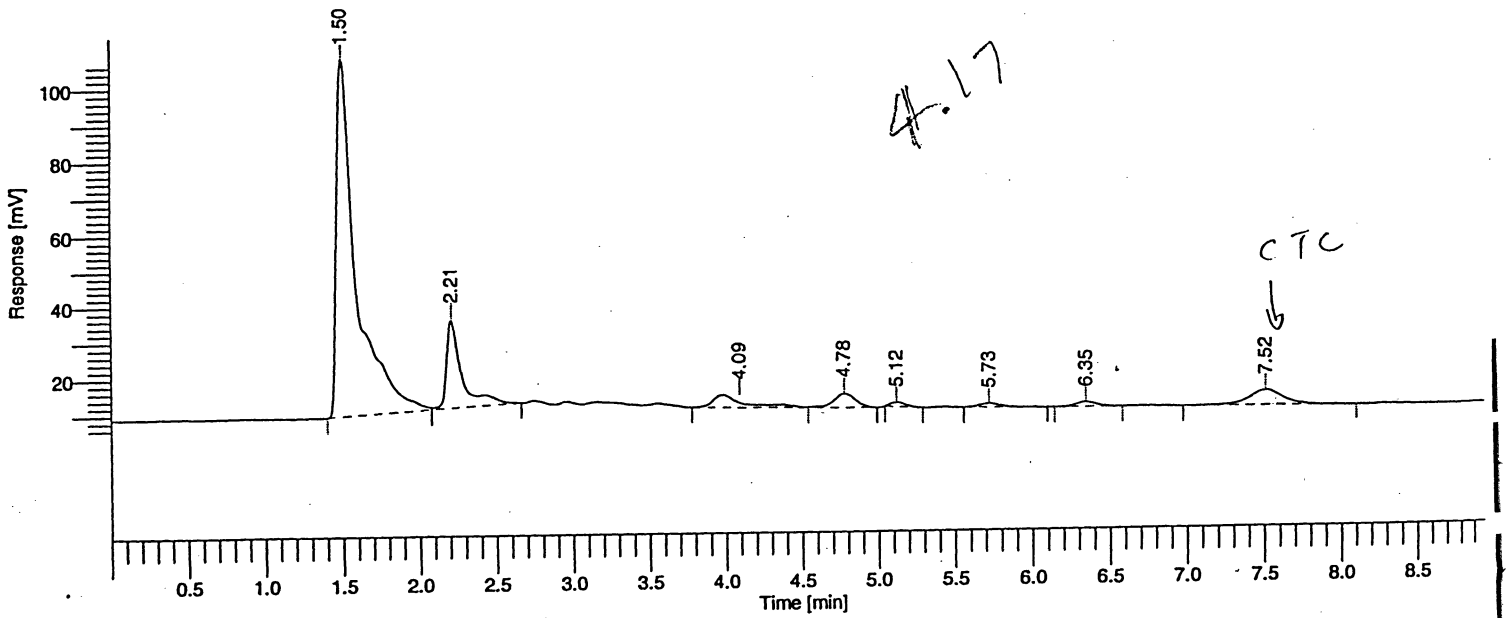
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 2:55:07 PM
Operator	: manager	Sample Name	: deg.,P32,EPS
Sample Number	: 145	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/30
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 3:53:39 PM	Cycle	: 151

Raw Data File : \\Poweredge\E drive\TC\dennis\data141.raw
Result File : \\Poweredge\E drive\TC\dennis\data141.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data141.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.501	887963.02	98277.16	71.72	71.72	BV	9.0353
2	2.211	168775.78	24537.11	13.63	13.63	VB	6.8784
3	4.091	48648.72	1247.82	3.93	3.93	BV	38.9869
4	4.778	38346.48	3844.34	3.10	3.10	VB	9.9748
5	5.124	8330.00	1234.31	0.67	0.67	BB	6.7487
6	5.726	10354.00	1085.23	0.84	0.84	BB	9.5408
7	6.351	11429.80	1291.14	0.92	0.92	BB	8.8525
8	7.516	64302.00	4156.64	5.19	5.19	BB	15.4697
		1238149.80	135673.75	100.00	100.00		

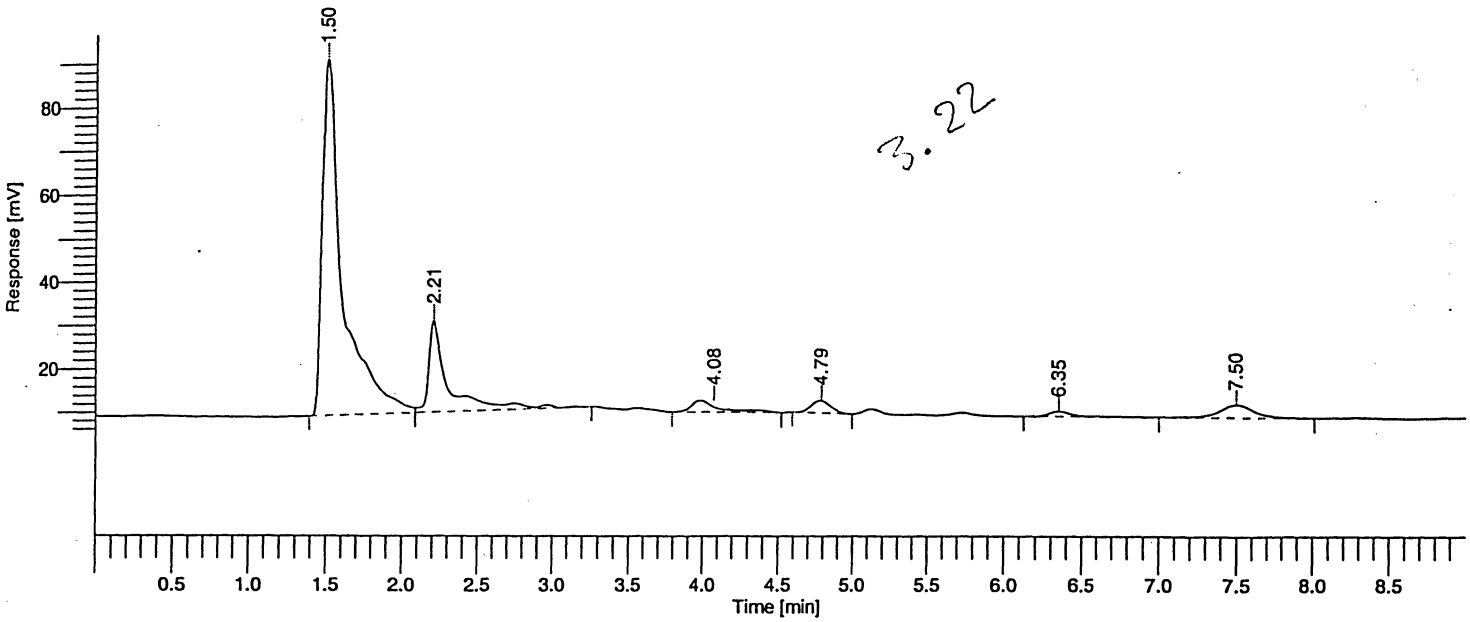
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 3:05:09 PM
Operator	: manager	Sample Name	: deg.,P33,EPS
Sample Number	: 146	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/31
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 4:03:41 PM	Cycle	: 152

Raw Data File : \\Poweredge\E drive\TC\dennis\data142.raw
Result File : \\Poweredge\E drive\TC\dennis\data142.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data142.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



3.22

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.504	771128.93	82182.25	70.31	70.31	BV	9.3832
2	2.209	196300.07	21278.58	17.90	17.90	VB	9.2252
3	4.078	36924.00	1210.96	3.37	3.37	BB	30.4914
4	4.789	27652.40	3006.98	2.52	2.52	BB	9.1961
5	6.349	15119.29	1237.86	1.38	1.38	BV	12.2140
6	7.497	49708.71	3161.49	4.53	4.53	VB	15.7232
		1096833.40	112078.13	100.00	100.00		

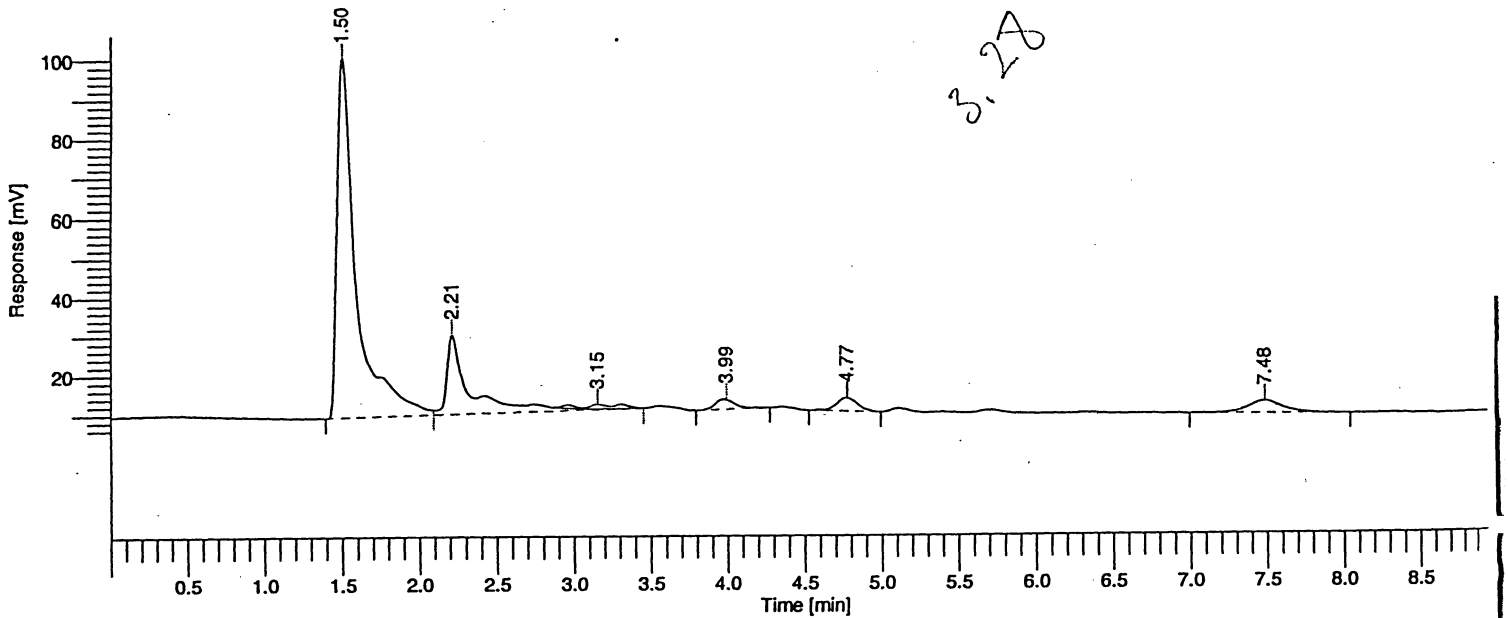
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 3:15:10 PM
Operator	: manager	Sample Name	: deg.,P41,EPS
Sample Number	: 147	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/32
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 4:13:41 PM	Cycle	: 153

Raw Data File : \\Poweredge\E drive\TC\dennis\data143.raw
Result File : \\Poweredge\E drive\TC\dennis\data143.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data143.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.503	792339.33	91058.48	69.85	69.85	BV	8.7014
2	2.212	213262.27	20272.70	18.80	18.80	VE	10.5197
3	3.155	21871.60	1195.52	1.93	1.93	EB	18.2947
4	3.993	23203.20	2452.65	2.05	2.05	BB	9.4605
5	4.774	33230.20	3441.78	2.93	2.93	BB	9.6550
6	7.483	50492.40	3182.48	4.45	4.45	BB	15.8658
		1134399.00	121603.60	100.00	100.00		

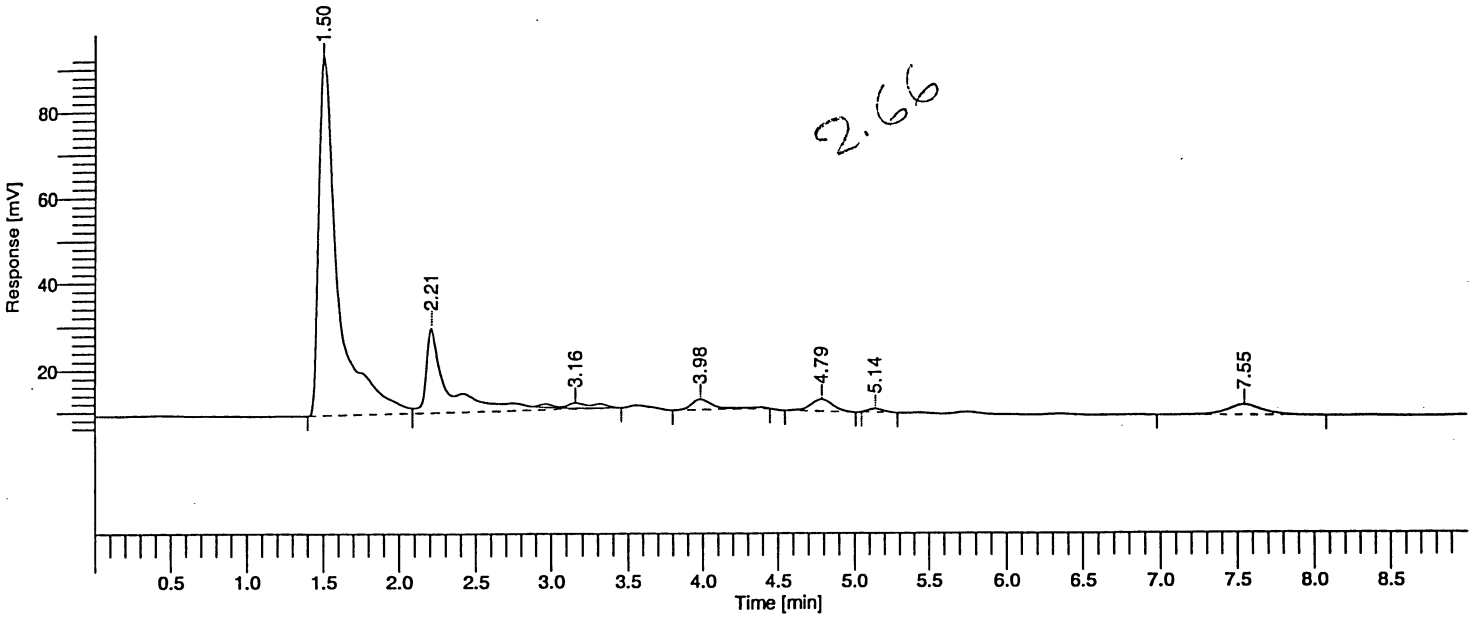
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 3:25:12 PM
Operator	: manager	Sample Name	: deg.,P42,EPS
Sample Number	: 148	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/33
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 4:23:41 PM	Cycle	: 154

Raw Data File : \\Poweredge\E drive\TC\dennis\data144.raw
Result File : \\Poweredge\E drive\TC\dennis\data144.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data144.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.503	738124.26	83602.33	68.60	68.60	BV	8.8290
2	2.210	211678.54	19875.50	19.67	19.67	VE	10.6502
3	3.159	21630.40	1258.03	2.01	2.01	EB	17.1939
4	3.984	27809.60	2503.24	2.58	2.58	BB	11.1094
5	4.788	29427.20	2917.73	2.74	2.74	BB	10.0856
6	5.139	6214.60	892.86	0.58	0.58	BB	6.9603
7	7.549	41054.00	2539.01	3.82	3.82	BB	16.1693
		1075938.60	113588.70	100.00	100.00		

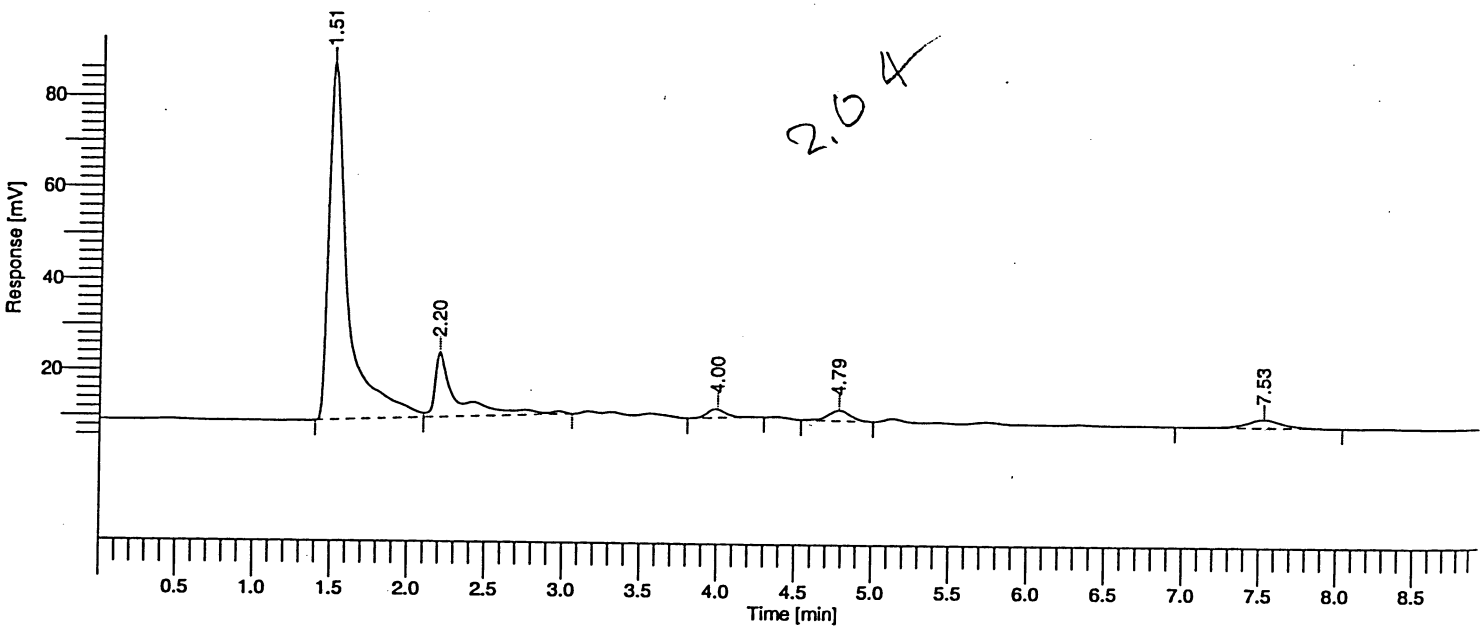
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 5/31/06 3:35:07 PM
Operator	: manager	Sample Name	: deg.,P43,EPS
Sample Number	: 149	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/34
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 5/31/06 4:33:42 PM	Cycle	: 155

Raw Data File : \\Poweredge\E drive\TC\dennis\data145.raw
Result File : \\Poweredge\E drive\TC\dennis\data145.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data145.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.506	669674.08	77954.26	76.01	76.01	BV	8.5906
2	2.202	140161.12	14162.42	15.91	15.91	VB	9.8967
3	4.005	16485.40	1796.09	1.87	1.87	BB	9.1785
4	4.785	23299.20	2268.87	2.64	2.64	BB	10.2691
5	7.531	31424.00	1880.35	3.57	3.57	BB	16.7118
		881043.80	98061.99	100.00	100.00		

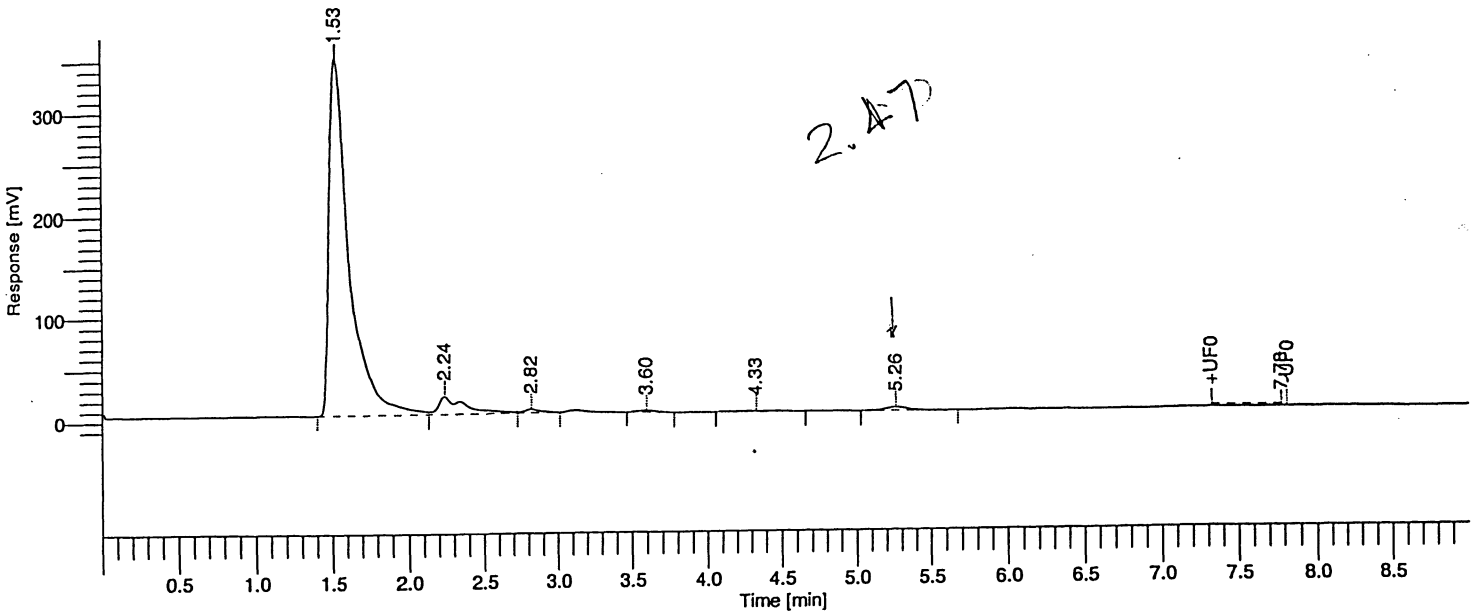
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 10:17:59 AM
Operator	: manager	Sample Name	: DEG., P11, CELLS
Sample Number	: 151	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/36
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 11:16:31 AM	Cycle	: 157

Raw Data File : \\Poweredge\E drive\TC\dennis\data147.raw
Result File : \\Poweredge\E drive\TC\dennis\data147.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data147.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



2.47

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.530	3206999.60	346502.08	90.94	90.94	BV	9.2554
2	2.239	224205.75	17685.94	6.36	6.36	VV	12.6771
3	2.819	28850.65	3640.44	0.82	0.82	VB	7.9250
4	3.595	15067.20	1923.92	0.43	0.43	BB	7.8315
5	4.330	13407.60	445.08	0.38	0.38	BB	30.1242
6	5.260	37988.40	3467.10	1.08	1.08	BB	10.9568
		3526519.20	373664.56	100.00	100.00		

Missing Component Report

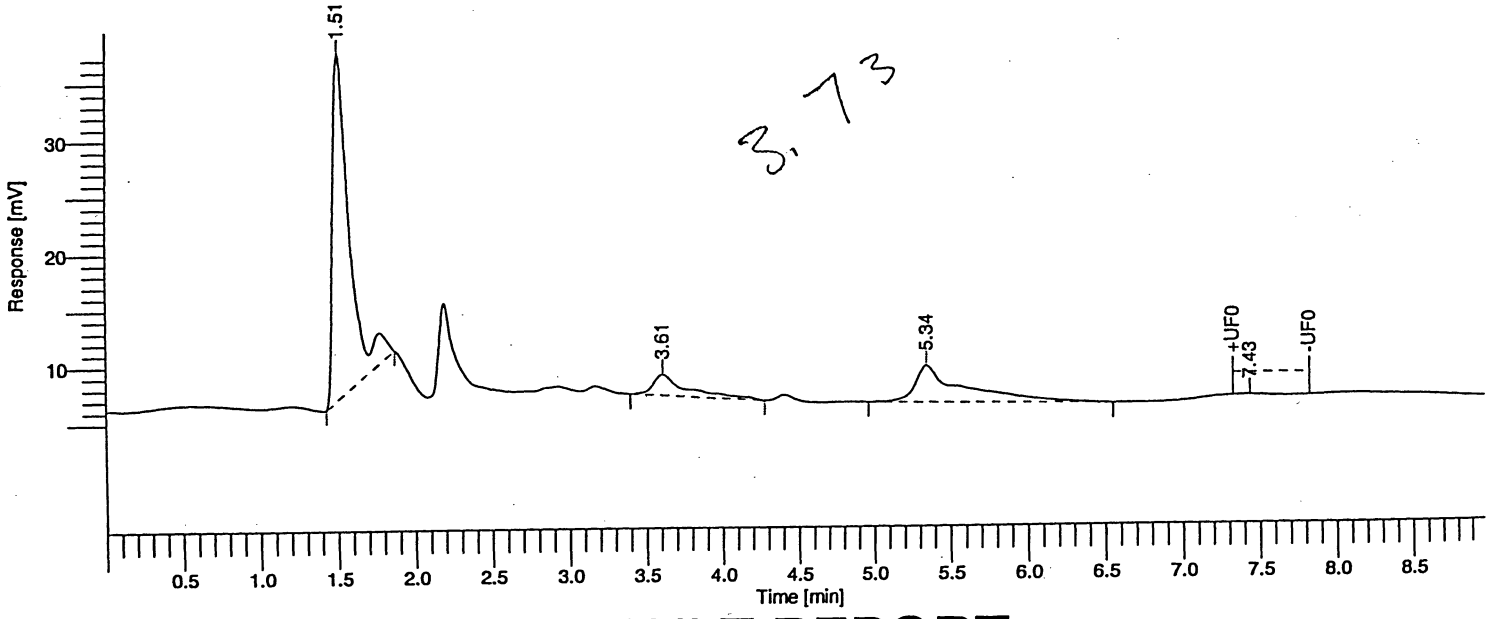
Component	Expected Retention (Calibration File)

All components were found

35124

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 10:28:01 AM
Operator	: manager	Sample Name	: DEG., P12 CELLS
Sample Number	: 152	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/37
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 11:26:31 AM	Cycle	: 158

Raw Data File : \\Poweredge\E drive\TC\dennis\data148.raw
Result File : \\Poweredge\E drive\TC\dennis\data148.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data148.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



3.73

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.512	230874.60	30189.97	70.01	70.01	BB	7.6474
2	3.605	28218.00	1839.43	8.56	8.56	BB	15.3407
3	5.342	70672.00	3182.62	21.43	21.43	BB	22.2056
		329764.60	35212.02	100.00	100.00		

Missing Component Report

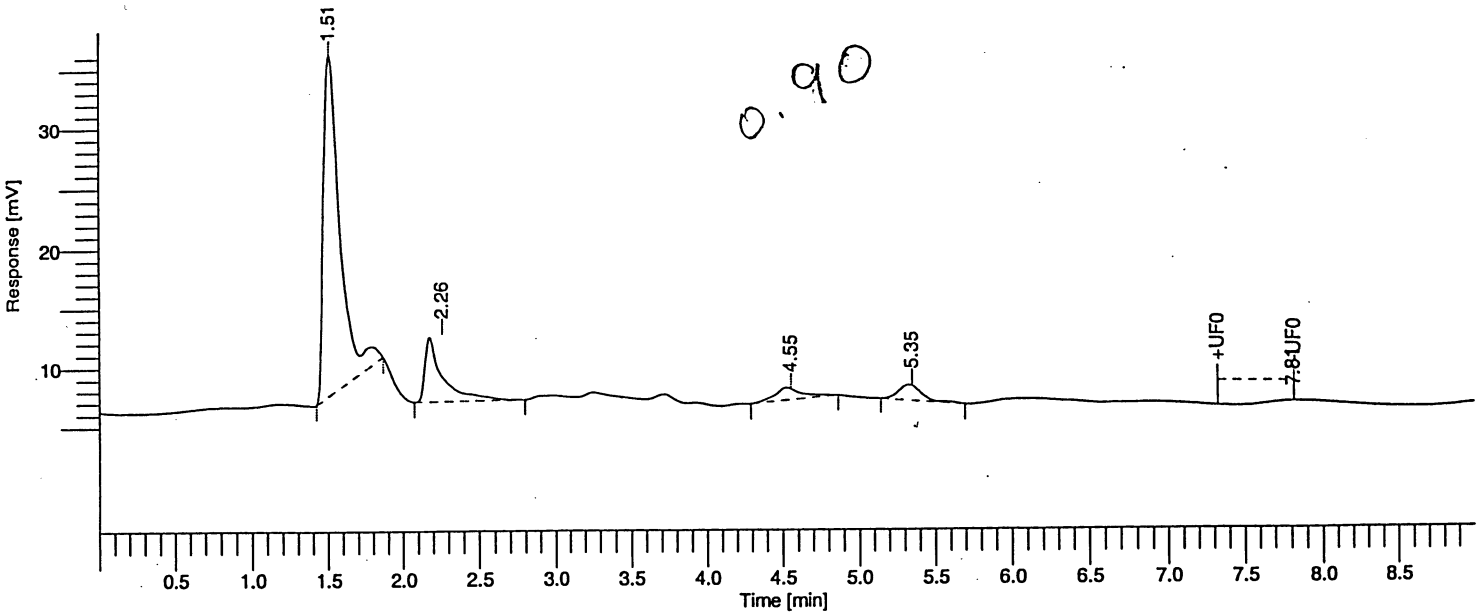
Component	Expected Retention (Calibration File)

All components were found

18910

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 10:38:03 AM
Operator	: manager	Sample Name	: DEG., P13 CELLS
Sample Number	: 153	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/38
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 11:36:31 AM	Cycle	: 159

Raw Data File : \\Poweredge\E drive\TC\dennis\data149.raw
Result File : \\Poweredge\E drive\TC\dennis\data149.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data149.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.510	222741.20	28555.54	75.53	75.53	BB	7.8003
2	2.259	45271.20	2108.08	15.35	15.35	BB	21.4751
3	4.550	13004.80	938.95	4.41	4.41	BB	13.8504
4	5.346	13905.60	1292.50	4.71	4.71	BB	10.7587
		294922.80	32895.07	100.00	100.00		

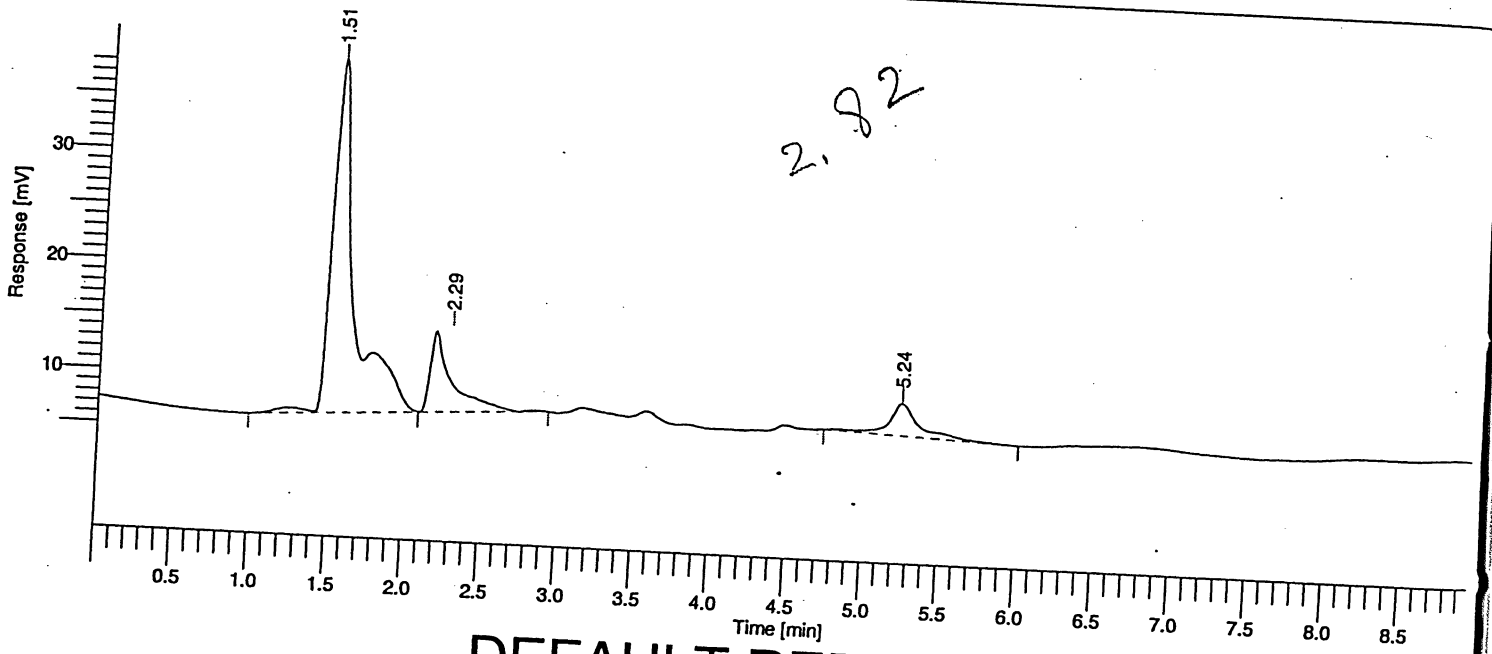
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 10:47:59 AM
Operator	: manager	Sample Name	: DEG., P21 CELLS
Sample Number	: 154	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/39
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 11:46:32 AM	Cycle	: 160

Raw Data File : \\Poweredge\E drive\TC\dennis\data150.raw
Result File : \\Poweredge\E drive\TC\dennis\data150.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data150.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.507	333381.16	32110.35	73.73	73.73	BV	10.3824
2	2.287	75229.04	2748.86	16.64	16.64	VB	27.3673
3	5.239	43533.20	2978.59	9.63	9.63	BB	14.6154
		452143.40	37837.80	100.00	100.00		

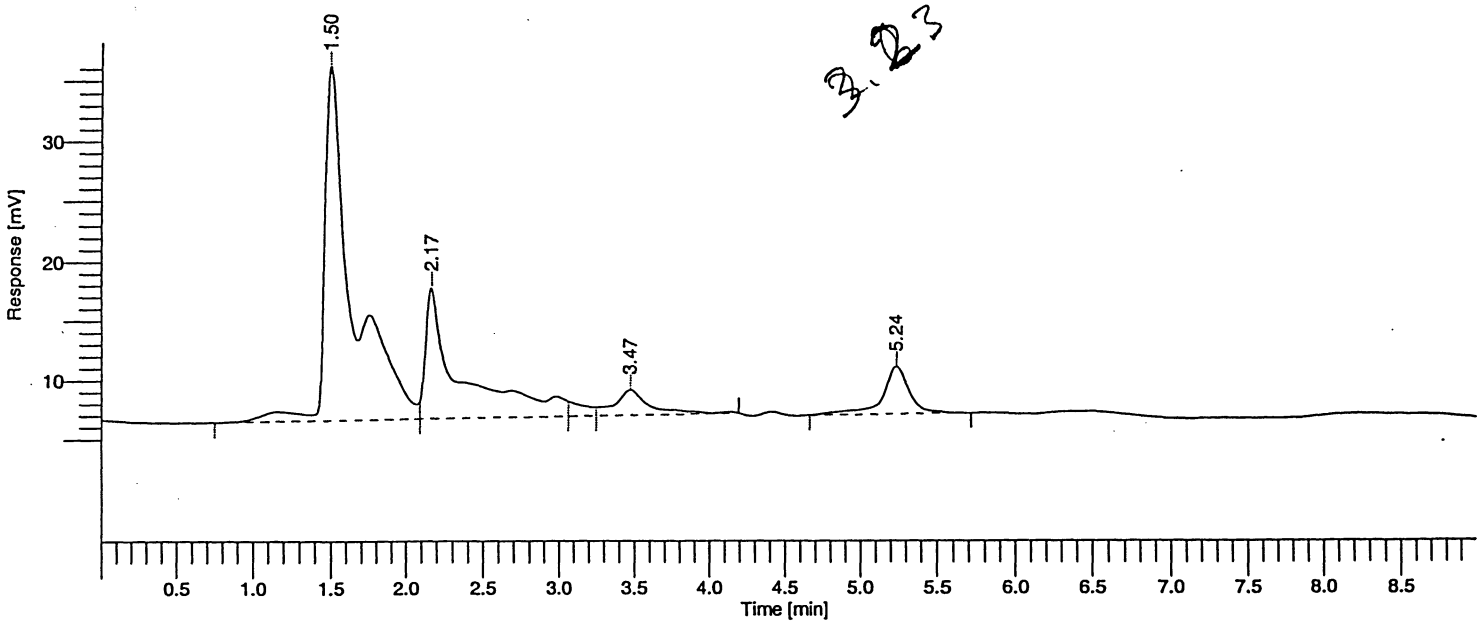
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 10:58:08 AM
Operator	: manager	Sample Name	: DEG., P22 CELLS
Sample Number	: 155	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/40
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 11:56:36 AM	Cycle	: 161

Raw Data File : \\Poweredge\E drive\TC\dennis\data151.raw
Result File : \\Poweredge\E drive\TC\dennis\data151.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data151.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.505	380252.88	29653.57	59.15	59.15	BV	12.8232
2	2.170	176797.59	11102.51	27.50	27.50	VV	15.9241
3	3.473	35990.11	2139.50	5.60	5.60	VB	16.8217
4	5.238	49832.40	3971.21	7.75	7.75	BB	12.5484
		642872.98	46866.80	100.00	100.00		

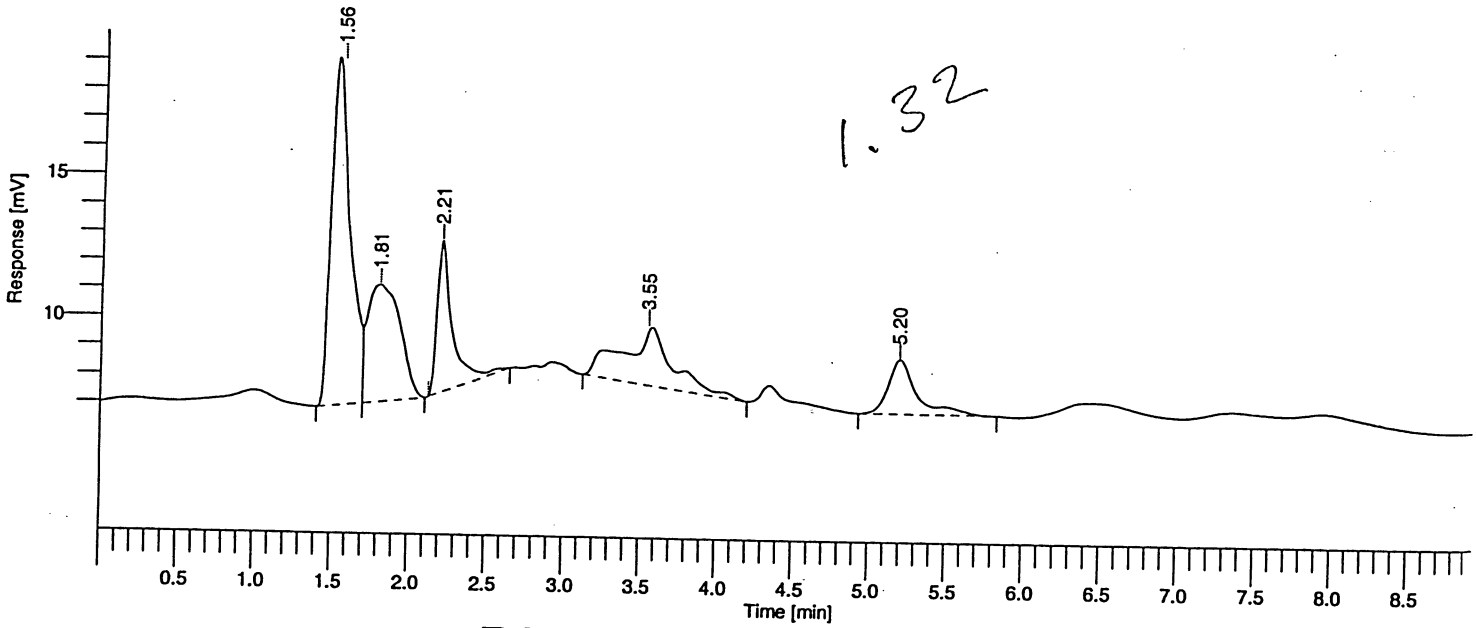
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 11:08:09 AM
Operator	: manager	Sample Name	: DEG., P23 CELLS
Sample Number	: 156	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/41
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 12:06:38 PM	Cycle	: 162

Raw Data File : \\Poweredge\E drive\TC\dennis\data152.raw
Result File : \\Poweredge\E drive\TC\dennis\data152.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data152.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



1.32

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.555	103571.55	9713.35	38.68	38.68	BV	10.6628
2	1.808	59297.25	4087.19	22.15	22.15	VB	14.5081
3	2.208	35484.80	5341.43	13.25	13.25	BB	6.6433
4	3.554	44405.60	1836.66	16.58	16.58	BB	24.1773
5	5.197	24997.60	1873.37	9.34	9.34	BB	13.3436
		267756.80	22852.01	100.00	100.00		

Missing Component Report

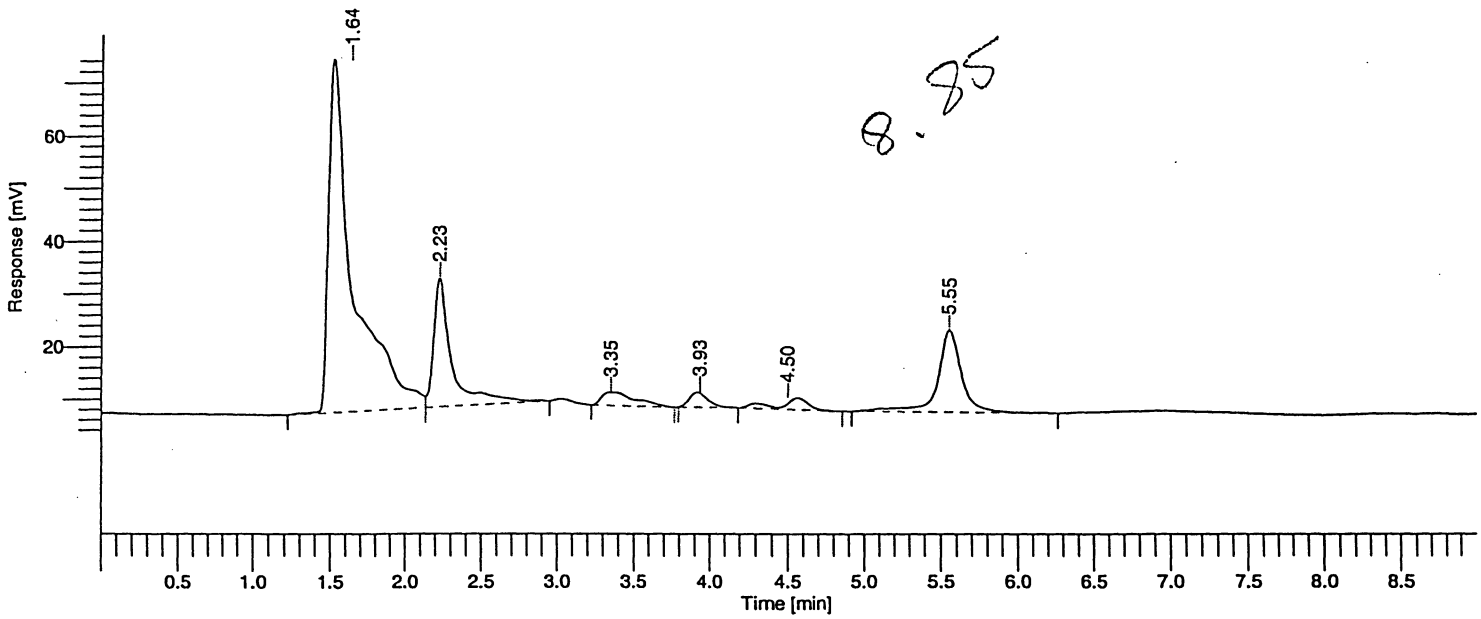
Component	Expected Retention (Calibration File)

All components were found

1747

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 12:18:05 PM
Operator	: manager	Sample Name	: DEG,P31CELL
Sample Number	: 157	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/42
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 1:16:36 PM	Cycle	: 163

Raw Data File : \\Poweredge\E drive\TC\dennis\data153.raw
Result File : \\Poweredge\E drive\TC\dennis\data153.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data153.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.642	774344.37	21589.85	62.34	62.34	BV	35.8661
2	2.228	205657.63	24552.80	16.56	16.56	VB	8.3761
3	3.349	40277.40	2600.30	3.24	3.24	BB	15.4895
4	3.933	25082.40	2834.68	2.02	2.02	BB	8.8484
5	4.497	28882.00	1117.61	2.33	2.33	BB	25.8427
6	5.553	167922.60	15733.23	13.52	13.52	BB	10.6731
		1242166.40	68428.47	100.00	100.00		

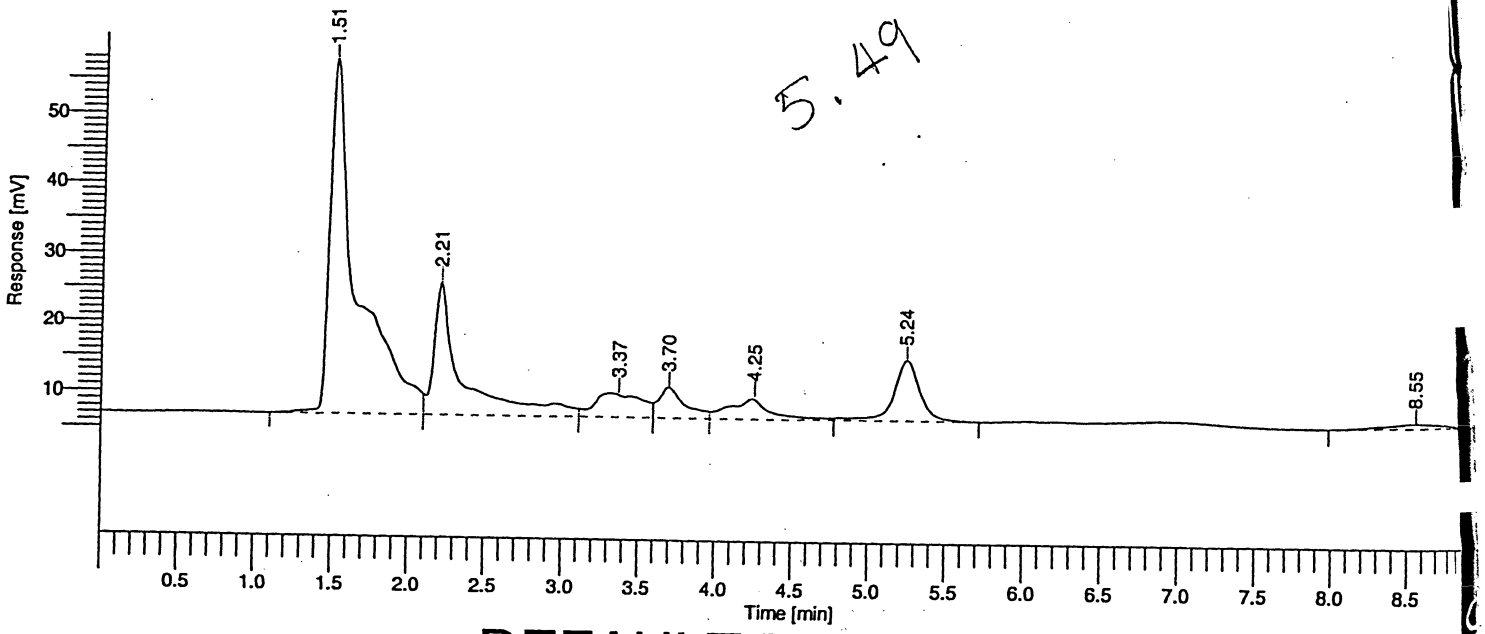
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 12:28:06 PM
Operator	: manager	Sample Name	: DEG,P32CELL
Sample Number	: 158	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/43
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 1:26:36 PM	Cycle	: 164

Raw Data File : \\Poweredge\E drive\TC\dennis\data154.raw
Result File : \\Poweredge\E drive\TC\dennis\data154.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data154.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.507	630312.82	50898.07	53.89	53.89	BV	12.3838
2	2.211	235825.31	19002.76	20.16	20.16	VV	12.4101
3	3.371	71830.90	3077.70	6.14	6.14	VV	23.3391
4	3.696	51147.67	4313.33	4.37	4.37	VV	11.8581
5	4.254	55953.48	2715.50	4.78	4.78	VV	20.6052
6	5.244	104096.61	8575.11	8.90	8.90	VB	12.1394
7	8.551	20469.00	685.42	1.75	1.75	BB	29.8632
		1169635.80	89267.90	100.00	100.00		

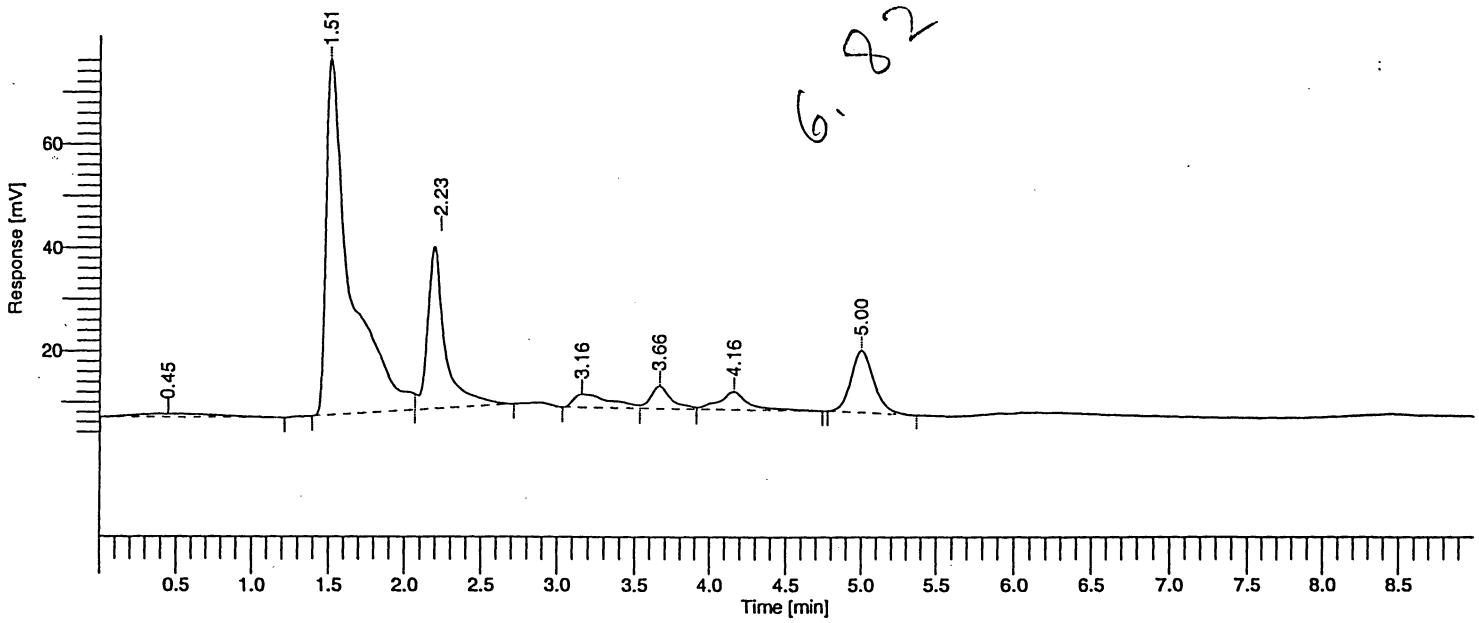
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 12:38:07 PM
Operator	: manager	Sample Name	: DEGP33 CELL
Sample Number	: 159	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/44
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 1:36:37 PM	Cycle	: 165

Raw Data File : \\Poweredge\E drive\TC\dennis\data155.raw
Result File : \\Poweredge\E drive\TC\dennis\data155.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data155.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.448	28096.80	704.98	2.12	2.12	BB	39.8547
2	1.513	771453.77	68704.78	58.28	58.28	BV	11.2285
3	2.231	256826.63	18801.40	19.40	19.40	VB	13.6600
4	3.158	44888.93	2661.31	3.39	3.39	BV	16.8673
5	3.660	42817.13	4514.19	3.23	3.23	VV	9.4850
6	4.158	50312.54	3643.01	3.80	3.80	VB	13.8107
7	4.996	129376.40	12144.57	9.77	9.77	BB	10.6530
		1323772.20	111174.23	100.00	100.00		

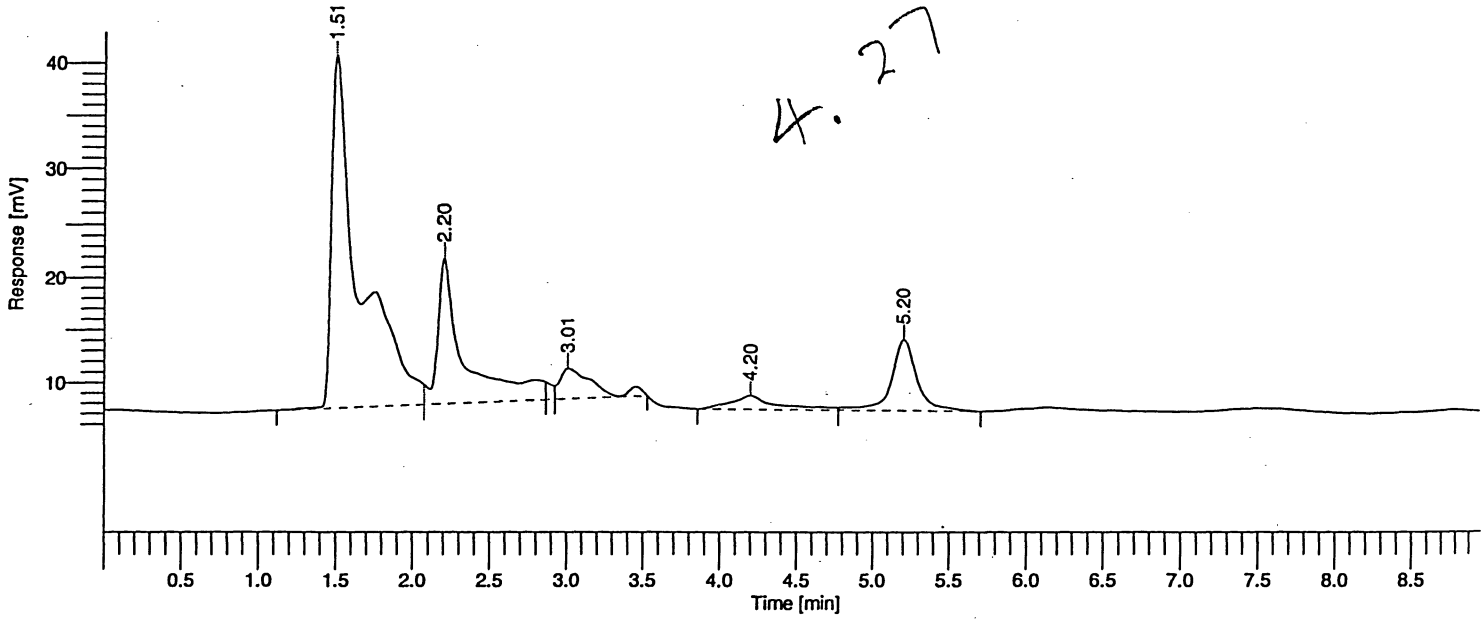
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 12:48:02 PM
Operator	: manager	Sample Name	: DEG P41 CELL
Sample Number	: 160	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/45
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 1:46:37 PM	Cycle	: 166

Raw Data File : \\Poweredge\E drive\TC\dennis\data156.raw
Result File : \\Poweredge\E drive\TC\dennis\data156.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data156.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.505	422534.18	33210.33	56.51	56.51	BV	12.7230
2	2.205	170342.07	13940.42	22.78	22.78	VV	12.2193
3	3.007	45363.60	2933.94	6.07	6.07	VB	15.4616
4	4.199	28552.00	1353.27	3.82	3.82	BV	21.0986
5	5.203	80962.60	6829.78	10.83	10.83	VB	11.8544
		747754.44	58267.73	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 12:58:04 PM
Operator	: manager	Sample Name	: DEG P42 CELL
Sample Number	: 161	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/46
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 1:56:37 PM	Cycle	: 167

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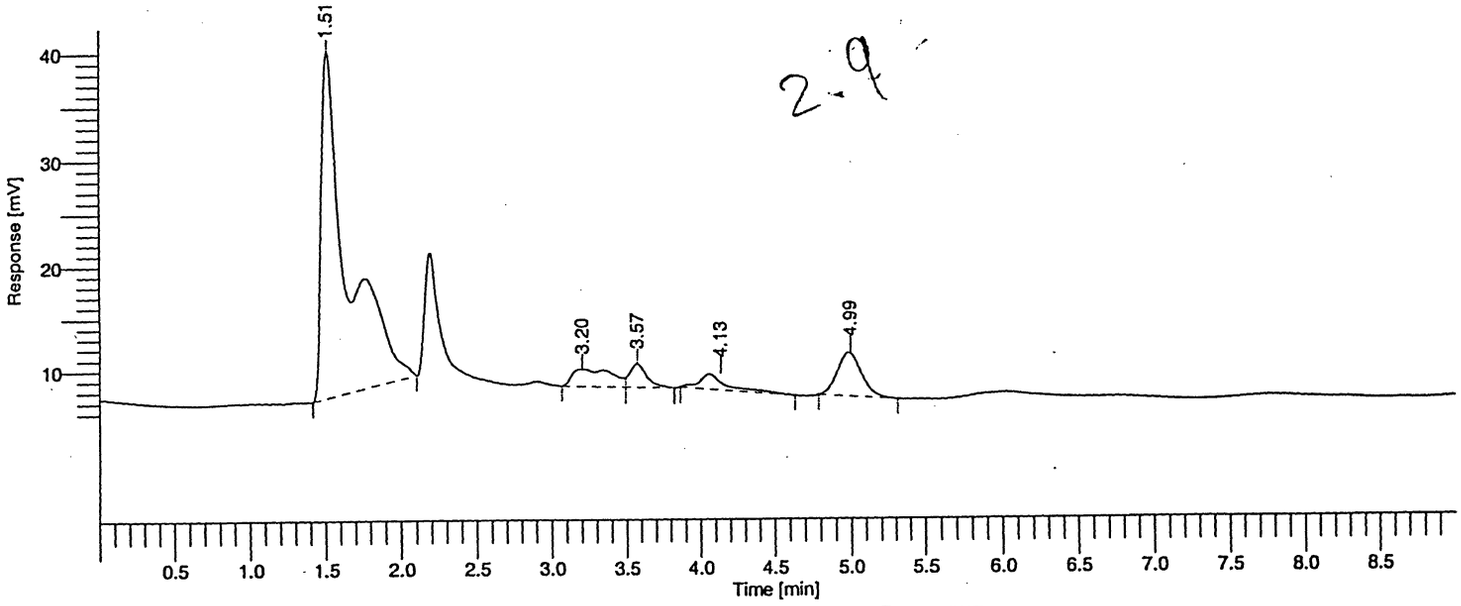

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.510	393713.60	32560.43	78.15	78.15	BB	12.0918
2	3.201	30357.47	1627.35	6.03	6.03	BV	18.6546
3	3.569	17578.93	2212.23	3.49	3.49	VB	7.9463
4	4.132	17414.00	653.93	3.46	3.46	BB	26.6299
5	4.993	44734.80	4016.67	8.88	8.88	BB	11.1373
		503798.80	41070.60	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/1/06 1:08:05 PM
Operator	: manager	Sample Name	: DEG P43 CELL
Sample Number	: 162	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/47
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/1/06 2:06:37 PM	Cycle	: 168

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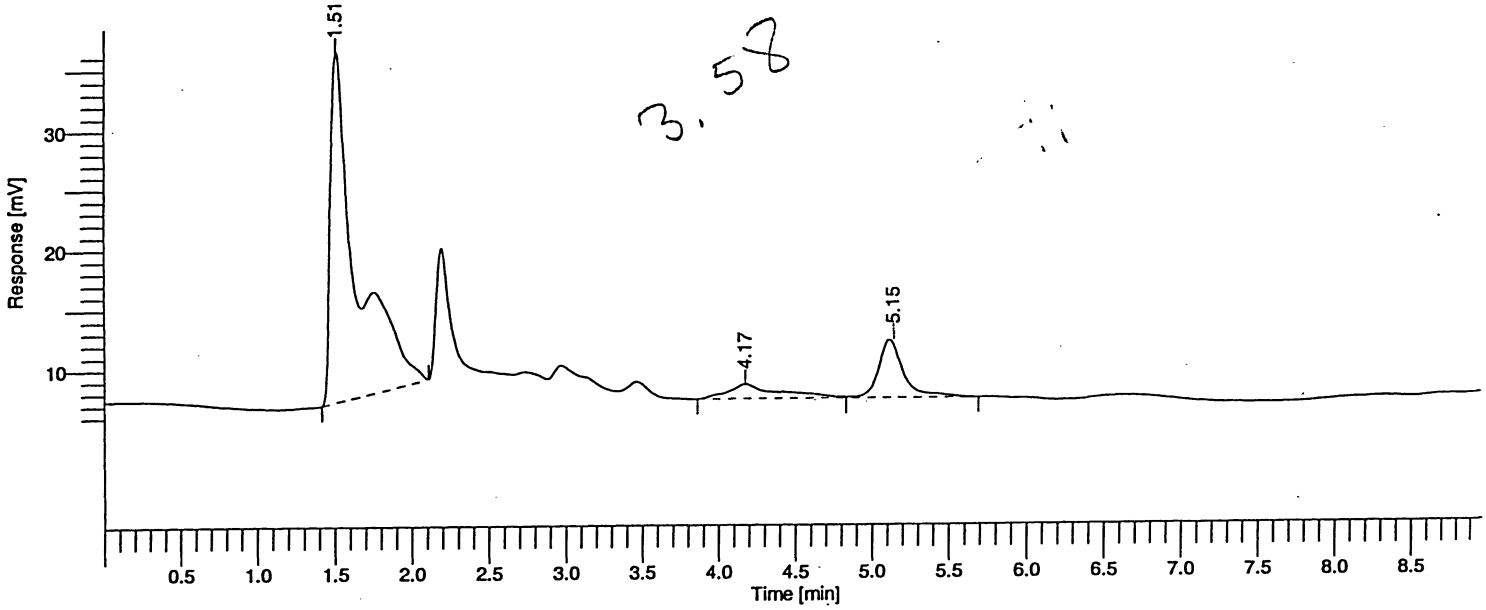
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.510	343230.80	29121.41	80.33	80.33	BB	11.7862
2	4.174	28818.46	1240.13	6.75	6.75	BV	23.2382
3	5.146	55203.54	4345.89	12.92	12.92	VB	12.7025
		427252.80	34707.43	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

APPENDIX B: PARAMETERS OF CHLORTETRACYCLINE REMOVAL UNDER ACTIVE AND INAACTIVE BIOMASS

Table B.1: Concentration of Chlortetracycline (μg/mL) in bulk water

sample	Chlortetracycline Concentration under inactive biomass (μg/mL)	Chlortetracycline Concentration under active biomass (μg/mL)
Sample 1	0.77	2.09
Sample 2	0.36	2.43
Sample 3	0.212	1.41
Sample4		1.08
Sample 5		2.74
Sample 6		2.29

Student t-test: two-sample unequal variance, one tailed distribution

$p = 0.03$

Table B.2: Concentration of Chlortetracycline (μg/mL) in extracellular polymeric substance

sample	Chlortetracycline Concentration under inactive biomass (μg/mL)	Chlortetracycline Concentration under active biomass (μg/mL)
Sample 1	0.358	0.225
Sample 2	0.357	0.362
Sample 3	0.306	0.305
Sample4		0.413
Sample 5		0.771

Student t-test: two-sample unequal variance, one tailed distribution

$p = 0.24$

Table B.3: Concentration of Chlortetracycline (µg/mL) in microbial cells

Chlortetracycline Concentration under inactive biomass (µg/mL)	Chlortetracycline Concentration under active biomass (µg/mL)
4.03	1.03
3.88	0.561

Student t-test: two-sample unequal variance, one tailed distribution
p= 0.003

Table B.4: Effect of substrate presence on Chlortetracycline concentration (µg/mL) in bulk water under inactive biomass

sample	Chlortetracycline Concentration under inactive biomass /no substrate (µg/mL)	Chlortetracycline Concentration under inactive biomass /w. substrate (µg/mL)
Sample 1	1.61	0.77
Sample 2	2.71	0.36
Sample 3	1.03	0.212
Sample4	1.83	
Sample 5	2.04	
Sample 6	3.29	

Student t-test: two-sample unequal variance, one tailed distribution
p= 0.002

Table B.5: Parameters of sludge samples under inactive biomass

sample	MLSS (mg/L)	SOUR (mg/g)/h	F/M (g BOD/ g MLSS)
Sample 1	2.04	2.50	No substrate
Sample 2	2.04	3.00	No substrate
Sample 3	0.78	2.30	1.54
Sample4	1.0	3.67	No substrate
Sample 5	2.62	1.67	0.46
Sample 6	2.58	1.68	0.47
Sample 7	0.74	2.96	1.62
Sample 8	0.74	2.92	1.62

Table B.6: Parameters of sludge samples under active biomass

sample	MLSS (mg/L)	SOUR (mg/g)/h	F/M (g BOD/ g MLSS)
Sample 1	1.24	5.28	0.97
Sample 2	1.24	4.62	0.97
Sample 3	1.8	4.30	0.533
Sample4	1.8	5.67	0.533
Sample 5	0.62	32.9	1.94
Sample 6	0.58	26.4	2.06

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 11:17:15 AM
Operator	: manager	Sample Name	: ctc20ppm,MEOH
Sample Number	: 295	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/1
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 12:17:29 PM	Cycle	: 295

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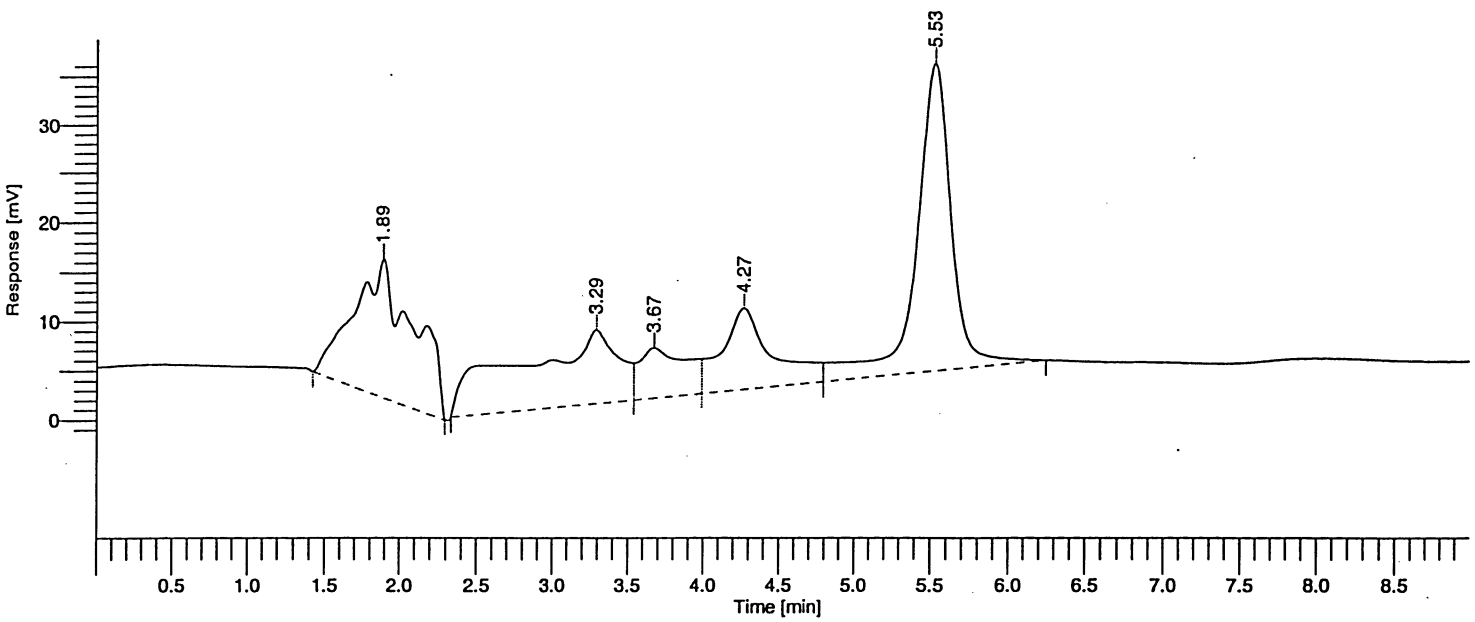

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.887	385310.40	14071.47	25.32	25.32	BB	27.3824
2	3.290	344516.32	7498.78	22.64	22.64	BV	45.9430
3	3.671	108615.89	5109.34	7.14	7.14	VV	21.2583
4	4.265	190404.32	8230.52	12.51	12.51	VV	23.1339
5	5.529	~~492739.07~~	31383.16	32.38	32.38	VB	15.7007
		1521586.00	66293.27	100.00	100.00		

Missing Component Report
Component Expected Retention (Calibration File)

All components were found

406409

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 11:27:10 AM
Operator	: manager	Sample Name	: -VE P11,OS
Sample Number	: 296	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/2
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 12:27:29 PM	Cycle	: 296

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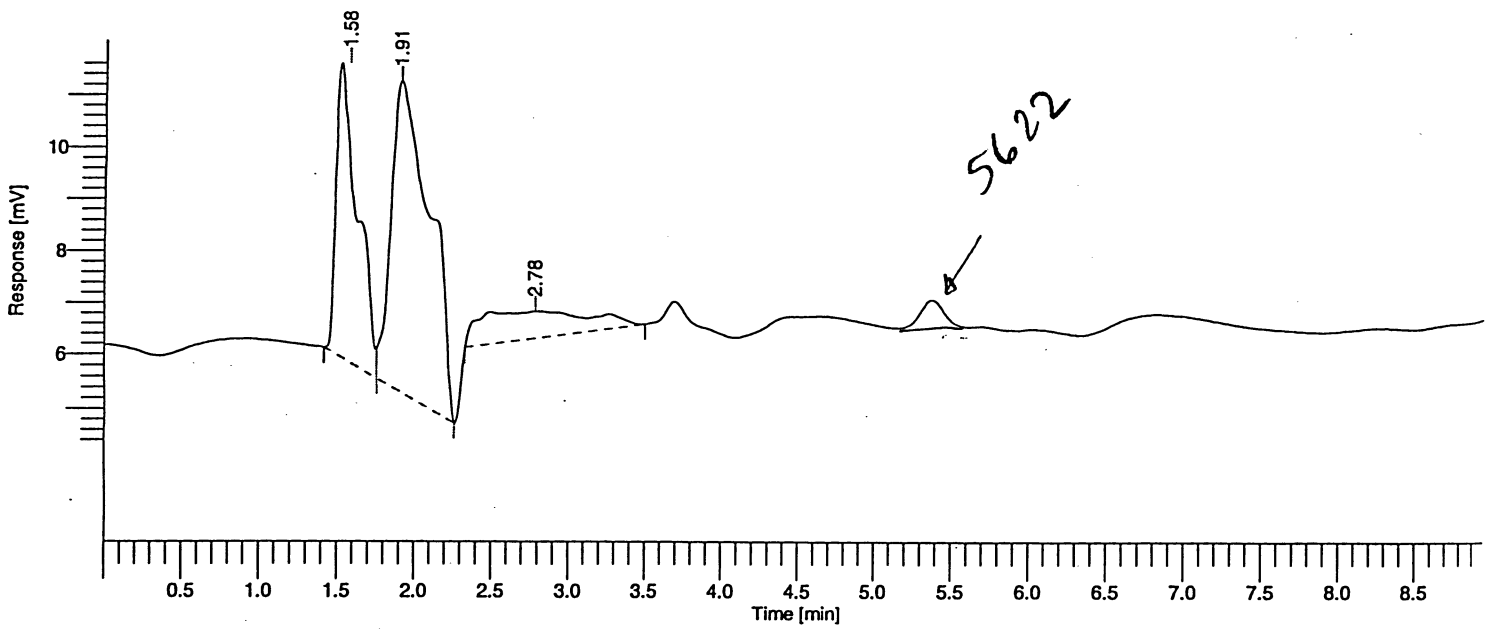
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.580	54548.35	3831.55	29.33	29.33	BV	14.2366
2	1.912	104617.65	5960.56	56.25	56.25	VB	17.5517
3	2.782	26820.00	529.26	14.42	14.42	BB	50.6750
		185986.00	10321.36	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 11:37:14 AM
Operator	: manager	Sample Name	: -VE P12,0S
Sample Number	: 297	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/3
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 12:37:29 PM	Cycle	: 297

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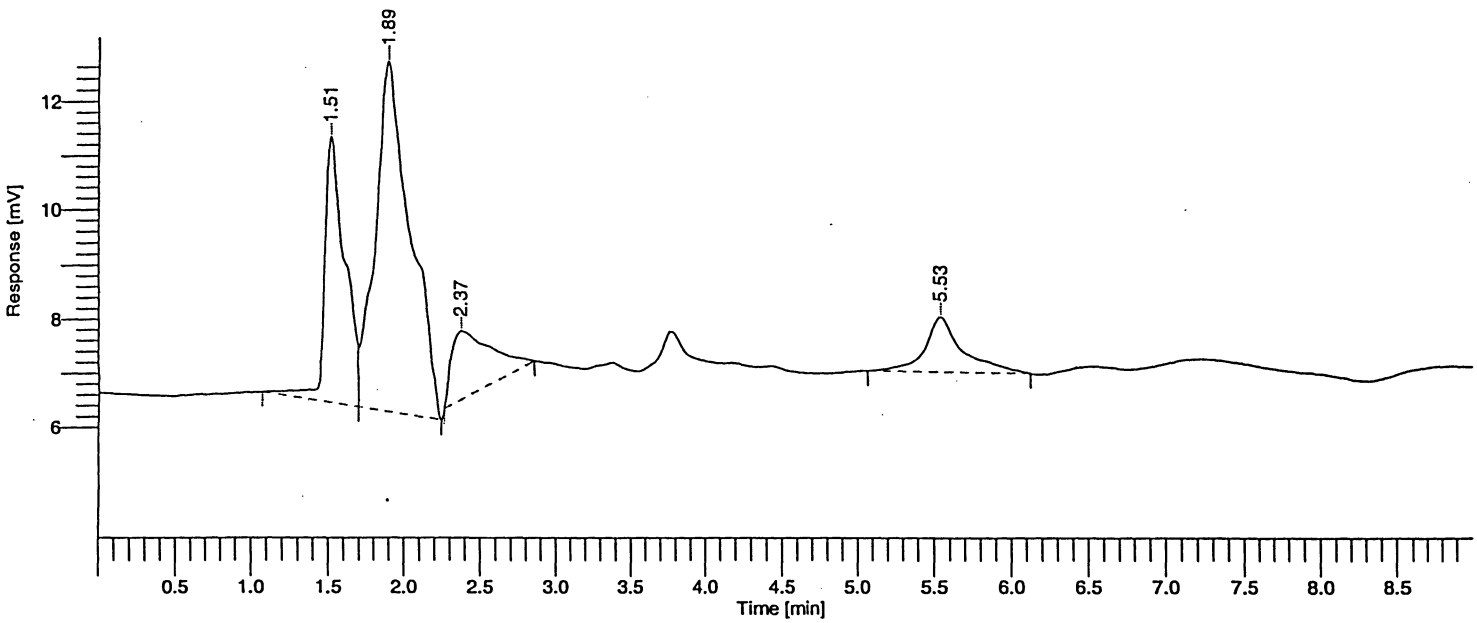

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.513	45490.70	4888.35	24.02	24.02	BV	9.3059
2	1.888	104304.70	6420.99	55.07	55.07	VB	16.2443
3	2.371	22331.60	1263.31	11.79	11.79	BB	17.6770
4	5.535	17266.80	1026.83	9.12	9.12	BB	16.8156
		189393.80	13599.49	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 11:47:10 AM
Operator	: manager	Sample Name	: -VEP11,50S
Sample Number	: 298	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/4
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 12:47:30 PM	Cycle	: 298

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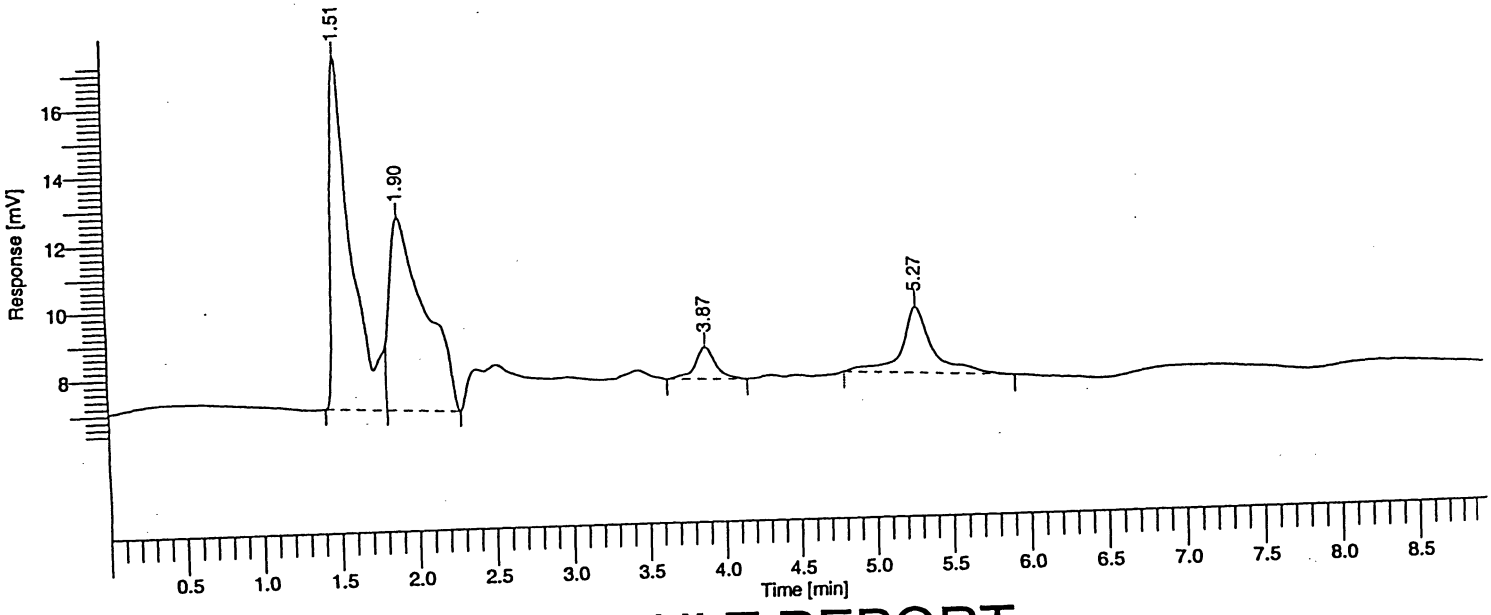

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.511	92767.77	10360.10	42.69	42.69	BV	8.9543
2	1.899	89516.63	5667.84	41.19	41.19	VB	15.7938
3	3.874	8338.00	904.54	3.84	3.84	BB	9.2179
4	5.270	26677.60	1933.74	12.28	12.28	BB	13.7959
		217300.00	18866.22	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 12:07:09 PM
Operator	: manager	Sample Name	: -VEP1,150S
Sample Number	: 300	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/6
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 1:07:30 PM	Cycle	: 300

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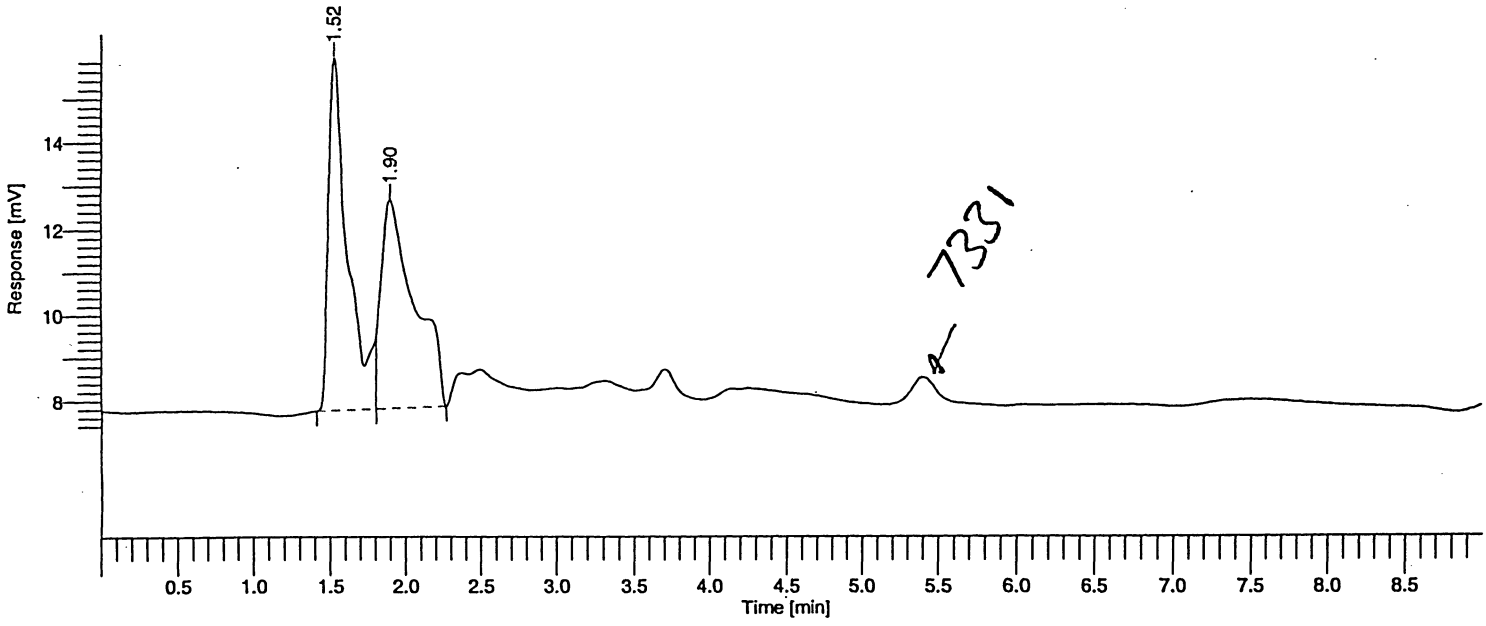

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.520	73219.97	8157.37	49.33	49.33	BV	8.9759
2	1.896	75213.23	4876.21	50.67	50.67	VB	15.4245
		148433.20	13033.58	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 12:17:13 PM
Operator	: manager	Sample Name	: P21,S
Sample Number	: 301	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/7
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 1:17:30 PM	Cycle	: 301

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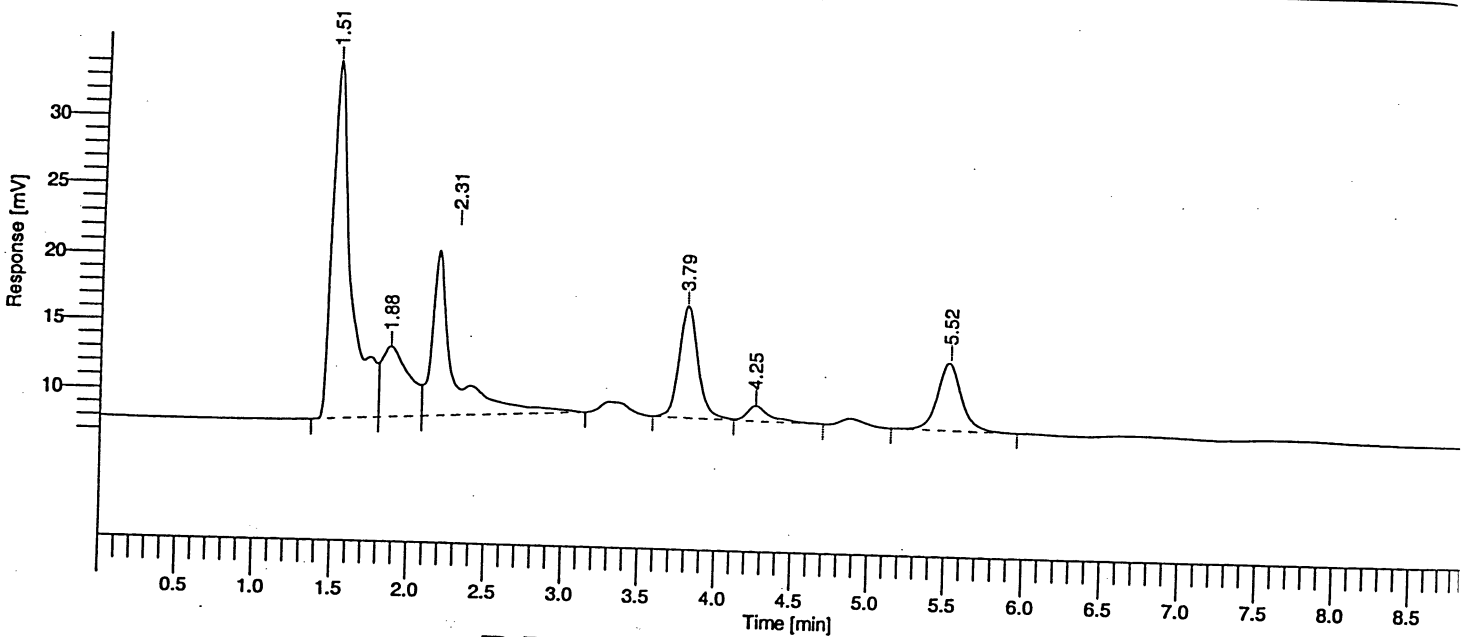

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.507	224988.31	26236.30	41.25	41.25	BV	8.5755
2	1.877	63271.09	5150.18	11.60	11.60	VV	12.2852
3	2.305	120277.40	2060.27	22.05	22.05	VB	58.3795
4	3.794	70767.30	8273.69	12.98	12.98	BV	8.5533
5	4.251	11695.50	1130.59	2.14	2.14	VB	10.3446
6	5.519	54407.40	4868.18	9.98	9.98	BB	11.1761
		545407.00	47719.21	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 12:27:16 PM
Operator	: manager	Sample Name	: P22,S
Sample Number	: 302	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/8
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 1:27:30 PM	Cycle	: 302

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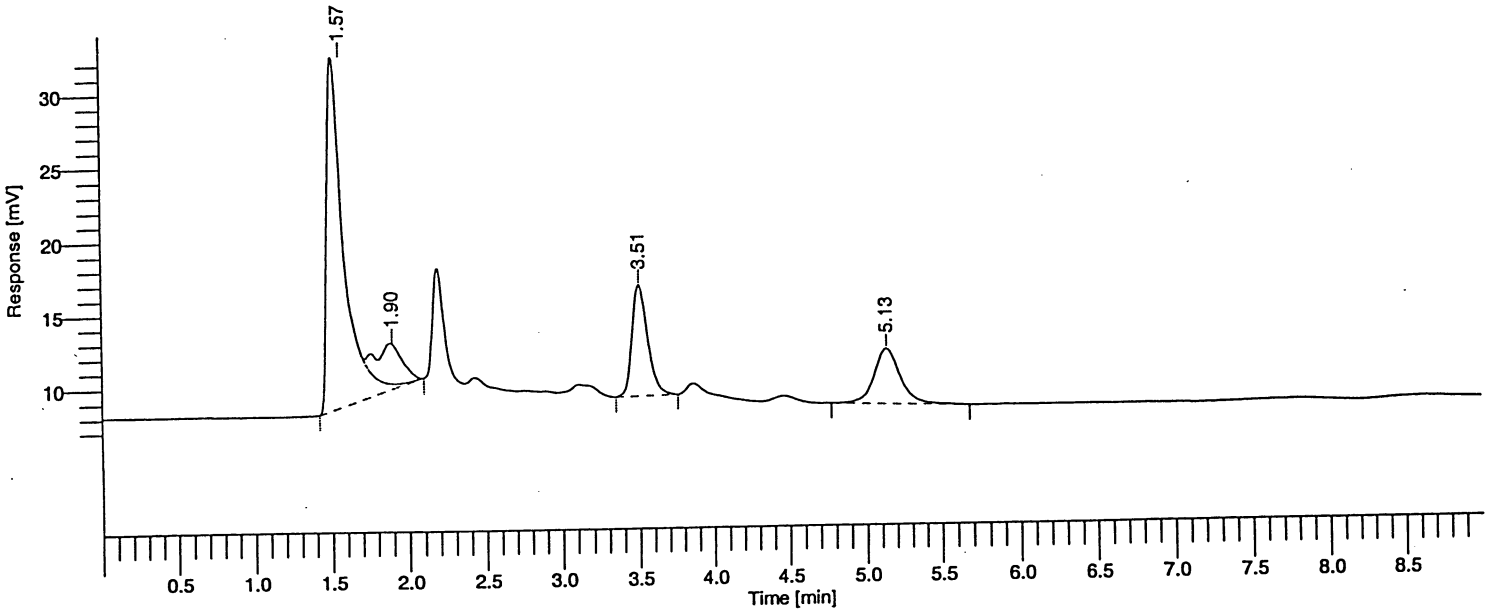

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.566	188845.40	14747.74	59.26	59.26	BE	12.8050
2	1.899	31341.20	2706.92	9.83	9.83	EB	11.5782
3	3.505	54090.40	7582.42	16.97	16.97	BB	7.1337
4	5.134	44420.20	3695.21	13.94	13.94	BB	12.0210
		318697.20	28732.28	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 12:37:12 PM
Operator	: manager	Sample Name	: P23,S
Sample Number	: 303	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/9
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 1:37:31 PM	Cycle	: 303

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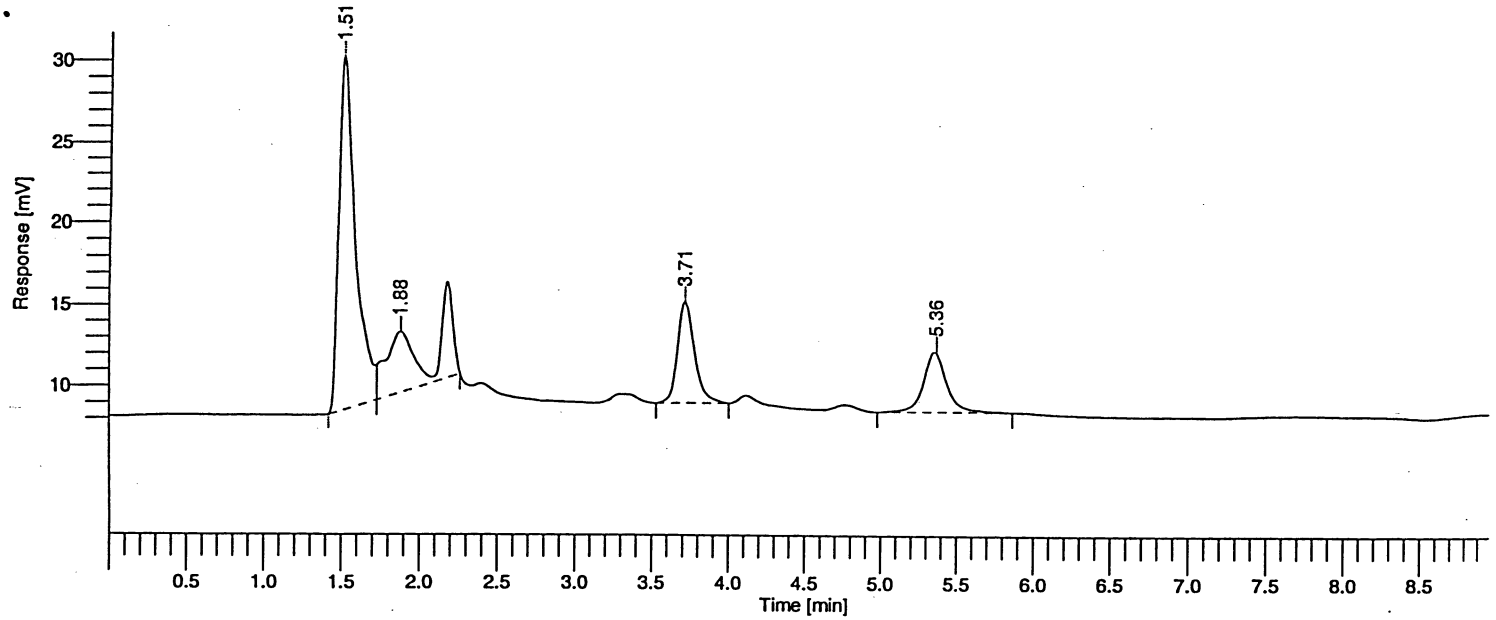


DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.512	160987.32	21785.72	49.79	49.79	BV	7.3896
2	1.878	74469.08	3742.43	23.03	23.03	VB	19.8986
3	3.711	48115.60	6282.40	14.88	14.88	BB	7.6588
4	5.362	39736.00	3597.83	12.29	12.29	BB	11.0444
		323308.00	35408.39	100.00	100.00		

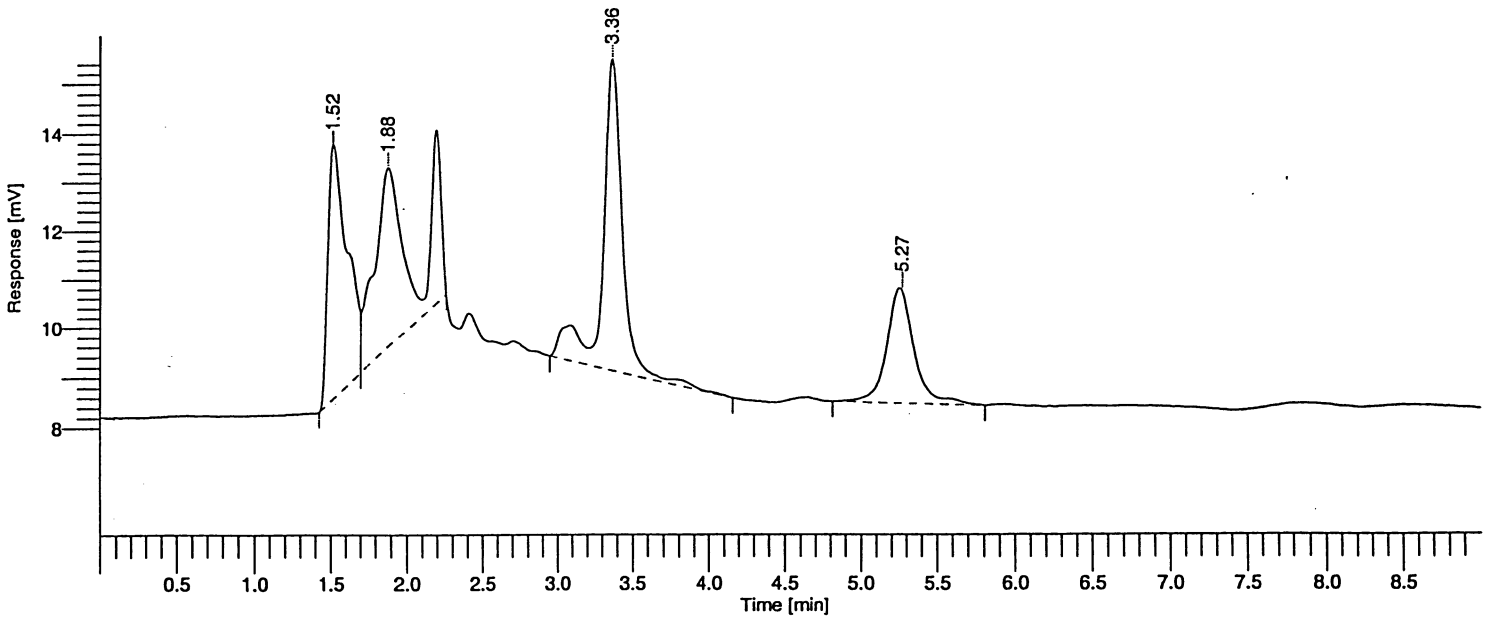
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 12:47:15 PM
Operator	: manager	Sample Name	: P31,0S
Sample Number	: 304	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/10
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 1:47:35 PM	Cycle	: 304

Raw Data File : \\Poweredge\E drive\TC\dennis\data293.raw
Result File : \\Poweredge\E drive\TC\dennis\data293.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data293.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.518	45872.07	5199.49	23.43	23.43	BV	8.8224
2	1.884	61846.73	3696.70	31.59	31.59	VB	16.7303
3	3.364	58799.60	6385.47	30.04	30.04	BB	9.2083
4	5.269	29238.40	2259.78	14.94	14.94	BB	12.9386
		195756.80	17541.45	100.00	100.00		

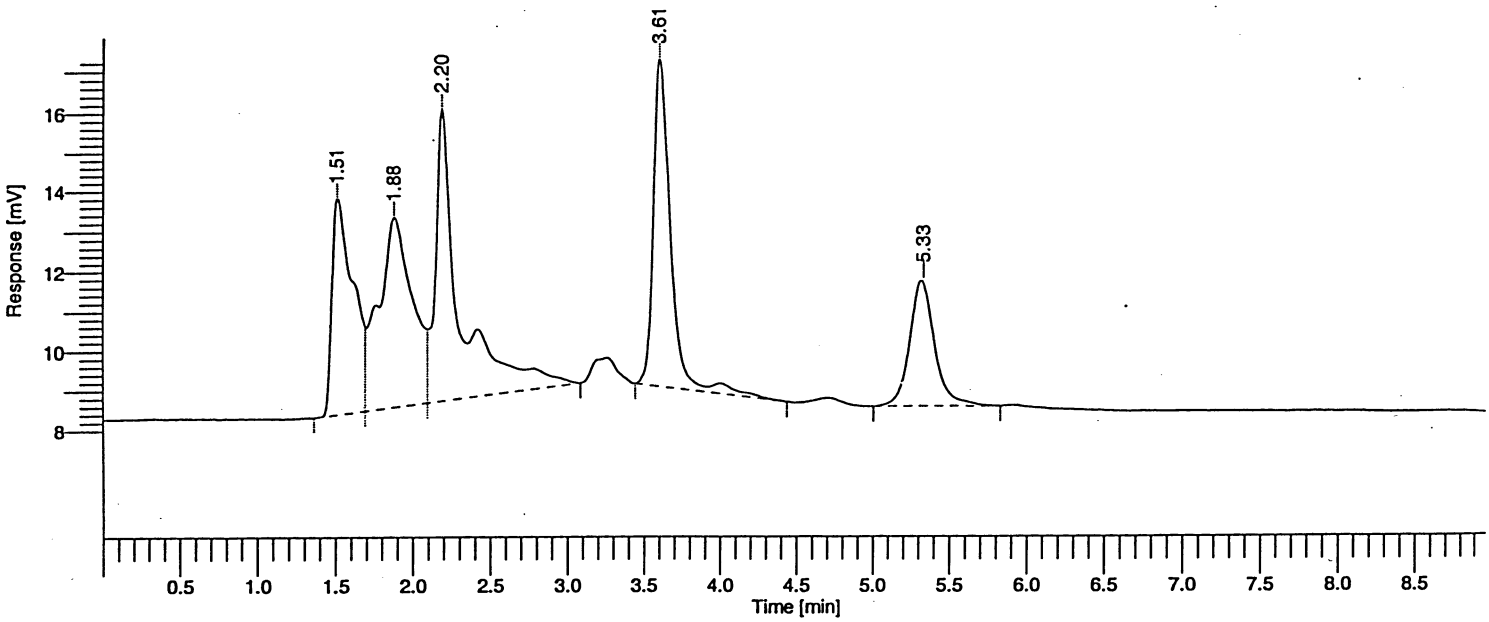
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 12:57:18 PM
Operator	: manager	Sample Name	: P32,0S
Sample Number	: 305	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/11
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 1:57:37 PM	Cycle	: 305

Raw Data File : \\Poweredge\E drive\TC\dennis\data294.raw
Result File : \\Poweredge\E drive\TC\dennis\data294.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data294.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.513	51635.57	5424.56	16.77	16.77	BV	9.5188
2	1.882	73843.74	4762.50	23.98	23.98	VV	15.5053
3	2.195	81236.69	7344.80	26.38	26.38	VB	11.0604
4	3.608	65540.40	8211.79	21.28	21.28	BB	7.9813
5	5.330	35697.00	3071.90	11.59	11.59	BB	11.6205
		307953.40	28815.54	100.00	100.00		

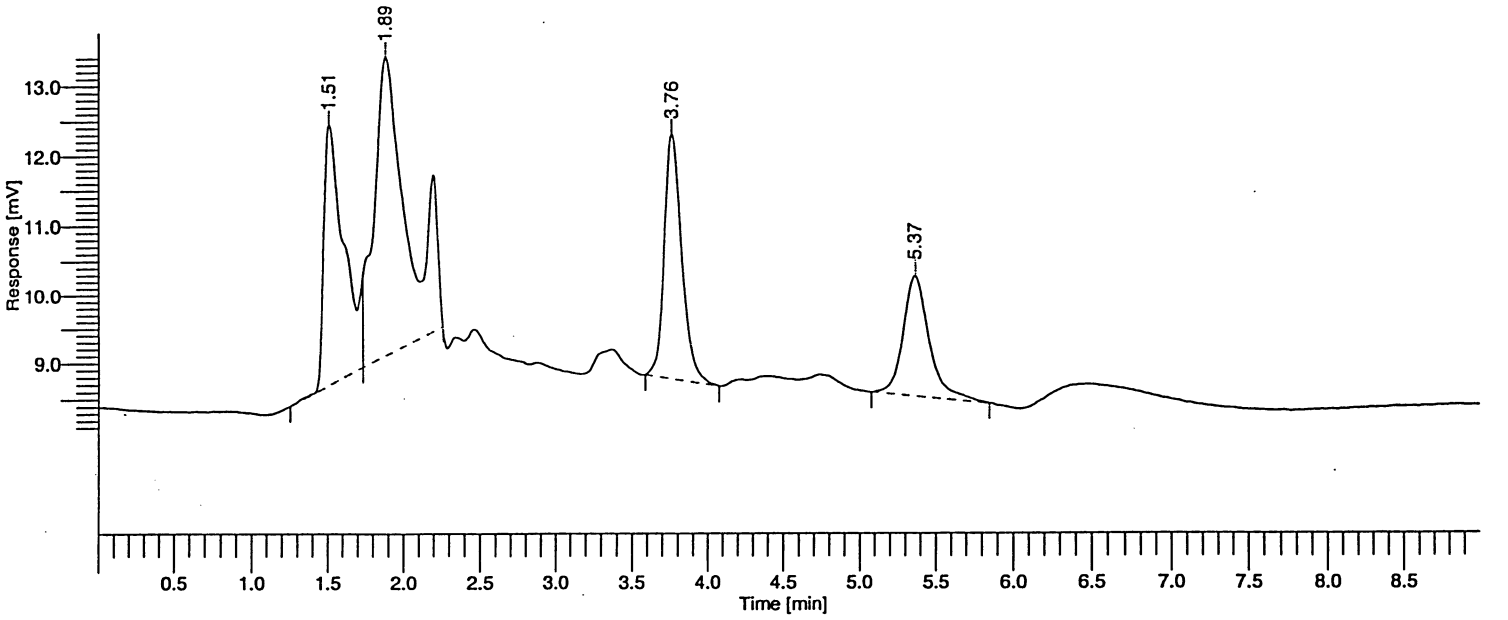
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 1:07:21 PM
Operator	: manager	Sample Name	: P31,ES
Sample Number	: 306	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/12
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 2:07:37 PM	Cycle	: 306

Raw Data File : \\Poweredge\E drive\TC\dennis\data295.raw
Result File : \\Poweredge\E drive\TC\dennis\data295.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data295.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.511	35490.37	3766.26	23.69	23.69	BV	9.4232
2	1.886	65772.43	4324.59	43.90	43.90	VB	15.2089
3	3.764	28157.20	3560.90	18.79	18.79	BB	7.9073
4	5.366	20402.00	1768.56	13.62	13.62	BB	11.5359
		149822.00	13420.32	100.00	100.00		

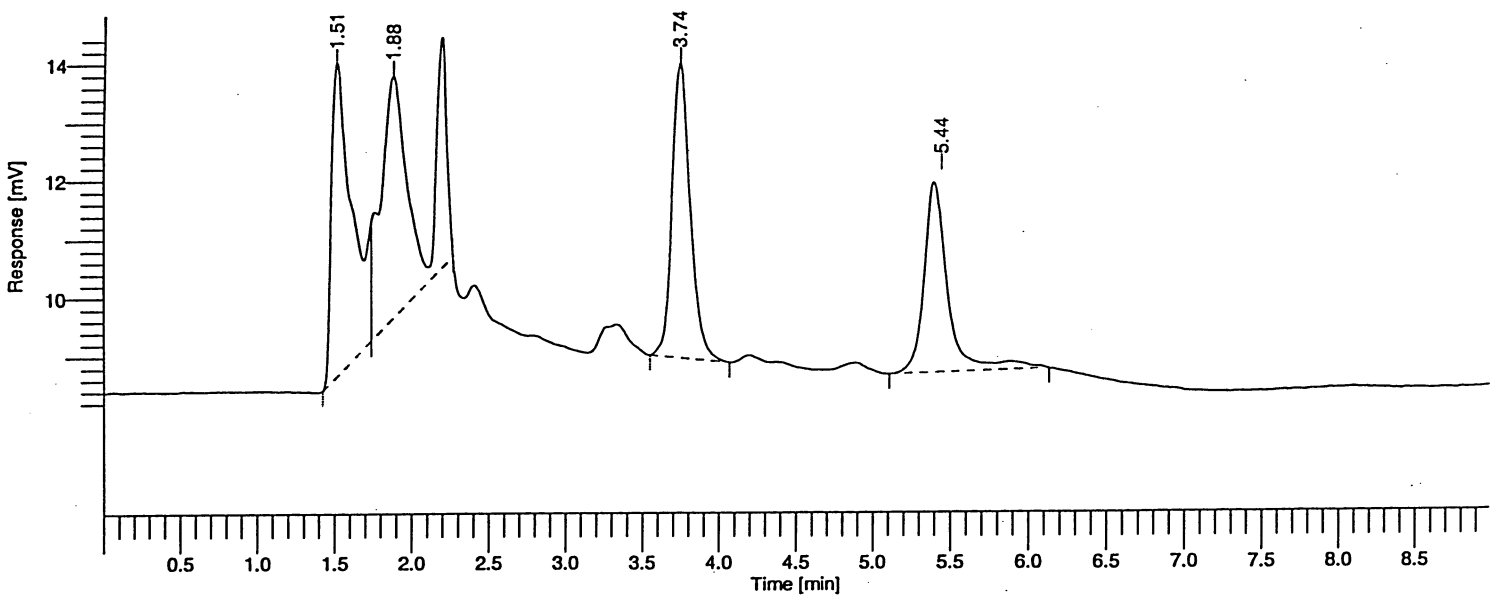
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 1:17:17 PM
Operator	: manager	Sample Name	: P3E2, S
Sample Number	: 307	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/13
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 2:17:37 PM	Cycle	: 307

Raw Data File : \\Poweredge\E drive\TC\dennis\data296.raw
Result File : \\Poweredge\E drive\TC\dennis\data296.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data296.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.511	49890.70	5355.44	25.91	25.91	BV	9.3159
2	1.879	66374.50	4139.69	34.47	34.47	VB	16.0337
3	3.738	41726.80	5041.10	21.67	21.67	BB	8.2773
4	5.440	34549.80	2342.99	17.94	17.94	BB	14.7460
		192541.80	16879.23	100.00	100.00		

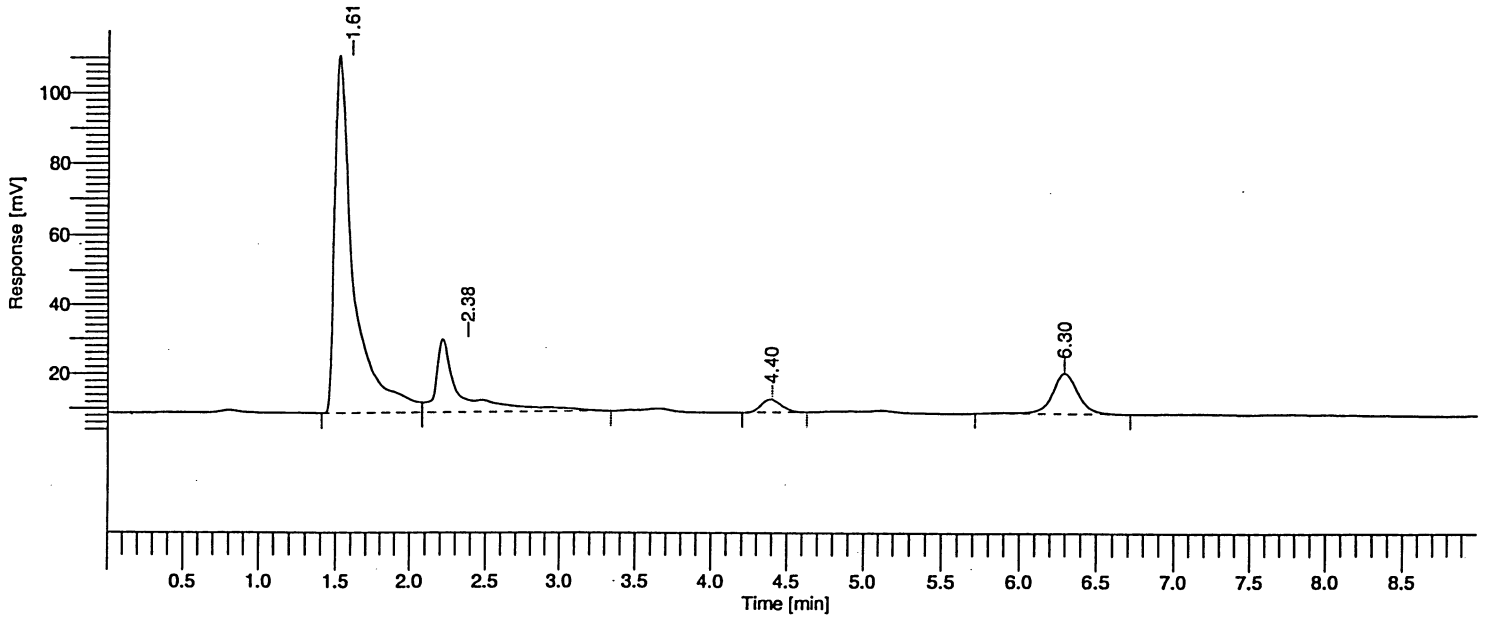
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 2:54:15 PM
Operator	: manager	Sample Name	: -VEP11,Z CELL
Sample Number	: 309	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/15
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 3:54:30 PM	Cycle	: 309

Raw Data File : \\Poweredge\E drive\TC\dennis\data298.raw
Result File : \\Poweredge\E drive\TC\dennis\data298.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data298.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.608	905741.22	40222.26	68.39	68.39	BV	22.5184
2	2.384	242267.58	3570.45	18.29	18.29	VB	67.8535
3	4.398	36055.00	3736.94	2.72	2.72	BB	9.6483
4	6.298	140383.00	11780.95	10.60	10.60	BB	11.9161
		1324446.80	59310.59	100.00	100.00		

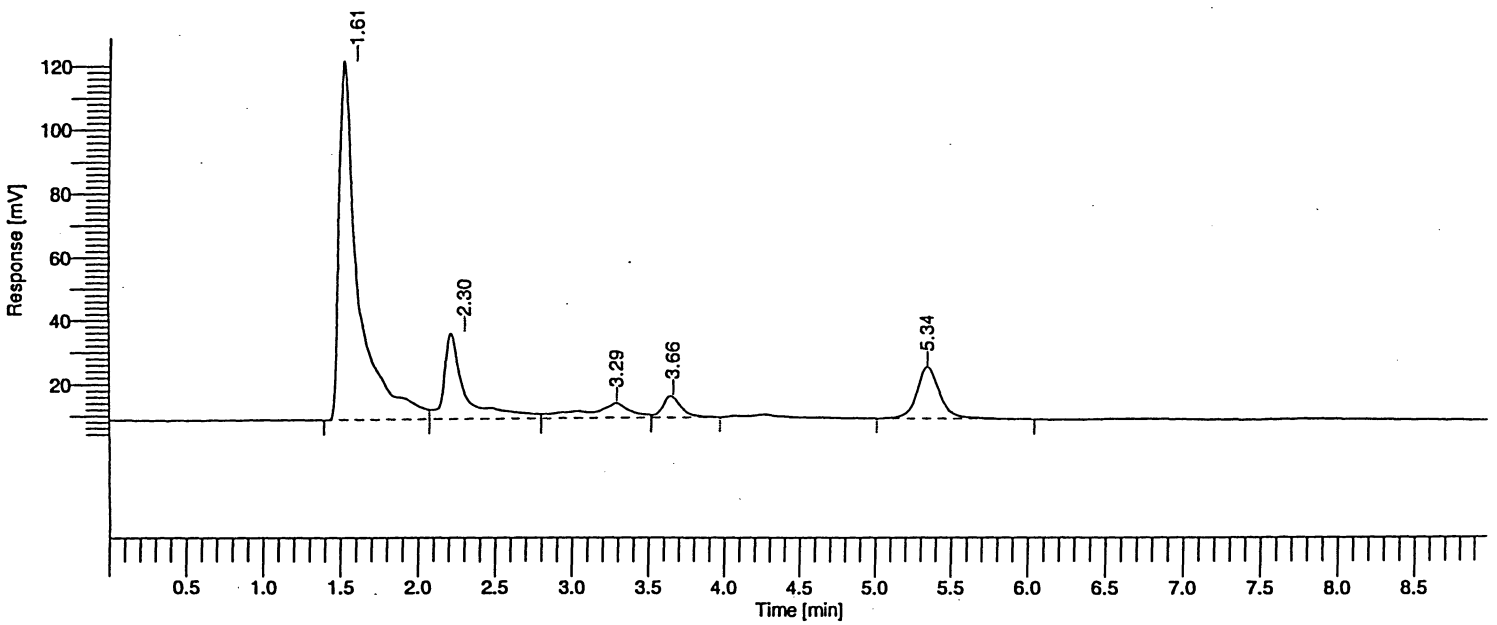
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 3:04:11 PM
Operator	: manager	Sample Name	: -VEP12,Z CELL
Sample Number	: 310	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/16
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 4:04:30 PM	Cycle	: 310

Raw Data File : \\Poweredge\E drive\TC\dennis\data299.raw
Result File : \\Poweredge\E drive\TC\dennis\data299.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data299.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.609	985959.83	42340.20	62.90	62.90	BV	23.2866
2	2.304	260469.90	7710.70	16.62	16.62	VV	33.7803
3	3.290	93429.41	4641.23	5.96	5.96	VV	20.1303
4	3.659	56903.27	6383.17	3.63	3.63	VB	8.9146
5	5.341	170699.20	16204.19	10.89	10.89	BB	10.5343
		1567461.60	77279.49	100.00	100.00		

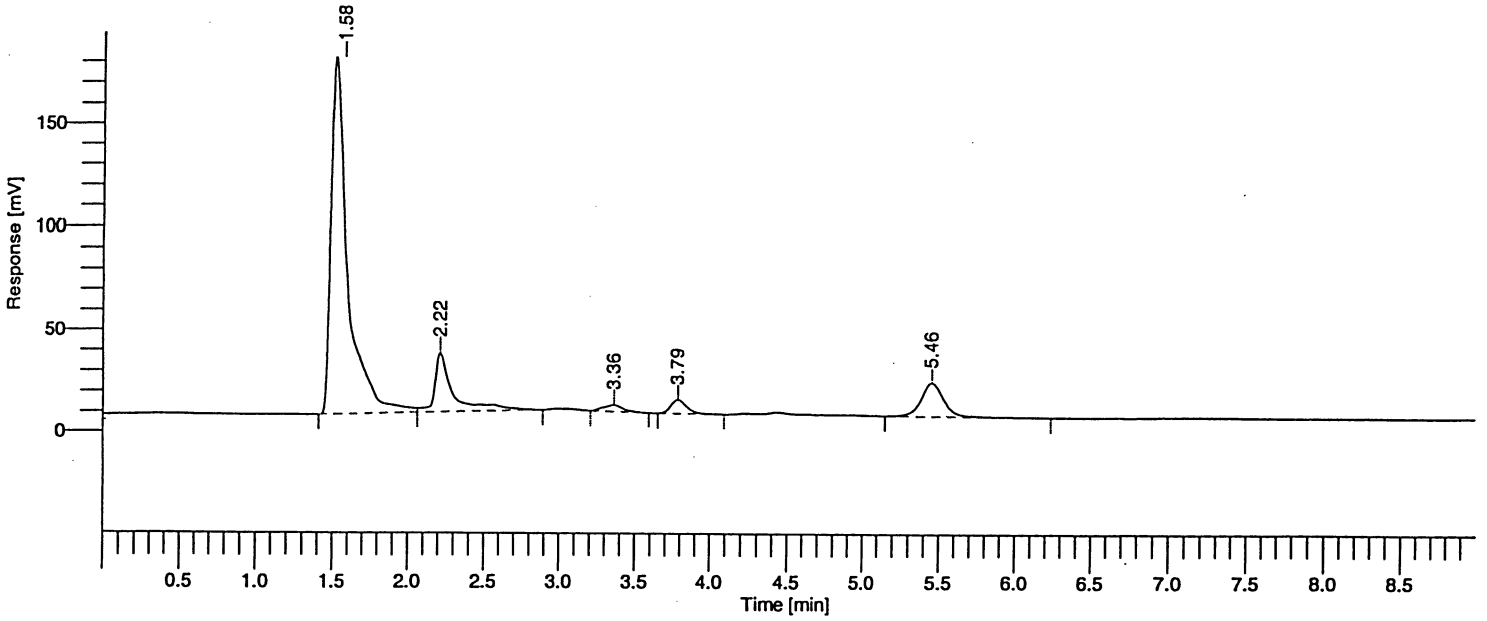
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 3:14:14 PM
Operator	: manager	Sample Name	: -VEP11,50 CELL
Sample Number	: 311	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/17
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 4:14:30 PM	Cycle	: 311

Raw Data File : \\Poweredge\E drive\TC\dennis\data300.raw
Result File : \\Poweredge\E drive\TC\dennis\data300.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data300.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.581	1347816.90	90218.07	73.27	73.27	BV	14.9395
2	2.218	242659.90	29076.60	13.19	13.19	VB	8.3455
3	3.363	29057.00	3298.21	1.58	1.58	BB	8.8099
4	3.785	51184.40	6774.32	2.78	2.78	BB	7.5557
5	5.457	168901.60	16476.00	9.18	9.18	BB	10.2514
		1839619.80	145843.20	100.00	100.00		

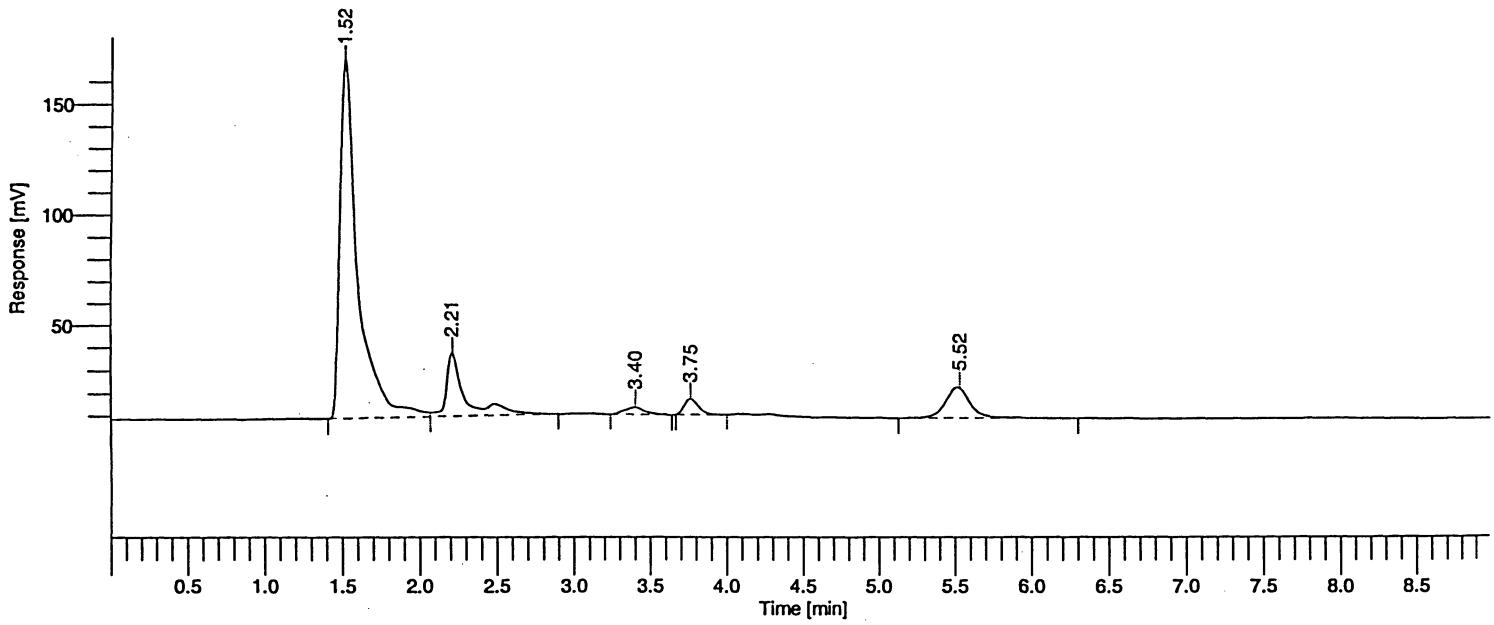
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 3:24:09 PM
Operator	: manager	Sample Name	: -VEP12,50 CELL
Sample Number	: 312	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/18
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 4:24:30 PM	Cycle	: 312

Raw Data File : \\Poweredge\E drive\TC\dennis\data301.raw
Result File : \\Poweredge\E drive\TC\dennis\data301.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data301.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.516	1285500.26	161426.11	73.39	73.39	BV	7.9634
2	2.206	237937.34	27733.10	13.58	13.58	VB	8.5795
3	3.396	28666.80	3262.49	1.64	1.64	BB	8.7868
4	3.755	45202.80	6962.77	2.58	2.58	BB	6.4921
5	5.523	154357.00	13014.85	8.81	8.81	BB	11.8601
		1751664.20	212399.32	100.00	100.00		

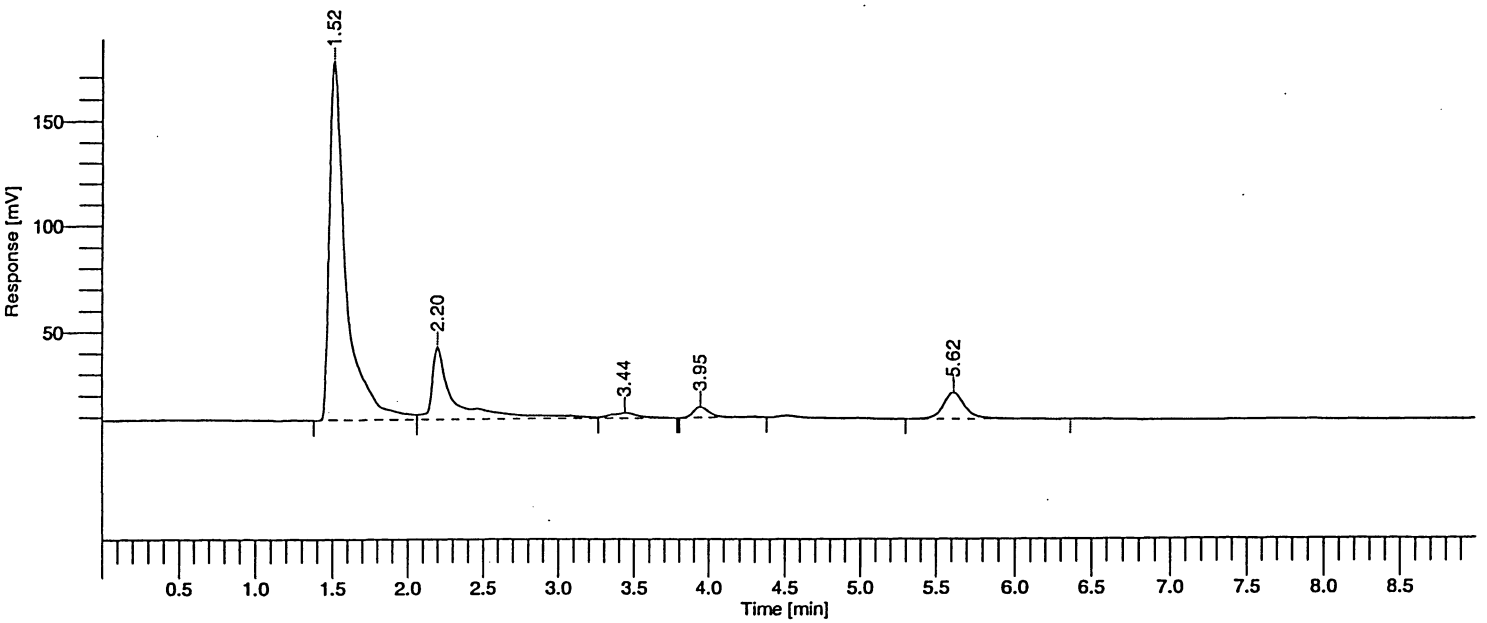
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 3:34:12 PM
Operator	: manager	Sample Name	: -VEP1,150 CELL
Sample Number	: 313	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/19
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 4:34:31 PM	Cycle	: 313

Raw Data File : \\Poweredge\E drive\TC\dennis\data302.raw
Result File : \\Poweredge\E drive\TC\dennis\data302.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data302.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.518	1310518.96	169251.41	70.12	70.12	BV	7.7430
2	2.201	359019.97	34037.35	19.21	19.21	VV	10.5478
3	3.440	32939.06	2491.78	1.76	1.76	VB	13.2191
4	3.946	39974.40	5111.48	2.14	2.14	BB	7.8205
5	5.617	126389.60	12439.01	6.76	6.76	BB	10.1607
		1868842.00	223331.02	100.00	100.00		

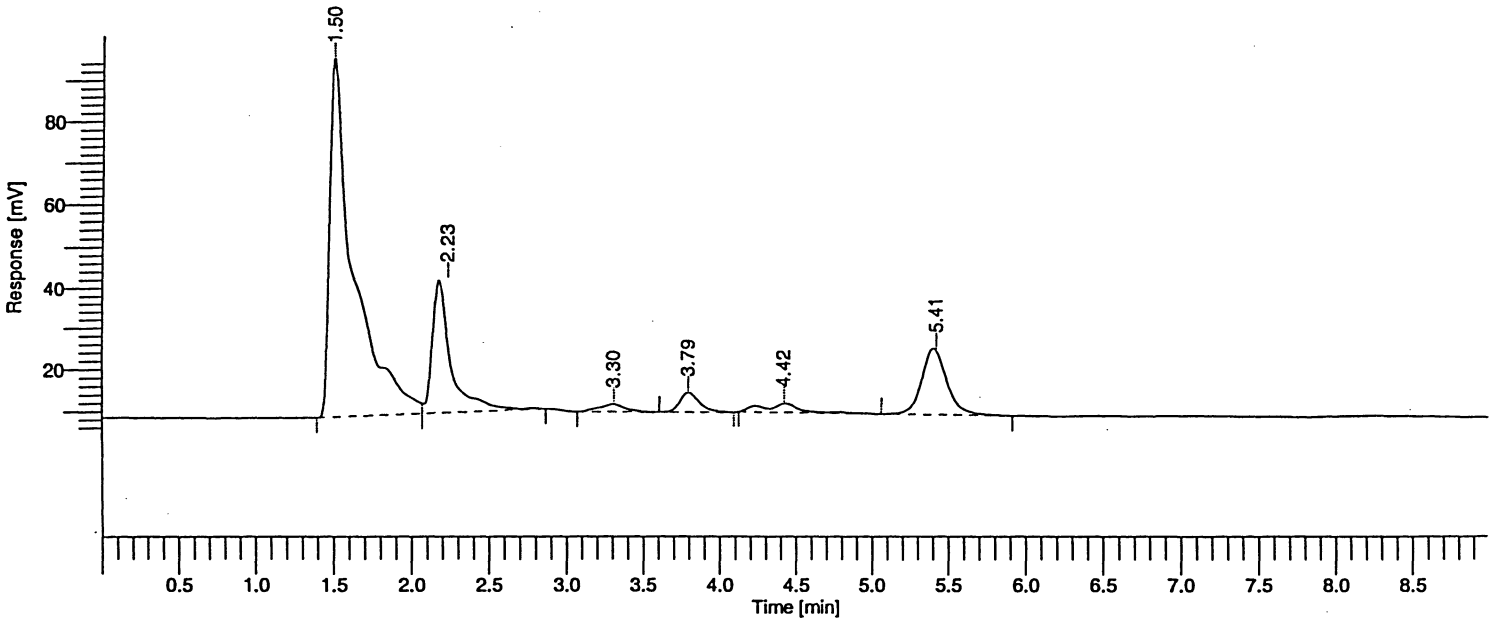
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 4:38:28 PM
Operator	: manager	Sample Name	: P21,CELL
Sample Number	: 314	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/20
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 5:38:49 PM	Cycle	: 314

Raw Data File : \\Poweredge\E drive\TC\dennis\data303.raw
Result File : \\Poweredge\E drive\TC\dennis\data303.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data303.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.504	926533.28	86960.82	62.91	62.91	BV	10.6546
2	2.230	269379.32	15674.95	18.29	18.29	VB	17.1853
3	3.305	20712.94	1803.17	1.41	1.41	BV	11.4870
4	3.790	40650.44	4555.19	2.76	2.76	VB	8.9240
5	4.416	37240.52	2132.46	2.53	2.53	BV	17.4636
6	5.410	178368.22	15290.06	12.11	12.11	VB	11.6656
		1472884.72	126416.64	100.00	100.00		

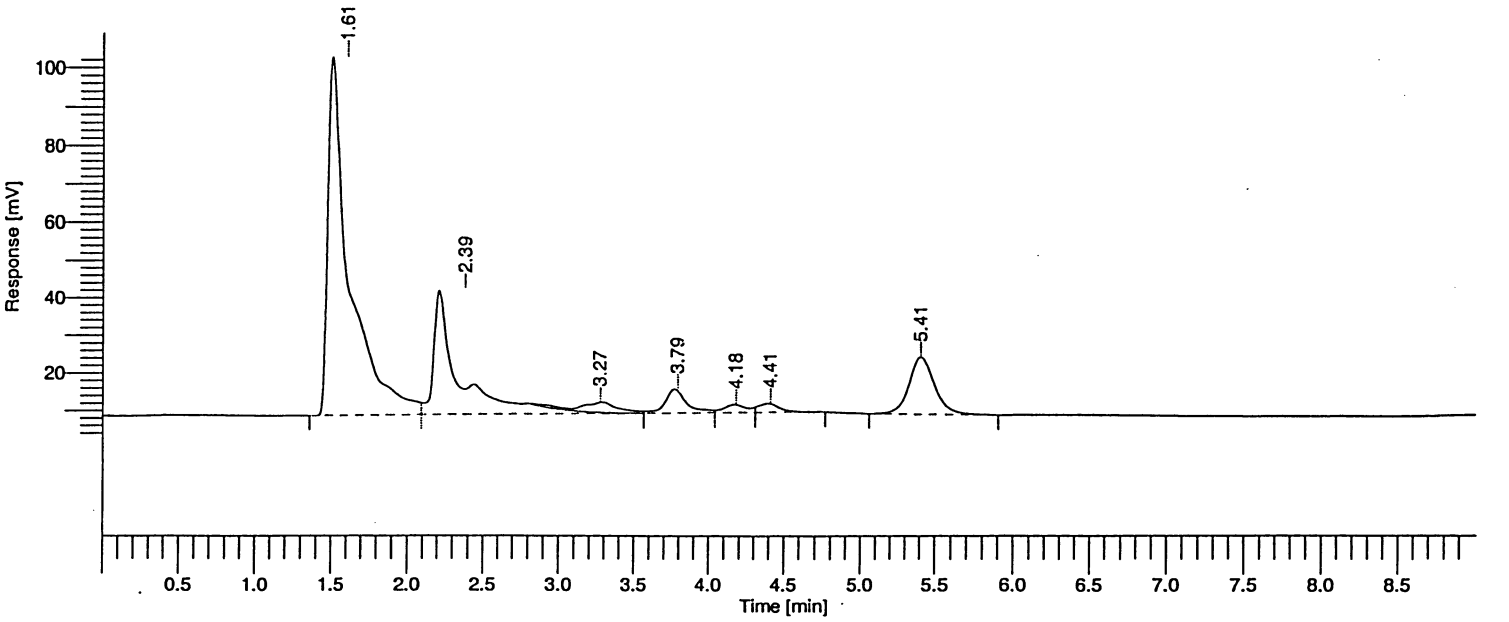
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 4:48:30 PM
Operator	: manager	Sample Name	: P22,CELL
Sample Number	: 315	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/21
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 5:48:51 PM	Cycle	: 315

Raw Data File : \\Poweredge\E drive\TC\dennis\data304.raw
Result File : \\Poweredge\E drive\TC\dennis\data304.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data304.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.614	950424.14	33861.25	56.36	56.36	BV	28.0682
2	2.387	392447.87	6695.99	23.27	23.27	VE	58.6094
3	3.273	51000.00	2744.26	3.02	3.02	EV	18.5842
4	3.791	64295.54	5866.70	3.81	3.81	VV	10.9594
5	4.180	22978.85	2169.93	1.36	1.36	VV	10.5897
6	4.410	23093.20	2328.10	1.37	1.37	VB	9.9193
7	5.409	182083.20	15421.53	10.80	10.80	BB	11.8071
		1686322.80	69087.77	100.00	100.00		

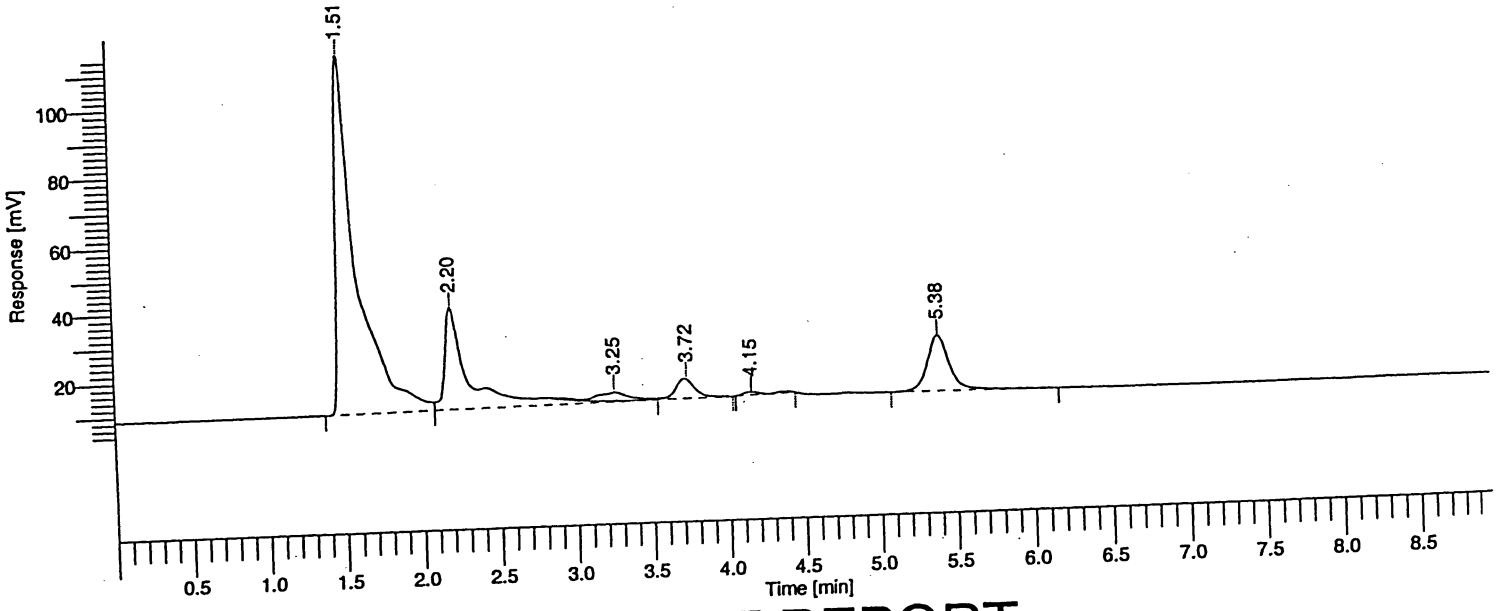
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 4:58:32 PM
Operator	: manager	Sample Name	: P23,CELL
Sample Number	: 316	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/22
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s	Area Reject	: 0.000000
Volume Injected	: 1.000000 ul	Dilution Factor	: 1.00
Sample Amount	: 1.0000	Cycle	: 316
Data Acquisition Time	: 6/27/06 5:58:51 PM		

Raw Data File : \\Poweredge\E drive\TC\dennis\data305.raw
Result File : \\Poweredge\E drive\TC\dennis\data305.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data305.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.509	975648.77	105558.21	61.87	61.87	BV	9.2428
2	2.195	334008.96	29864.34	21.18	21.18	VE	11.1842
3	3.249	41318.00	2479.89	2.62	2.62	EV	16.6612
4	3.723	51492.47	5650.80	3.27	3.27	VB	9.1124
5	4.149	9512.00	960.85	0.60	0.60	BB	9.8996
6	5.381	164966.40	16261.97	10.46	10.46	BB	10.1443
		1576946.60	160776.07	100.00	100.00		

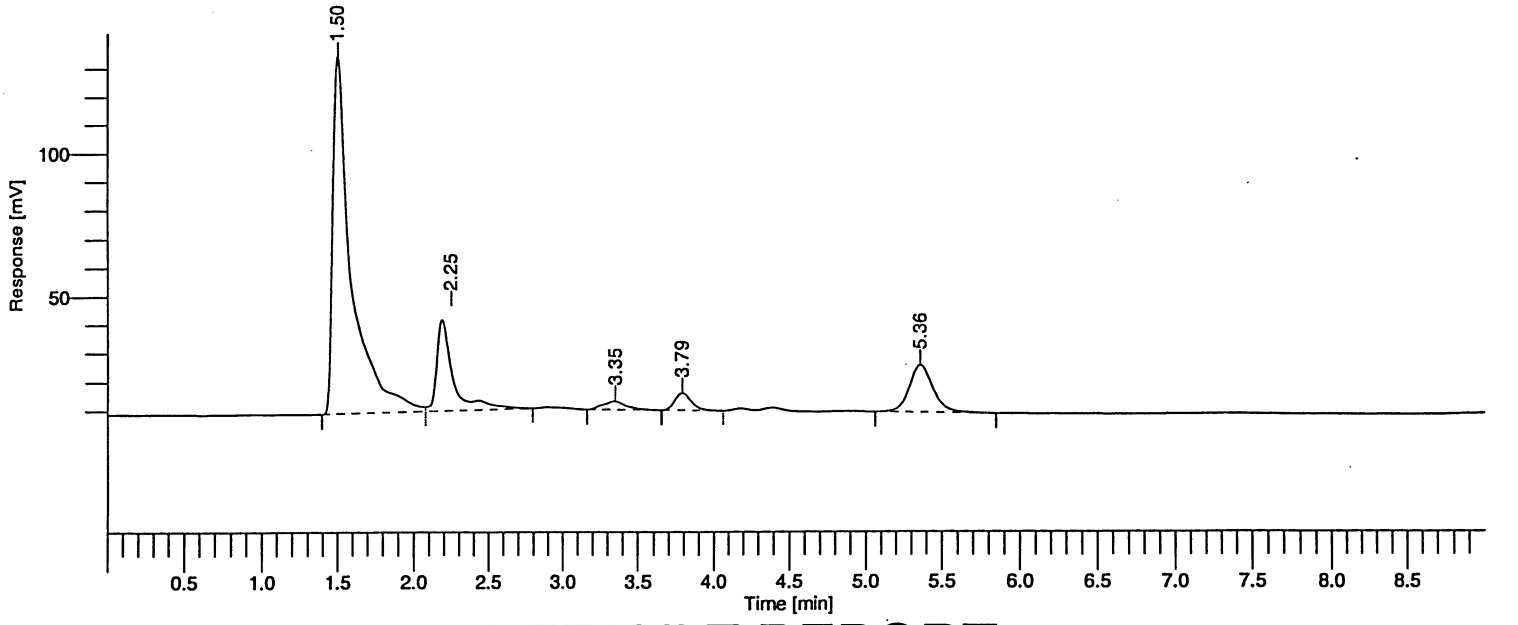
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 5:08:35 PM
Operator	: manager	Sample Name	: P30,CELL
Sample Number	: 317	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/23
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 6:08:51 PM	Cycle	: 317

Raw Data File : \\Poweredge\E drive\TC\dennis\data306.raw
Result File : \\Poweredge\E drive\TC\dennis\data306.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data306.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.503	1095513.28	125578.52	68.66	68.66	BV	8.7237
2	2.249	243672.72	13141.30	15.27	15.27	VB	18.5425
3	3.350	31644.80	2947.37	1.98	1.98	BB	10.7366
4	3.786	45675.20	5961.01	2.86	2.86	BB	7.6623
5	5.358	179086.60	16454.71	11.22	11.22	BB	10.8836
		1595592.60	164082.92	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 4:58:32 PM
Operator	: manager	Sample Name	: P23,CELL
Sample Number	: 316	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/22
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s	Area Reject	: 0.000000
Volume Injected	: 1.000000 ul	Dilution Factor	: 1.00
Sample Amount	: 1.0000	Cycle	: 316
Data Acquisition Time	: 6/27/06 5:58:51 PM		

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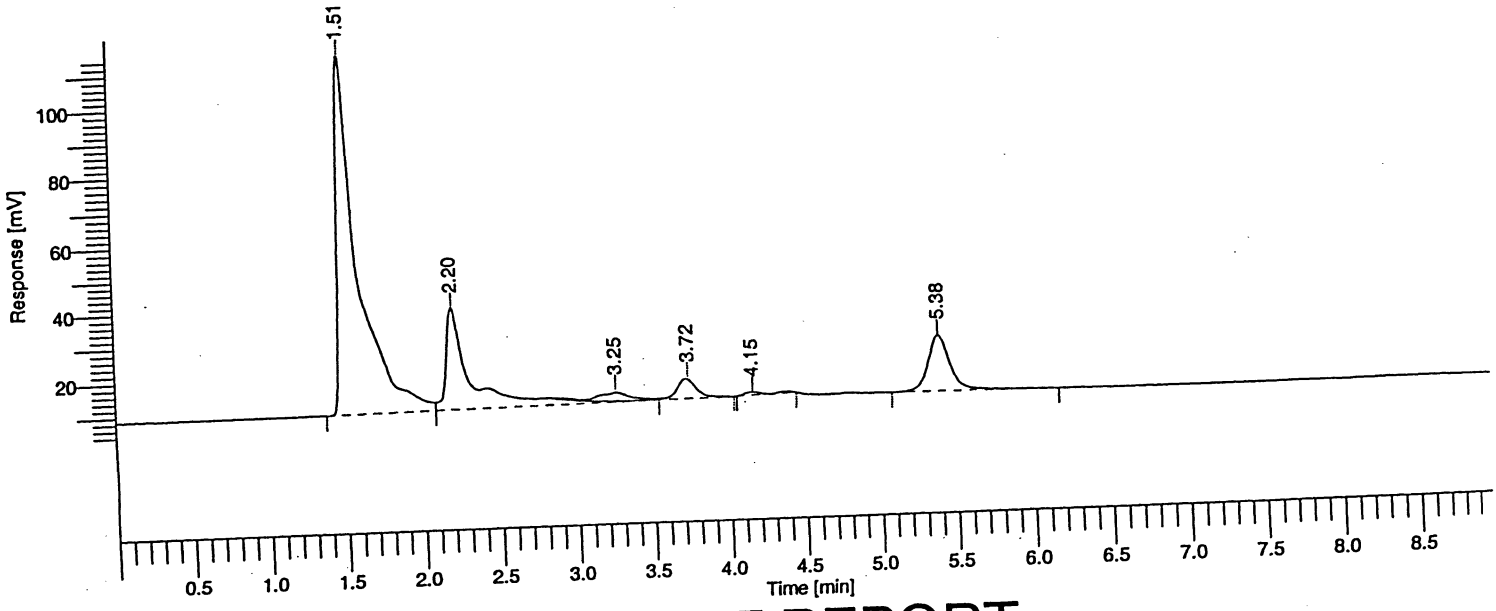
Result File : \\Poweredge\E drive\TC\dennis\data305.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data305.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.509	975648.77	105558.21	61.87	61.87	BV	9.2428
2	2.195	334008.96	29864.34	21.18	21.18	VE	11.1842
3	3.249	41318.00	2479.89	2.62	2.62	EV	16.6612
4	3.723	51492.47	5650.80	3.27	3.27	VB	9.1124
5	4.149	9512.00	960.85	0.60	0.60	BB	9.8996
6	5.381	164966.40	16261.97	10.46	10.46	BB	10.1443
		1576946.60	160776.07	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 5:08:35 PM
Operator	: manager	Sample Name	: P30,CELL
Sample Number	: 317	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/23
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 6:08:51 PM	Cycle	: 317

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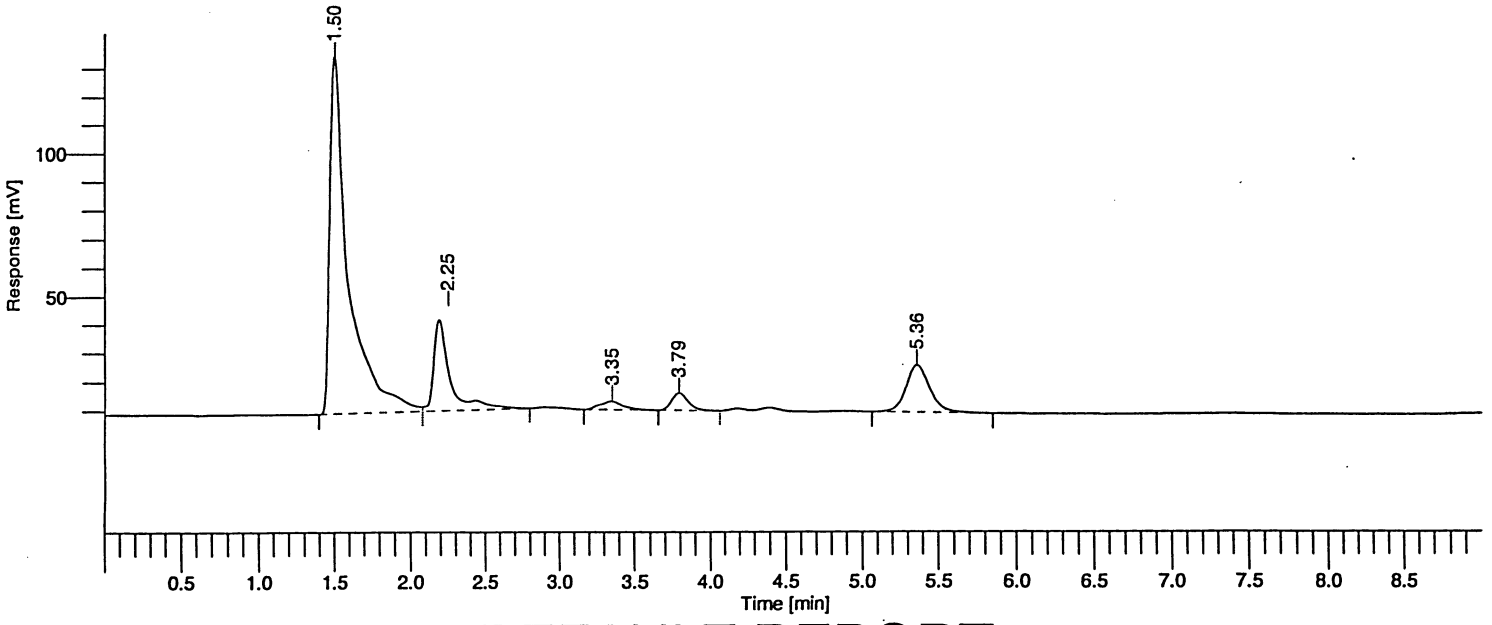
Result File : \\Poweredge\E drive\TC\dennis\data306.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data306.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.503	1095513.28	125578.52	68.66	68.66	BV	8.7237
2	2.249	243672.72	13141.30	15.27	15.27	VB	18.5425
3	3.350	31644.80	2947.37	1.98	1.98	BB	10.7366
4	3.786	45675.20	5961.01	2.86	2.86	BB	7.6623
5	5.358	179086.60	16454.71	11.22	11.22	BB	10.8836
		1595592.60	164082.92	100.00	100.00		

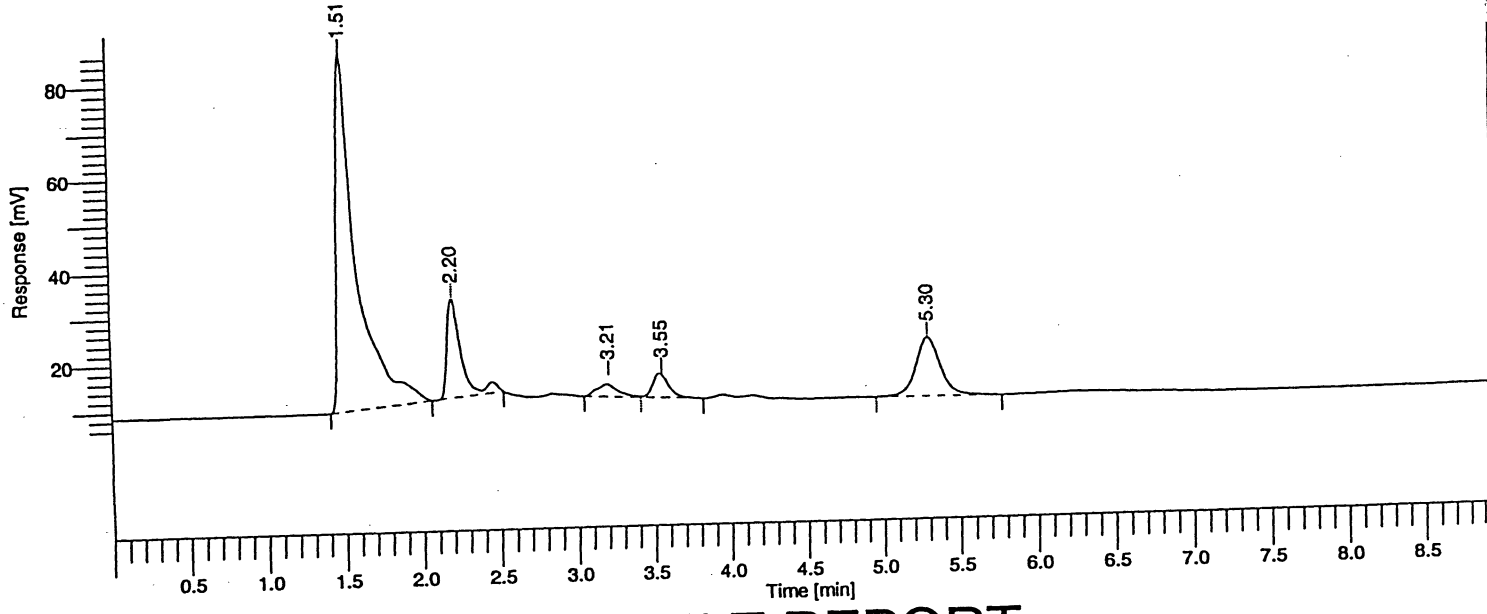
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 5:18:31 PM
Operator	: manager	Sample Name	: P30A,CELL
Sample Number	: 318	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/24
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 6:18:52 PM	Cycle	: 318

Raw Data File : \\Poweredge\E drive\TC\dennis\data307.raw
Result File : \\Poweredge\E drive\TC\dennis\data307.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data307.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.508	682905.30	76952.96	66.87	66.87	BV	8.8743
2	2.202	135702.57	21430.88	13.29	13.29	VB	6.3321
3	3.207	25442.16	2579.18	2.49	2.49	BV	9.8644
4	3.554	37318.64	4939.43	3.65	3.65	VB	7.5553
5	5.298	139813.20	12587.73	13.69	13.69	BB	11.1071
		1021181.87	118490.18	100.00	100.00		

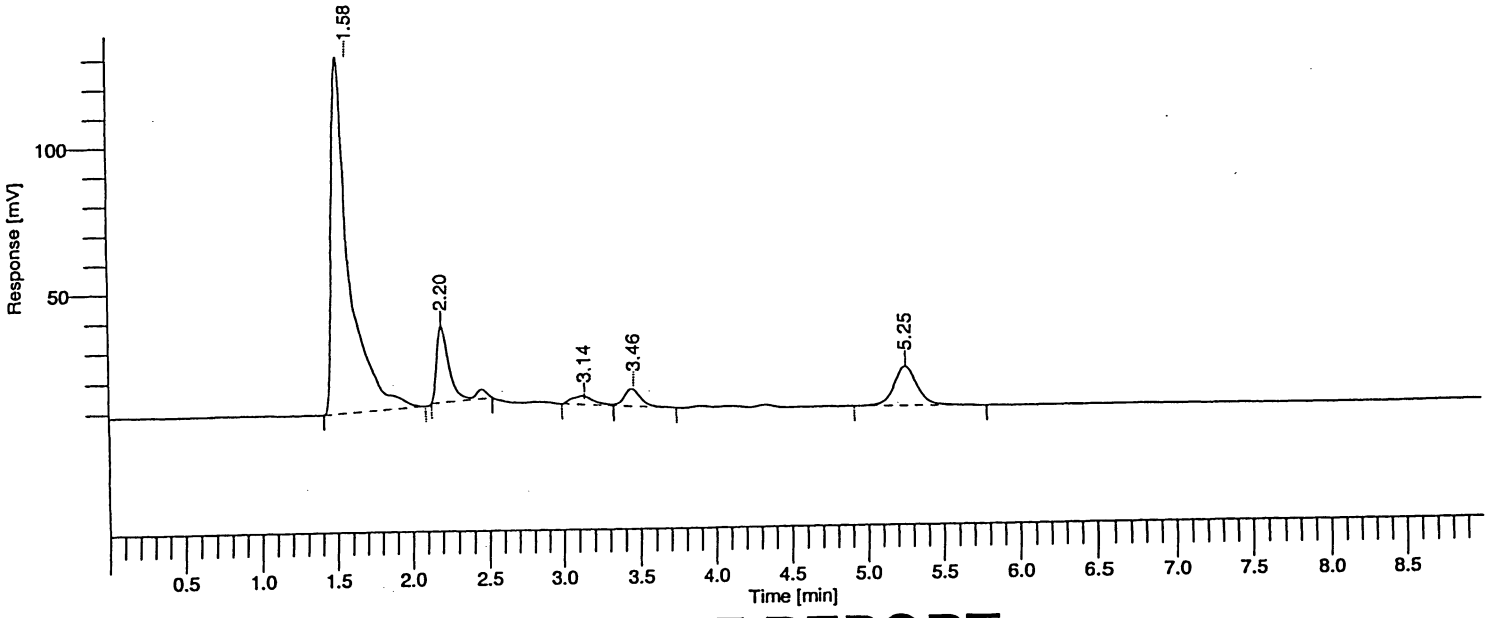
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 5:28:34 PM
Operator	: manager	Sample Name	: P3E,CELL
Sample Number	: 319	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/25
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 6:28:52 PM	Cycle	: 319

Raw Data File : \\Poweredge\E drive\TC\dennis\data308.raw
Result File : \\Poweredge\E drive\TC\dennis\data308.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data308.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.578	998872.60	56745.87	73.25	73.25	BB	17.6026
2	2.200	153379.60	25336.89	11.25	11.25	BB	6.0536
3	3.143	28689.29	2878.34	2.10	2.10	BV	9.9673
4	3.461	43057.91	5546.64	3.16	3.16	VB	7.7629
5	5.254	139568.00	12970.56	10.24	10.24	BB	10.7604
		1363567.40	103478.29	100.00	100.00		

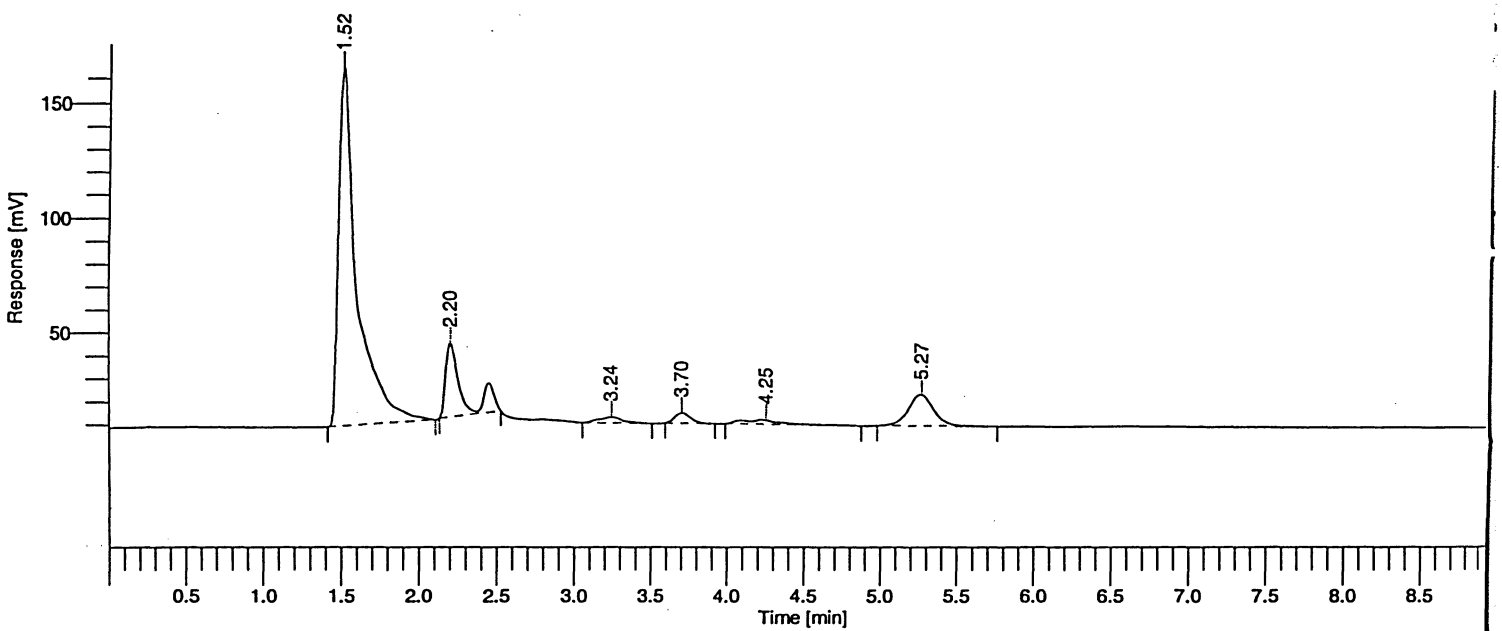
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/27/06 5:38:31 PM
Operator	: manager	Sample Name	: P3EA,CELL
Sample Number	: 320	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/26
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/06 6:38:52 PM	Cycle	: 320

Raw Data File : \\Poweredge\E drive\TC\dennis\data309.raw
Result File : \\Poweredge\E drive\TC\dennis\data309.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data309.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.516	1291829.00	155524.09	72.46	72.46	BB	8.3063
2	2.203	236272.00	32328.46	13.25	13.25	BB	7.3085
3	3.243	28467.80	2632.15	1.60	1.60	BB	10.8154
4	3.703	33558.80	4617.94	1.88	1.88	BB	7.2671
5	4.253	33701.60	1862.35	1.89	1.89	BB	18.0963
6	5.272	159064.40	13537.50	8.92	8.92	BB	11.7499
		1782893.60	210502.48	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 2:55:11 PM
Operator	: manager	Sample Name	: CTC 20PPM,MEOH
Sample Number	: 321	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/27
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 3:55:28 PM	Cycle	: 321

Raw Data File : \\Poweredge\E drive\TC\dennis\data310.raw
Result File : \\Poweredge\E drive\TC\dennis\data310.rst
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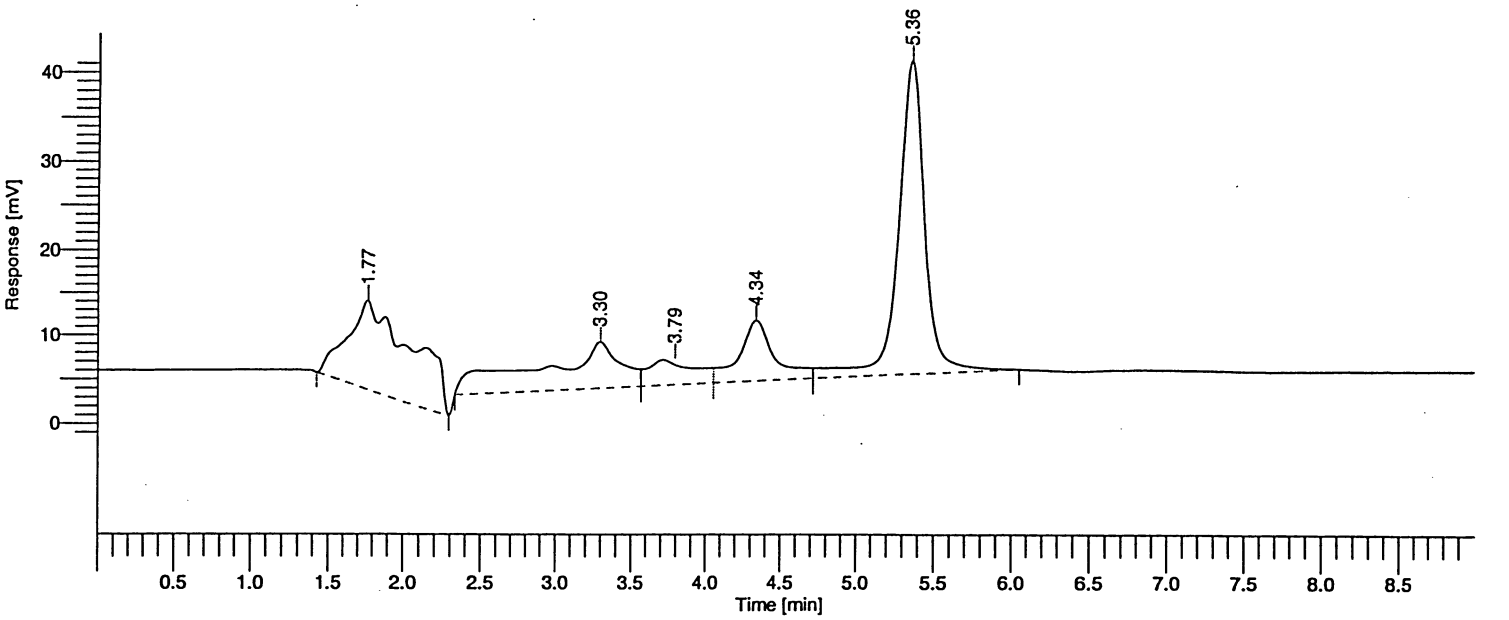
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.772	304261.60	10375.05	27.41	27.41	BB	29.3263
2	3.299	198738.43	5312.97	17.90	17.90	BV	37.4063
3	3.795	61802.35	2262.72	5.57	5.57	VV	27.3133
4	4.337	113650.29	7039.46	10.24	10.24	VV	16.1447
5	5.359	431596.73	36152.47	38.88	38.88	VB	11.9382
		1110049.40	61142.67	100.00	100.00		

Missing Component Report

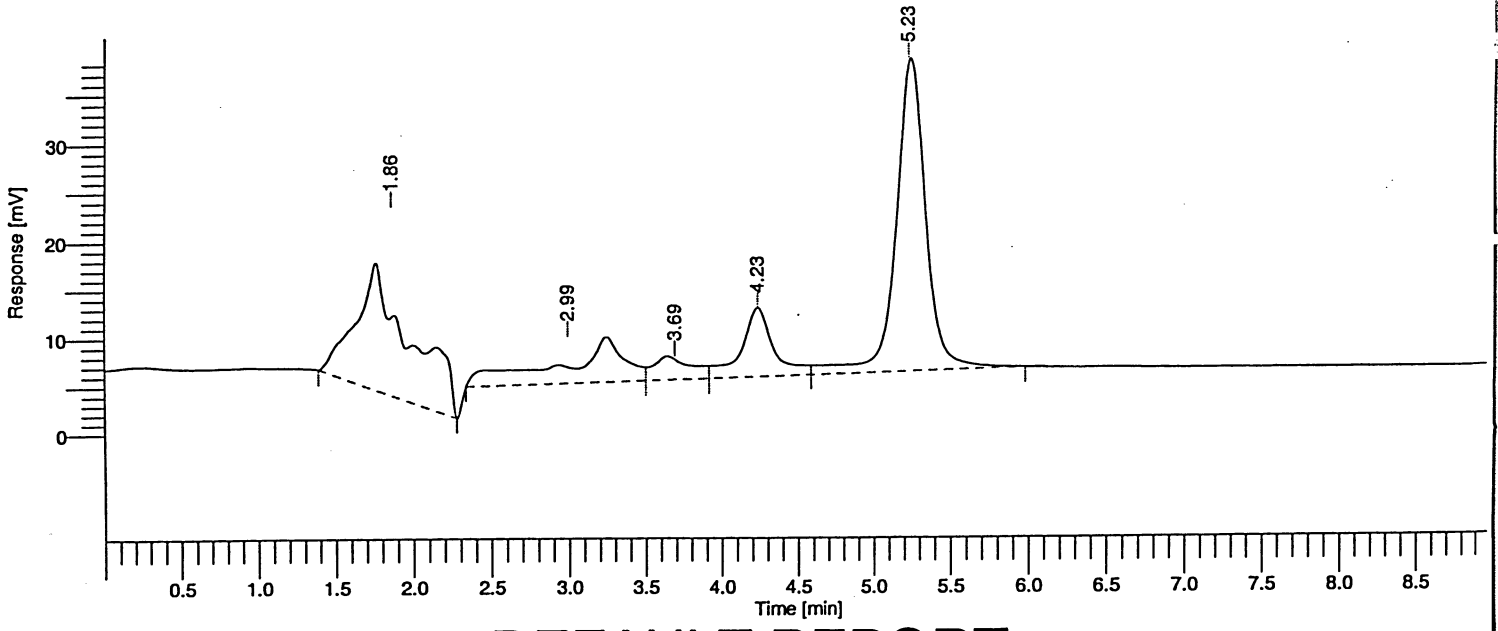
Component	Expected Retention (Calibration File)

All components were found

380964

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 4:05:12 PM
Operator	: manager	Sample Name	: CTC20PPM,MEOH
Sample Number	: 328	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/34
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 5:05:37 PM	Cycle	: 328

Raw Data File : \\Poweredge\E drive\TC\dennis\data317.raw
Result File : \\Poweredge\E drive\TC\dennis\data317.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data317.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.856	322766.00	8123.85	30.87	30.87	BB	39.7307
2	2.992	135822.44	1656.86	12.99	12.99	BV	81.9760
3	3.688	41850.89	2078.41	4.00	4.00	VV	20.1360
4	4.231	105660.68	7129.17	10.11	10.11	VV	14.8209
5	5.231	439511.99	31404.51	42.03	42.03	VB	13.9952
		1045612.00	50392.79	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

395 229

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 3:05:06 PM
Operator	: manager	Sample Name	: -VEP11,0EPS
Sample Number	: 322	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/28
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 4:05:29 PM	Cycle	: 322

Raw Data File : \\Poweredge\E drive\TC\dennis\data311.raw
Result File : \\Poweredge\E drive\TC\dennis\data311.rst
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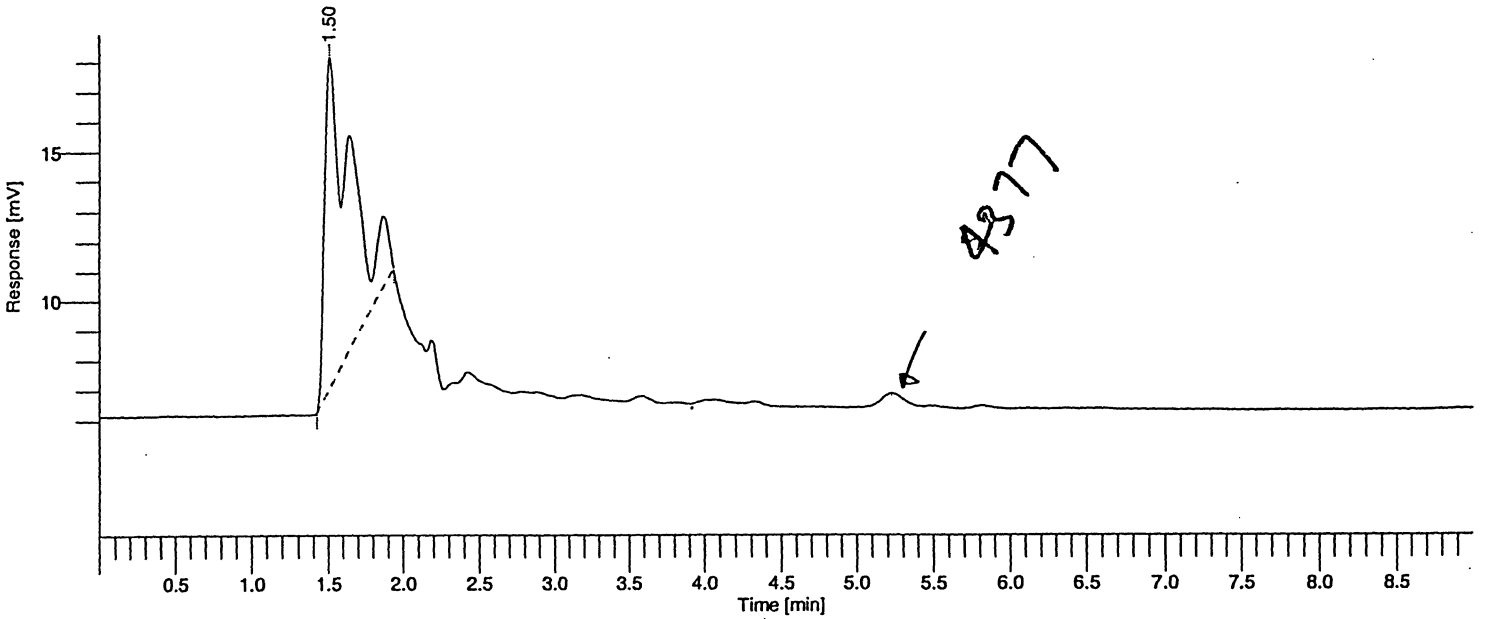
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.505	135903.20	11142.53	100.00	100.00	BB	12.1968
		135903.20	11142.53	100.00	100.00		

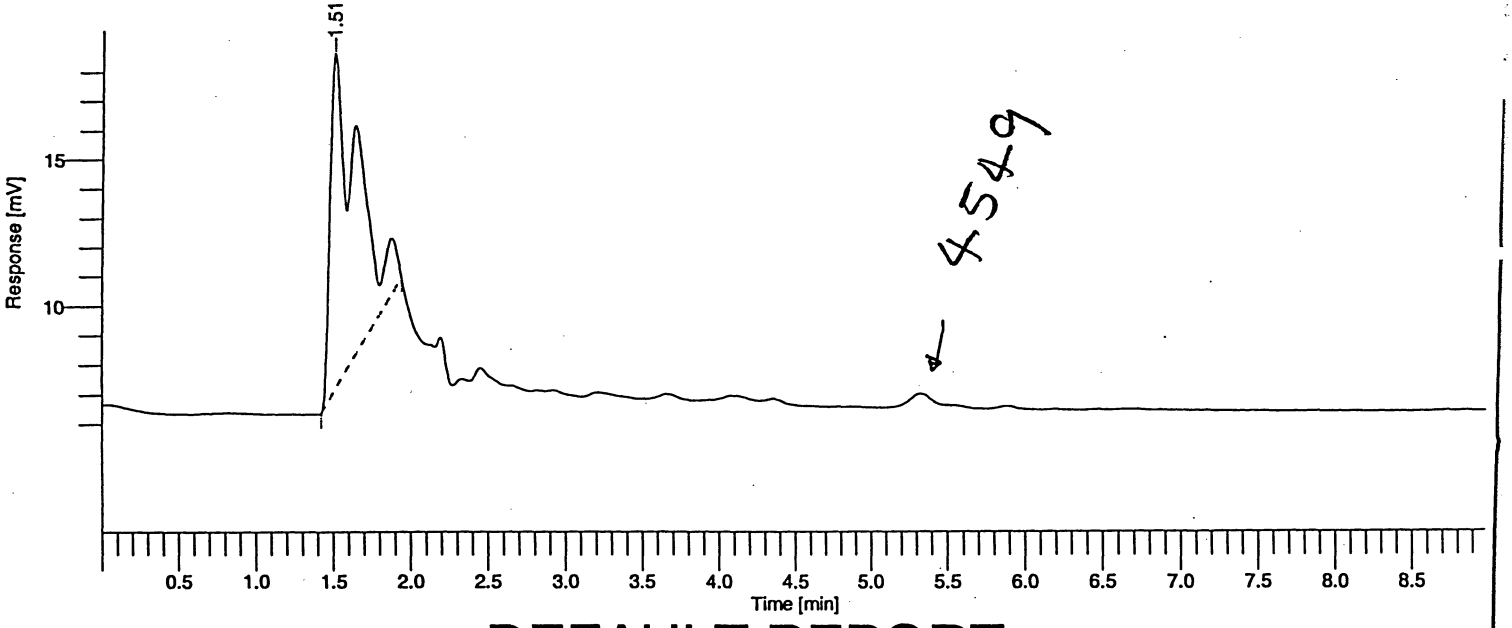
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 3:15:10 PM
Operator	: manager	Sample Name	: -VEPP12,0EPS
Sample Number	: 323	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/29
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 4:15:30 PM	Cycle	: 323

Raw Data File : \\Poweredge\E drive\TC\dennis\data312.raw
Result File : \\Poweredge\E drive\TC\dennis\data312.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data312.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.507	138896.40	11449.54	100.00	100.00	BB	12.1312
		138896.40	11449.54	100.00	100.00		

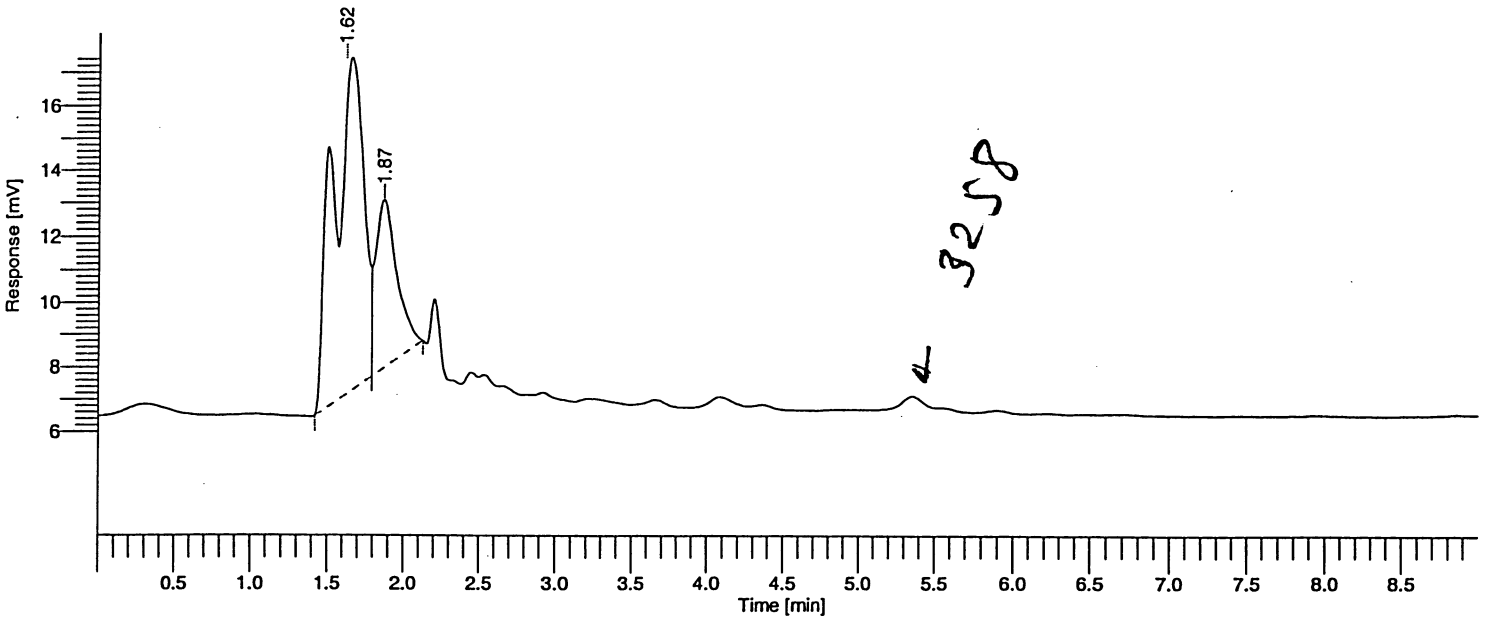
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 3:25:13 PM
Operator	: manager	Sample Name	: -VEP11,50EPS
Sample Number	: 324	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/30
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 4:25:34 PM	Cycle	: 324

Raw Data File : \\Poweredge\E drive\TC\dennis\data313.raw
Result File : \\Poweredge\E drive\TC\dennis\data313.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data313.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.622	139284.03	8637.07	73.45	73.45	BV	16.1263
2	1.873	50335.57	5128.91	26.55	26.55	VB	9.8141
		189619.60	13765.98	100.00	100.00		

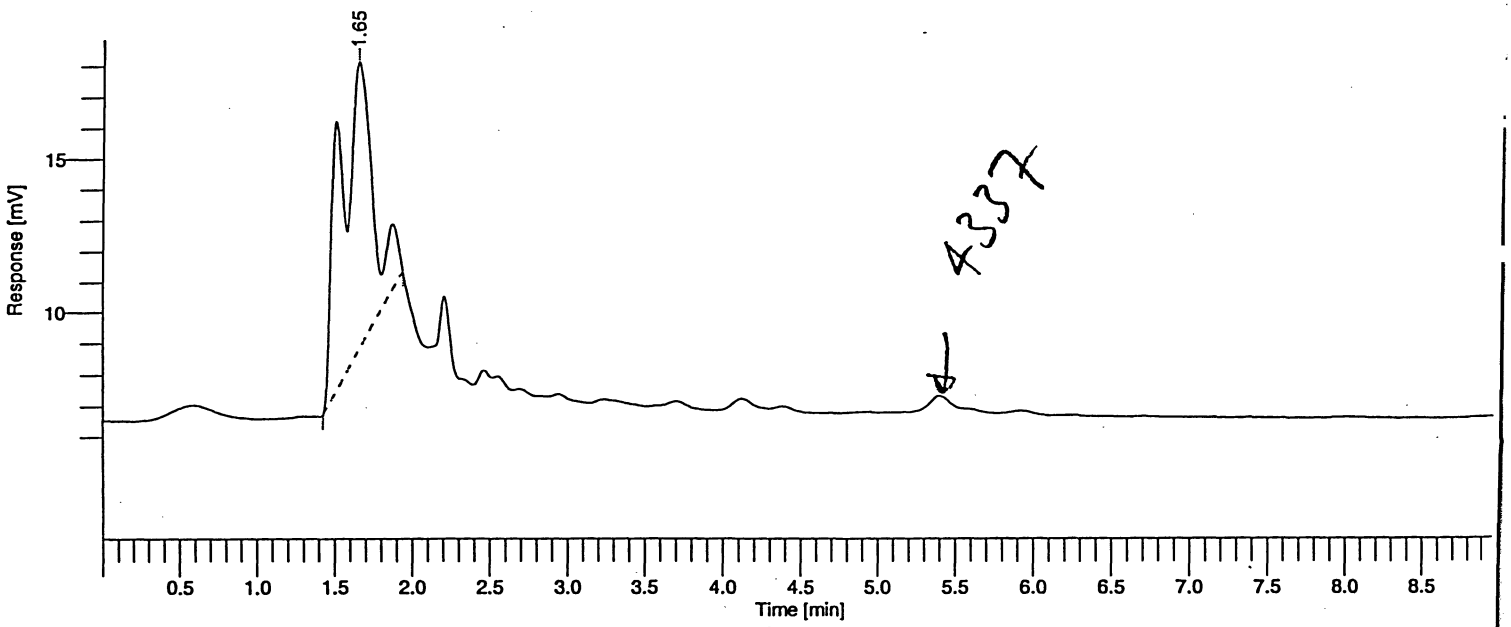
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 3:35:18 PM
Operator	: manager	Sample Name	: -VEP12,50EPS
Sample Number	: 325	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/31
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 4:35:36 PM	Cycle	: 325

Raw Data File : \\Poweredge\E drive\TC\dennis\data314.raw
Result File : \\Poweredge\E drive\TC\dennis\data314.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data314.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.653	138203.20	9309.70	100.00	100.00	BB	14.8451
		138203.20	9309.70	100.00	100.00		

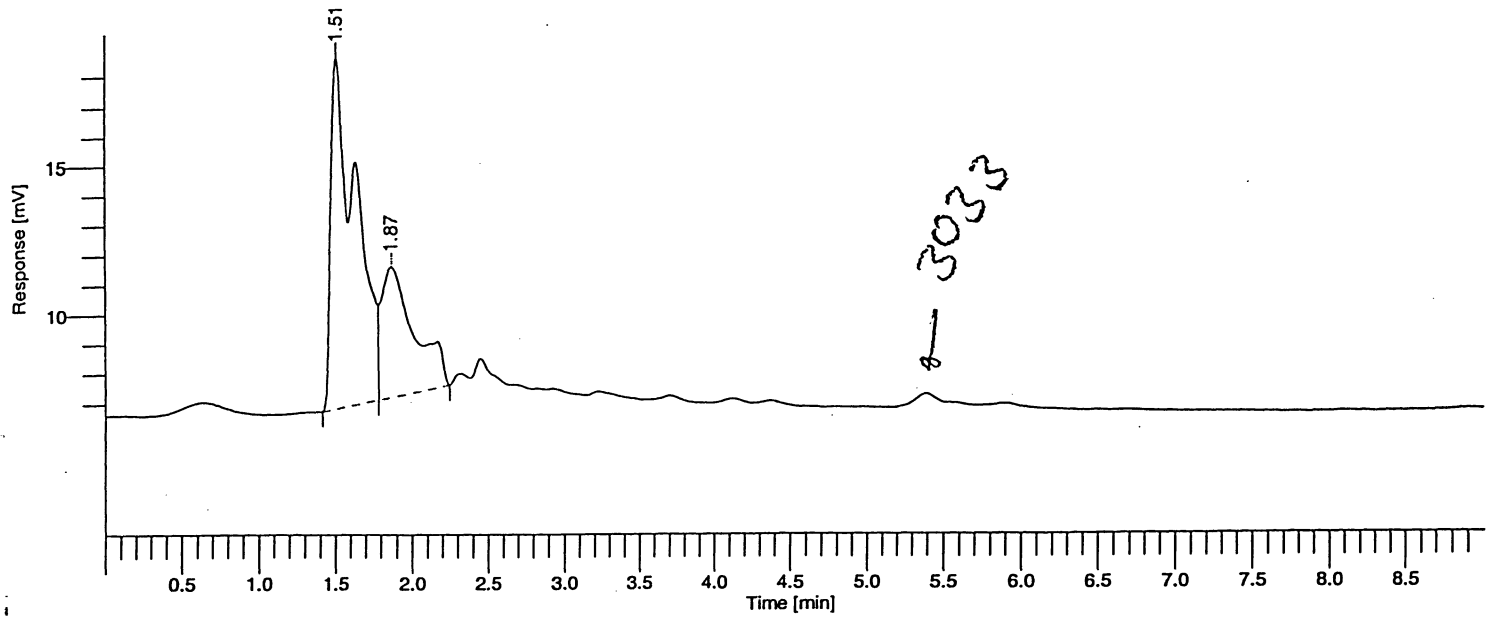
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 3:45:14 PM
Operator	: manager	Sample Name	: -VEP1,150EPS
Sample Number	: 326	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/32
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 4:45:36 PM	Cycle	: 326

Raw Data File : \\Poweredge\E drive\TC\dennis\data315.raw
Result File : \\Poweredge\E drive\TC\dennis\data315.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data315.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.510	137230.71	11788.36	67.73	67.73	BV	11.6412
2	1.874	65394.49	4392.97	32.27	32.27	VB	14.8862
		202625.20	16181.33	100.00	100.00		

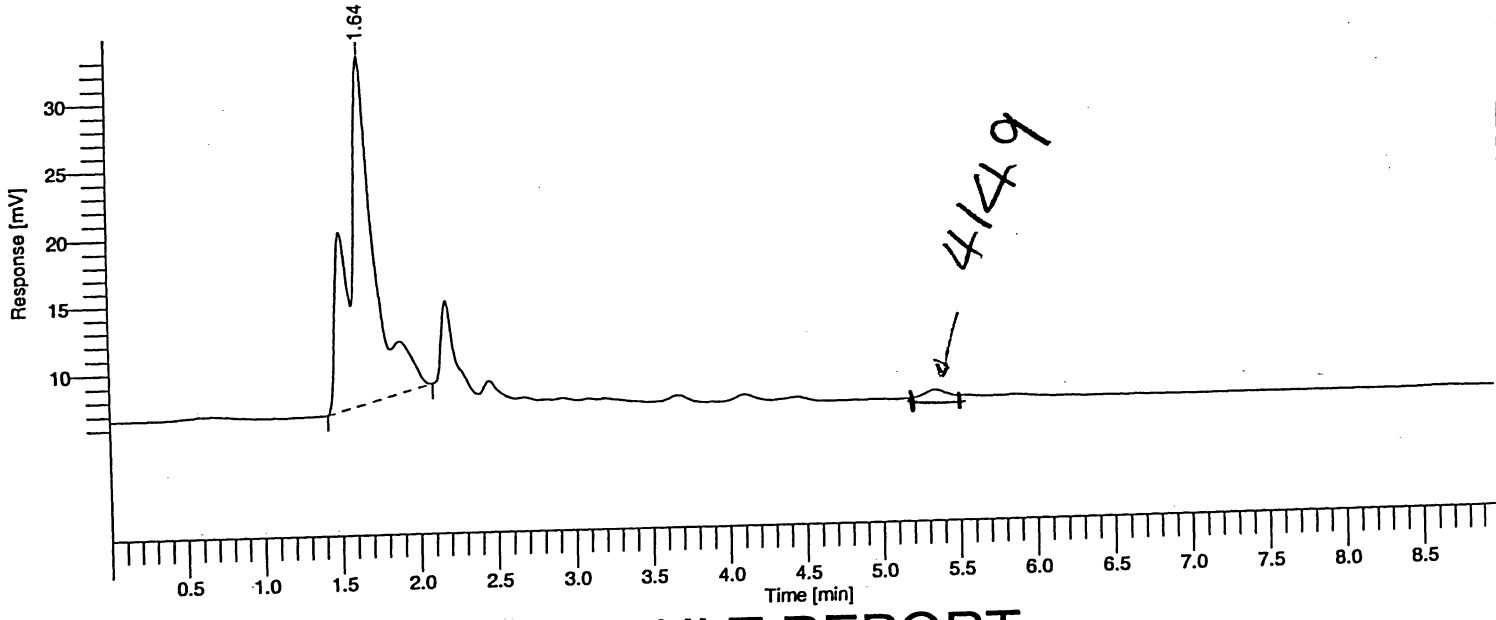
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 3:55:17 PM
Operator	: manager	Sample Name	: P3,0 EPS
Sample Number	: 327	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/33
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 4:55:36 PM	Cycle	: 327

Raw Data File : \\Poweredge\E drive\TC\dennis\data316.raw
Result File : \\Poweredge\E drive\TC\dennis\data316.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data316.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.645	301334.00	25779.95	100.00	100.00	BB	11.6887
		301334.00	25779.95	100.00	100.00		

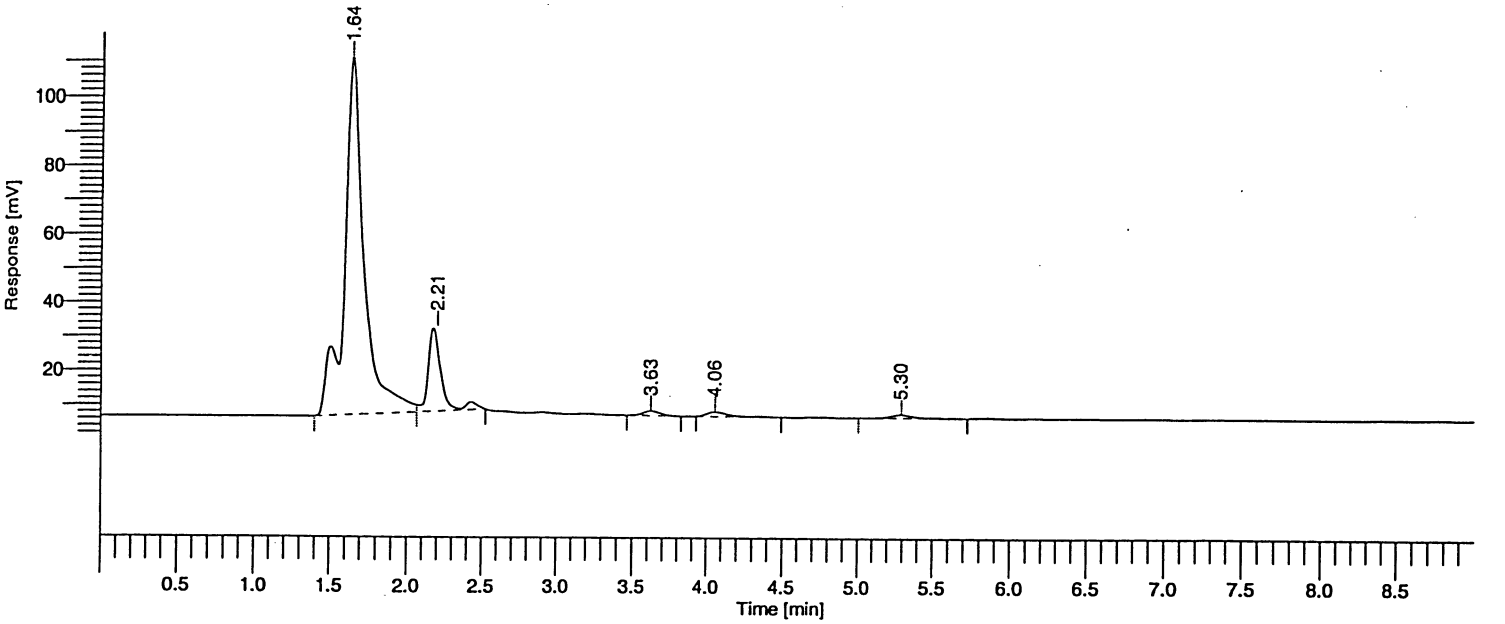
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 4:19:28 PM
Operator	: manager	Sample Name	: P21,EPS
Sample Number	: 329	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/35
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 5:19:46 PM	Cycle	: 329

Raw Data File : \\Poweredge\E drive\TC\dennis\data318.raw
Result File : \\Poweredge\E drive\TC\dennis\data318.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data318.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.643	947201.39	104384.94	83.73	83.73	BV	9.0741
2	2.207	148978.61	17622.32	13.17	13.17	VB	8.4540
3	3.626	10418.80	1377.32	0.92	0.92	BB	7.5645
4	4.058	13317.60	1327.90	1.18	1.18	BB	10.0291
5	5.295	11329.20	1035.22	1.00	1.00	BB	10.9438
		1131245.60	125747.70	100.00	100.00		

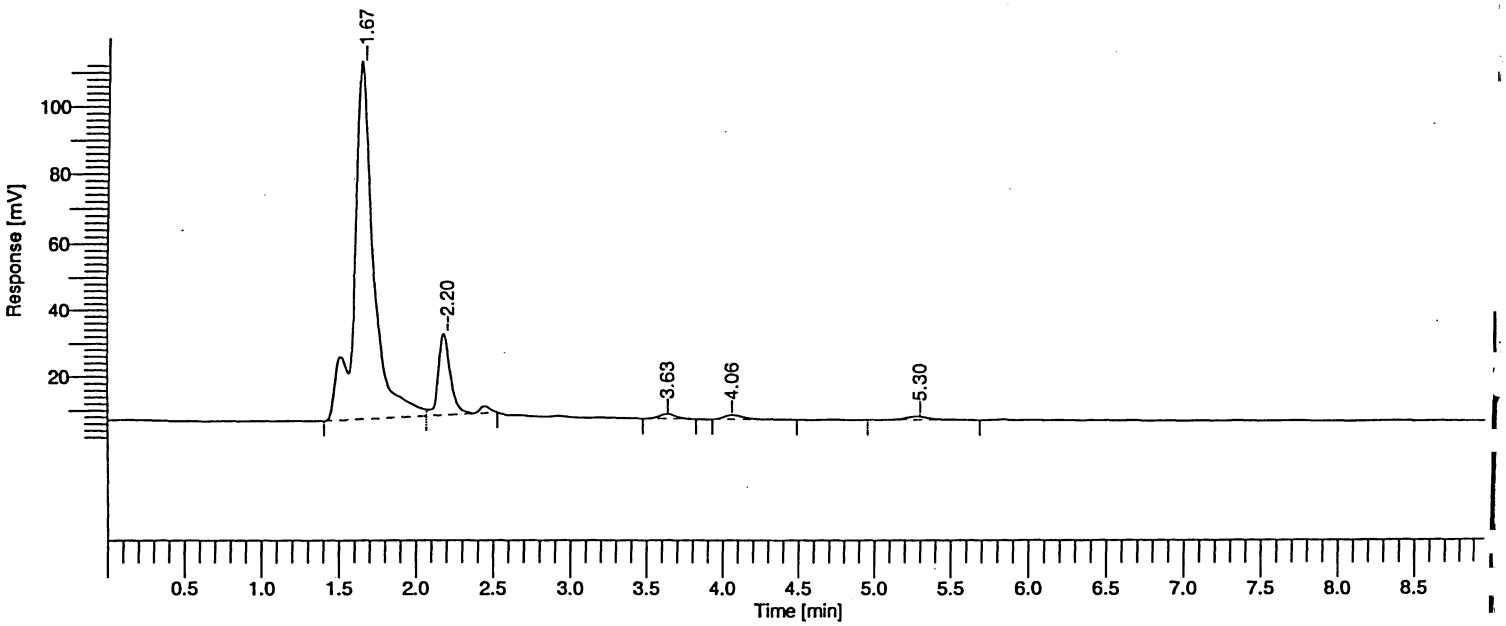
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 4:29:24 PM
Operator	: manager	Sample Name	: P22,EPS
Sample Number	: 330	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/36
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 5:29:47 PM	Cycle	: 330

Raw Data File : \\Poweredge\E drive\TC\dennis\data319.raw
Result File : \\Poweredge\E drive\TC\dennis\data319.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data319.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.673	933182.85	88440.36	83.99	83.99	BV	10.5515
2	2.205	142233.95	18173.83	12.80	12.80	VB	7.8263
3	3.635	10624.20	1401.15	0.96	0.96	BB	7.5825
4	4.060	12499.60	1257.64	1.12	1.12	BB	9.9390
5	5.297	12563.20	1010.55	1.13	1.13	BB	12.4320
		1111103.80	110283.53	100.00	100.00		

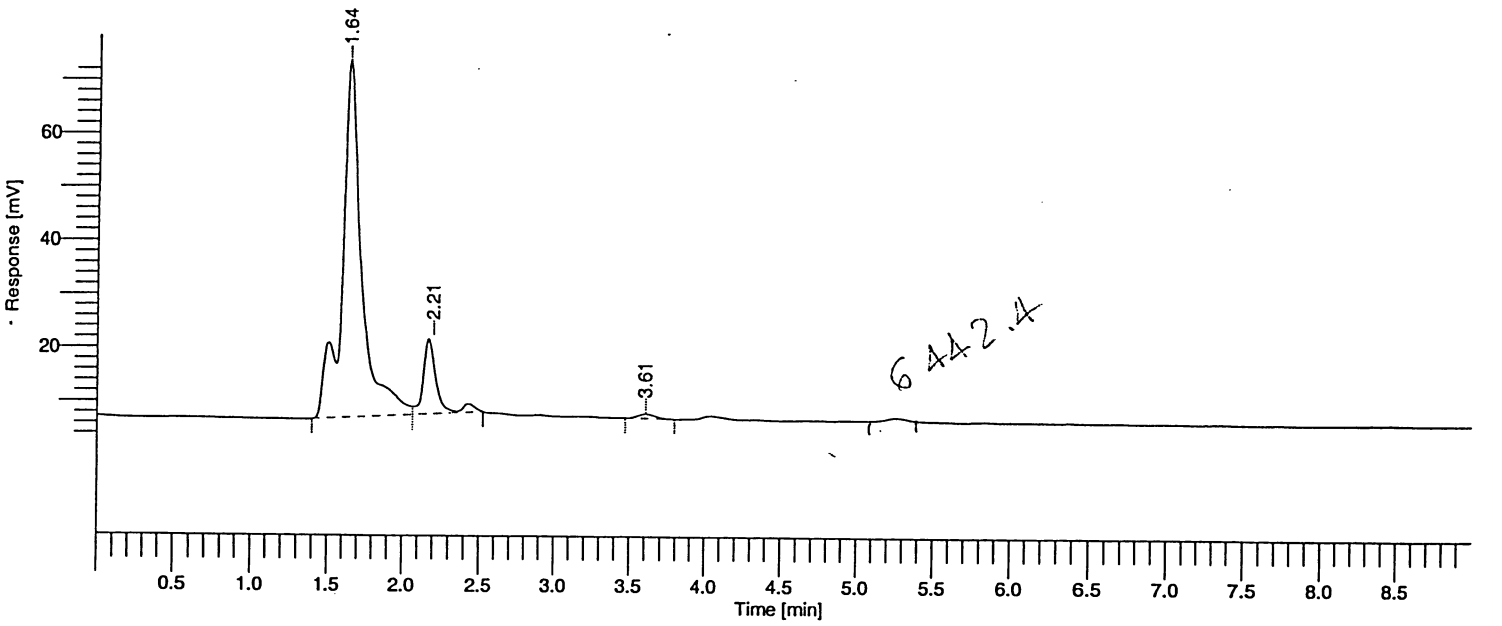
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 4:39:26 PM
Operator	: manager	Sample Name	: P23,EPS
Sample Number	: 331	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/37
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 5:39:47 PM	Cycle	: 331

Raw Data File : \\Poweredge\E drive\TC\dennis\data320.raw
Result File : \\Poweredge\E drive\TC\dennis\data320.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data320.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.644	616128.74	67103.22	86.88	86.88	BV	9.1818
2	2.205	86755.66	9166.06	12.23	12.23	VB	9.4649
3	3.608	6290.60	843.21	0.89	0.89	BB	7.4603
		709175.00	77112.49	100.00	100.00		

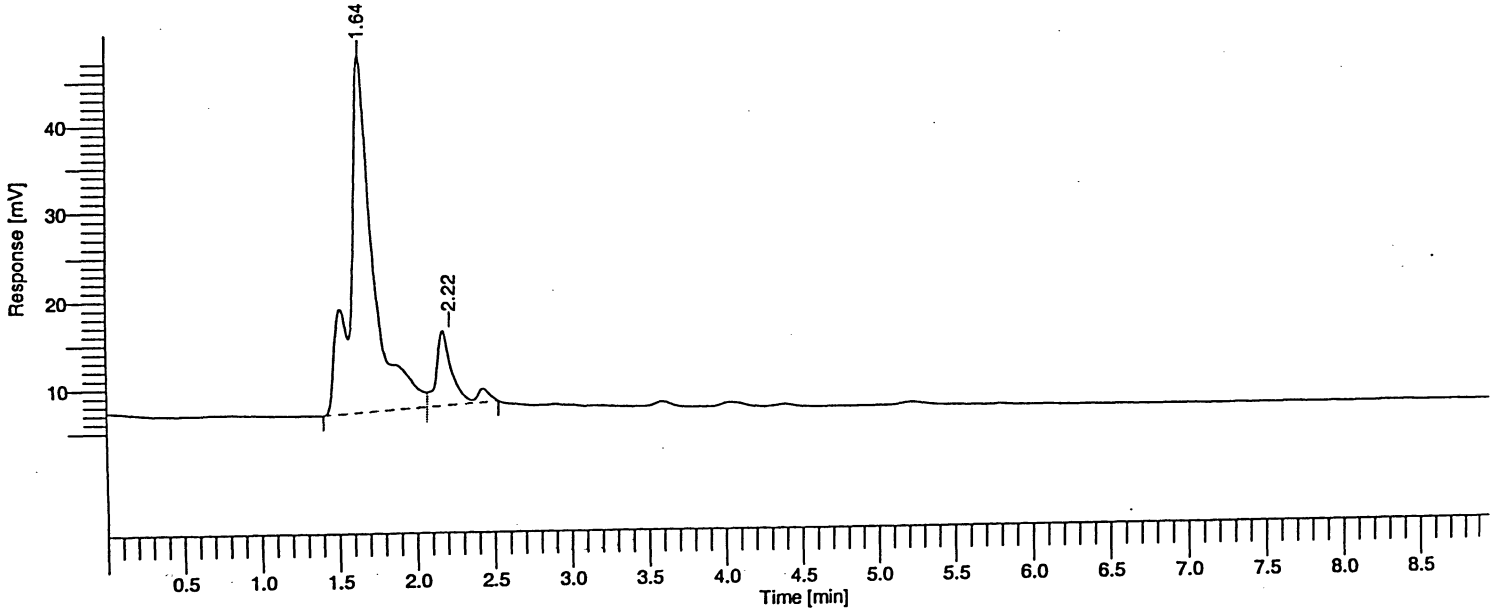
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 4:49:29 PM
Operator	: manager	Sample Name	: P3E,EPS
Sample Number	: 332	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/38
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 5:49:47 PM	Cycle	: 332

Raw Data File : \\Poweredge\E drive\TC\dennis\data321.raw
Result File : \\Poweredge\E drive\TC\dennis\data321.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data321.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.643	429145.84	40709.60	86.78	86.78	BV	10.5416
2	2.216	65364.96	4762.41	13.22	13.22	VB	13.7252
		494510.80	45472.01	100.00	100.00		

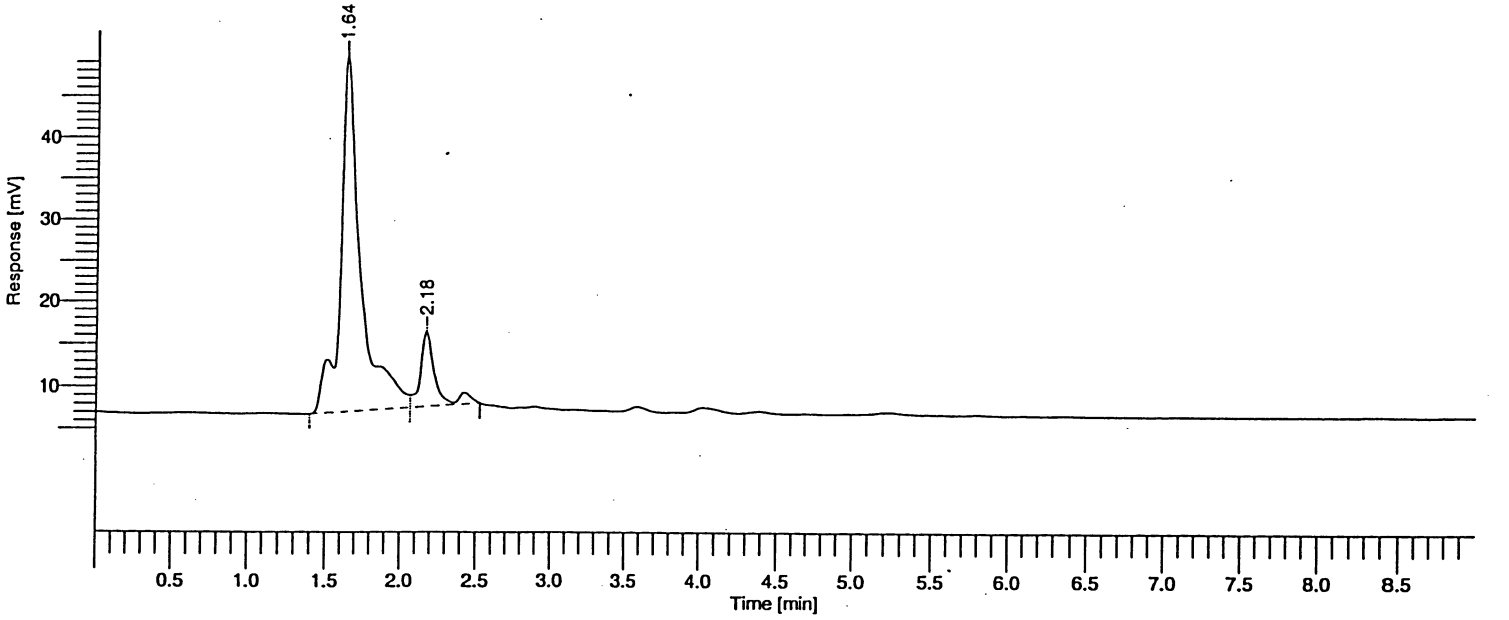
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/28/06 4:59:25 PM
Operator	: manager	Sample Name	: P3M,EPS
Sample Number	: 333	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/39
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/28/06 5:59:48 PM	Cycle	: 333

Raw Data File : \\Poweredge\E drive\TC\dennis\data322.raw
Result File : \\Poweredge\E drive\TC\dennis\data322.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data322.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.643	404939.78	42900.33	87.01	87.01	BV	9.4391
2	2.176	60465.82	8706.29	12.99	12.99	VB	6.9451
		465405.60	51606.62	100.00	100.00		

Missing Component Report

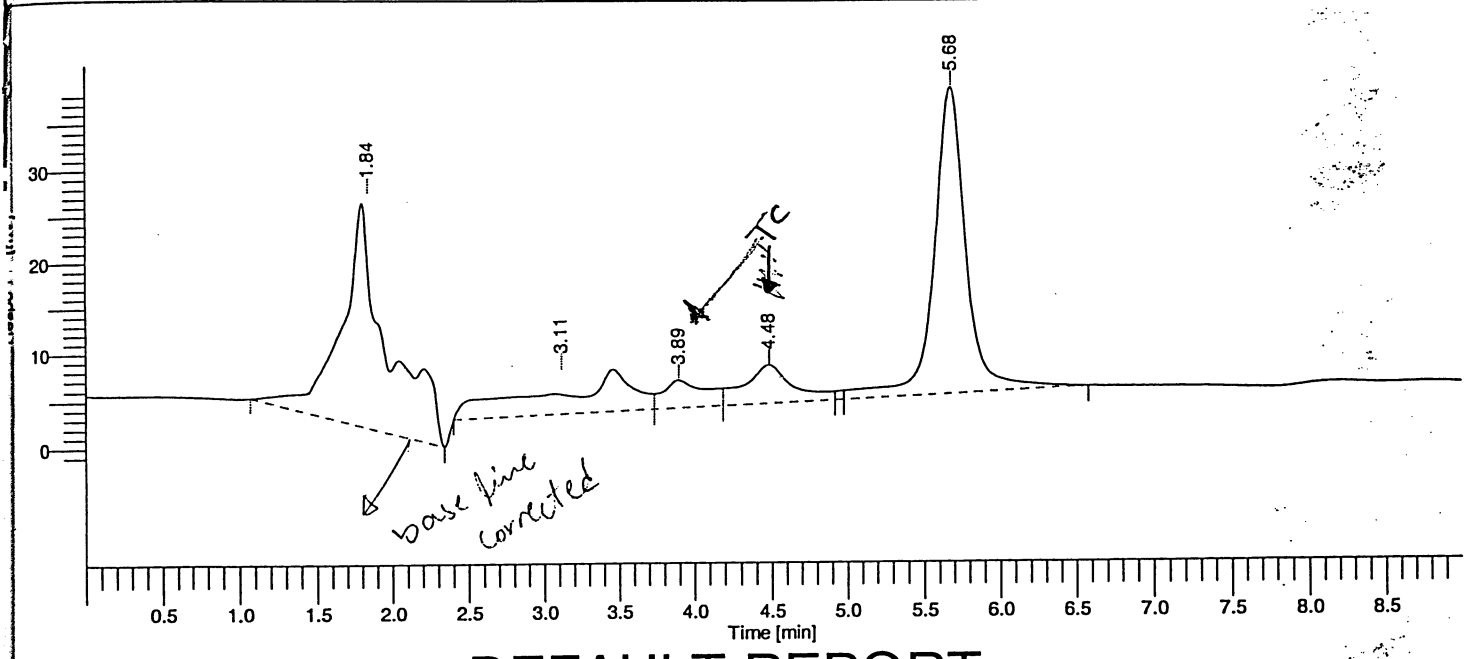
Component	Expected Retention (Calibration File)

All components were found

APPENDIX C: PARAMETERS OF CHLORTETRACYCLINE REMOVAL UNDER DIFFERETNT EDTA CONCENTRATION EXPERIMENTS

oftware Version	: 6.1.2.0.1:D19	Date	: 6/21/06 11:28:15 AM
perator	: manager	Sample Name	: ctc20ppm,meoh
ample Number	: 225	Study	: antibiotic
utoSampler	: SER200	Rack/Vial	: 1/1
strument Name	: LC	Channel	: A
strument Serial #	: None	A/D mV Range	: 1000
elay Time	: 0.00 min	End Time	: 8.99 min
ampling Rate	: 2.5000 pts/s		
olume Injected	: 1.000000 ul	Area Reject	: 0.000000
ample Amount	: 1.0000	Dilution Factor	: 1.00
ata Acquisition Time	: 6/21/06 12:28:18 PM	Cycle	: 230

aw Data File : \\Poweredge\E drive\TC\dennis\data219.raw
esult File : \\Poweredge\E drive\TC\dennis\data219.rst
st Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data219.rst
roc Method : \\Poweredge\E drive\TC\dennis\antibiotics
alib Method : \\Poweredge\E drive\TC\dennis\antibiotics
equence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.840	501944.00	16679.67	38.34	38.34	BB	30.0932
2	3.111	172570.89	2045.21	13.18	13.18	BV	84.3781
3	3.891	55921.31	2836.14	4.27	4.27	VV	19.7174
4	4.479	87227.90	3992.79	6.66	6.66	VV	21.8463
5	5.680	~~491379.11~~	33424.37	37.54	37.54	VB	14.7012
		1309043.21	58978.18	100.00	100.00		

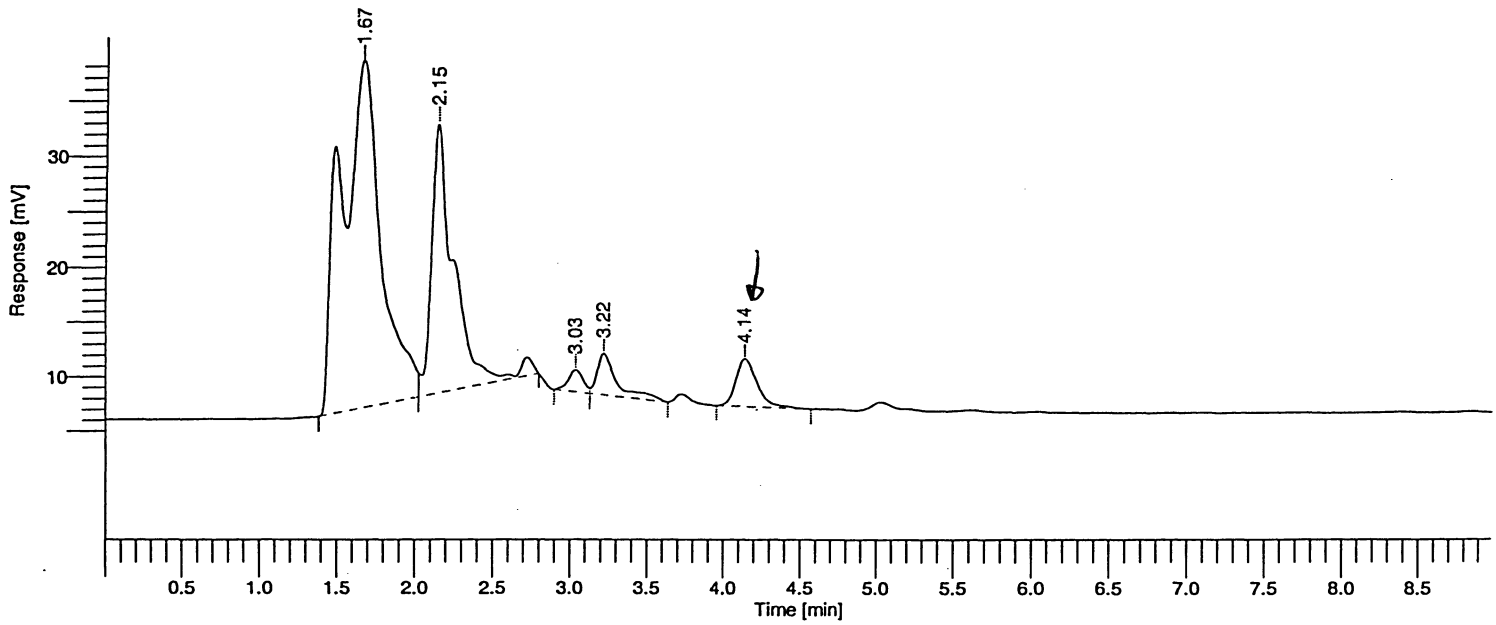
Missing Component Report
Component Expected Retention (Calibration File)

All components were found

435955

Software Version	: 6.1.2.0.1:D19	Date	: 6/13/06 12:20:36 PM
Operator	: manager	Sample Name	: -veP1,S,1
Sample Number	: 165	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/22
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/13/06 1:20:15 PM	Cycle	: 171

Raw Data File : \\Poweredge\E drive\TC\dennis\data161.raw
Result File : \\Poweredge\E drive\TC\dennis\data161.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data161.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.670	553452.66	31369.11	62.09	62.09	BV	17.6432
2	2.152	247537.34	24562.69	27.77	27.77	VB	10.0778
3	3.034	13852.03	2049.74	1.55	1.55	BV	6.7579
4	3.215	34368.77	3814.27	3.86	3.86	VB	9.0106
5	4.135	42161.20	4393.78	4.73	4.73	BB	9.5957
		891372.00	66189.60	100.00	100.00		

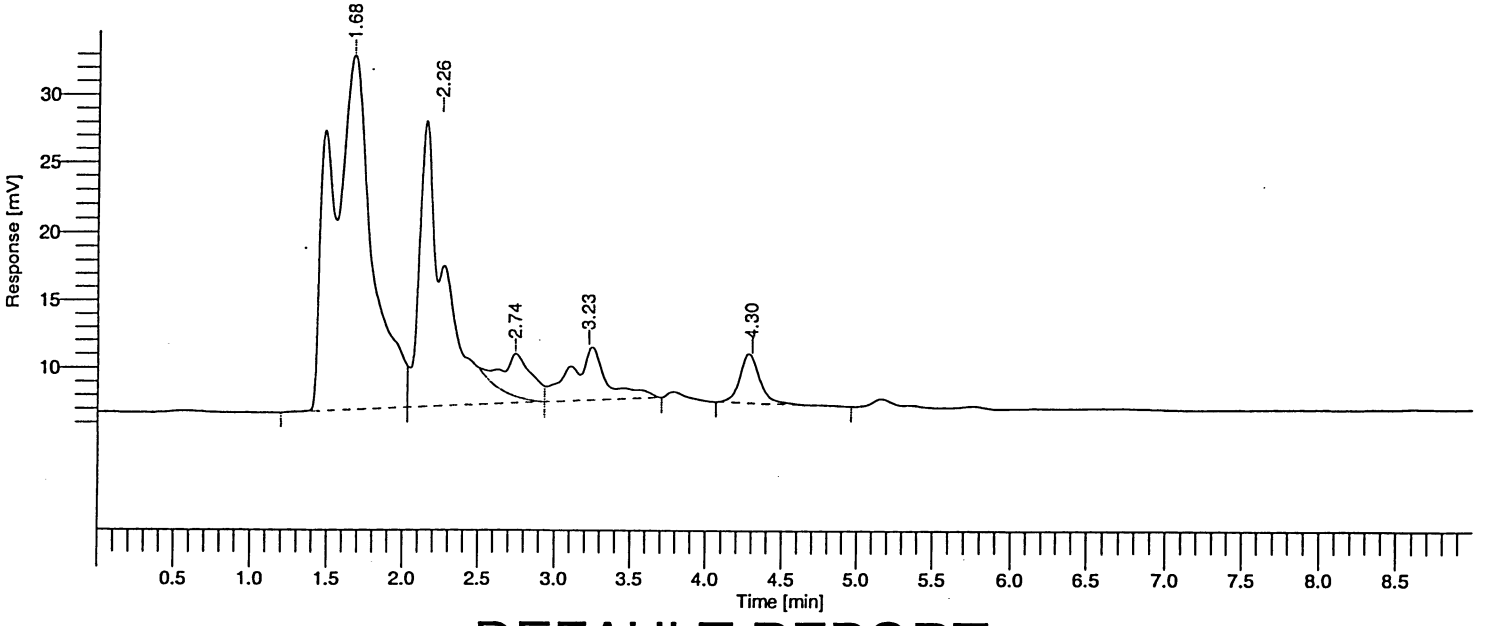
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/13/06 12:30:32 PM
Operator	: manager	Sample Name	: -VEP1,S 2
Sample Number	: 166	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/23
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/13/06 1:30:15 PM	Cycle	: 172

Raw Data File : \\Poweredge\E drive\TC\dennis\data162.raw
Result File : \\Poweredge\E drive\TC\dennis\data162.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data162.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.676	486549.81	26060.47	54.86	54.86	BV	18.6700
2	2.256	257530.28	10048.17	29.04	29.04	VE	25.6296
3	2.745	42522.80	3096.76	4.79	4.79	EV	13.7314
4	3.226	66427.51	3567.33	7.49	7.49	VB	18.6211
5	4.302	33932.20	3319.59	3.83	3.83	BB	10.2218
		886962.60	46092.32	100.00	100.00		

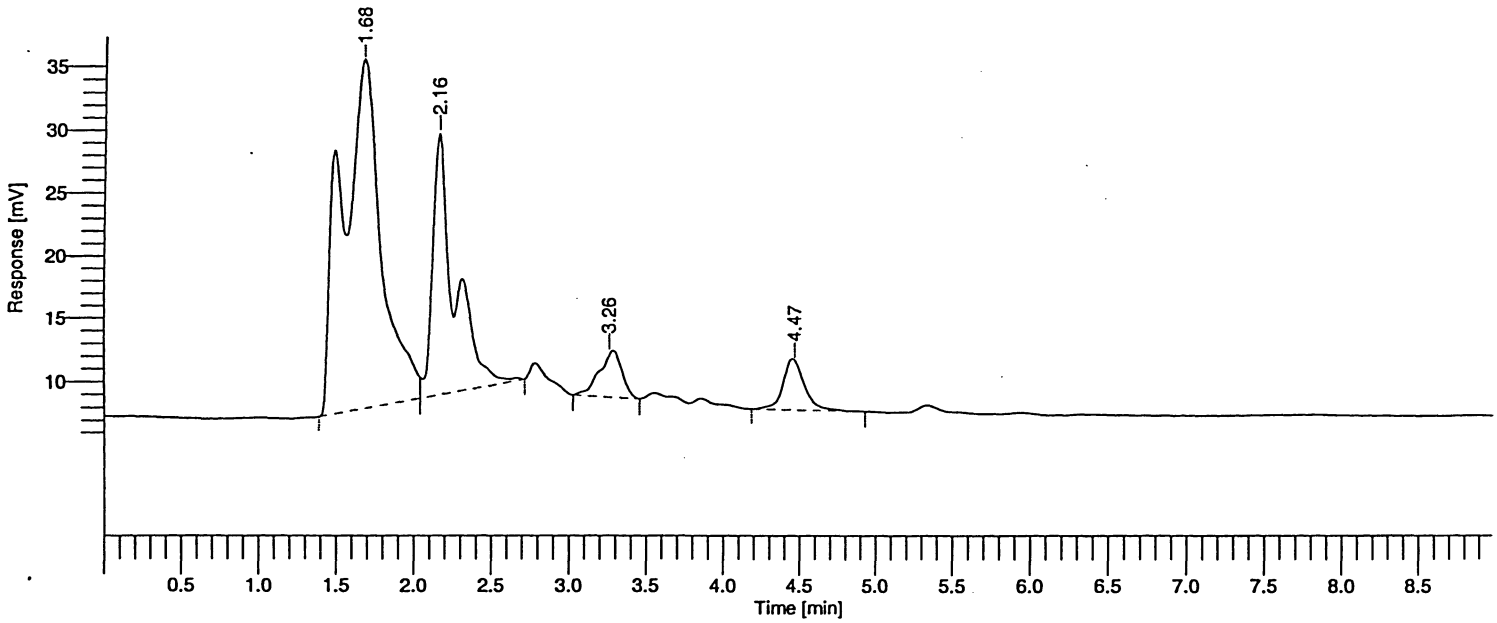
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/13/06 12:40:34 PM
Operator	: manager	Sample Name	: -VEP1S3
Sample Number	: 167	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/24
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/13/06 1:40:16 PM	Cycle	: 173

Raw Data File : \\Poweredge\E drive\TC\dennis\data163.raw
Result File : \\Poweredge\E drive\TC\dennis\data163.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data163.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.679	482704.70	27589.35	63.15	63.15	BV	17.4961
2	2.165	206054.50	20901.75	26.96	26.96	VB	9.8582
3	3.258	36599.20	3342.92	4.79	4.79	BB	10.9483
4	4.465	39039.40	3846.50	5.11	5.11	BB	10.1493
		764397.80	55680.53	100.00	100.00		

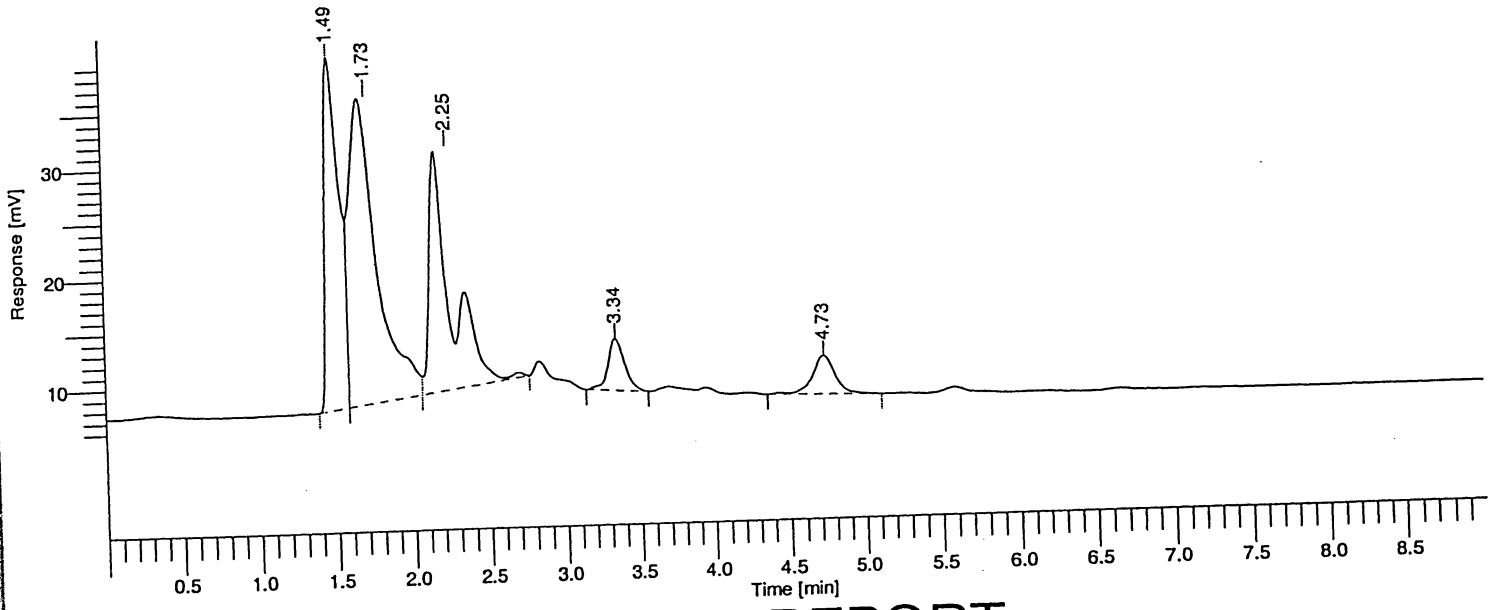
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/13/06 12:50:31 PM
Operator	: manager	Sample Name	: -VE P2 S1
Sample Number	: 168	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/25
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/13/06 1:50:16 PM	Cycle	: 174

Raw Data File : \\Poweredge\E drive\TC\dennis\data164.raw
Result File : \\Poweredge\E drive\TC\dennis\data164.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data164.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.486	211219.94	32025.50	24.96	24.96	BV	6.5954
2	1.726	346558.32	23178.27	40.96	40.96	VV	14.9519
3	2.247	215214.13	6974.77	25.43	25.43	VB	30.8561
4	3.344	36450.00	4696.74	4.31	4.31	BB	7.7607
5	4.729	36719.20	3489.17	4.34	4.34	BB	10.5238
		846161.60	70364.46	100.00	100.00		

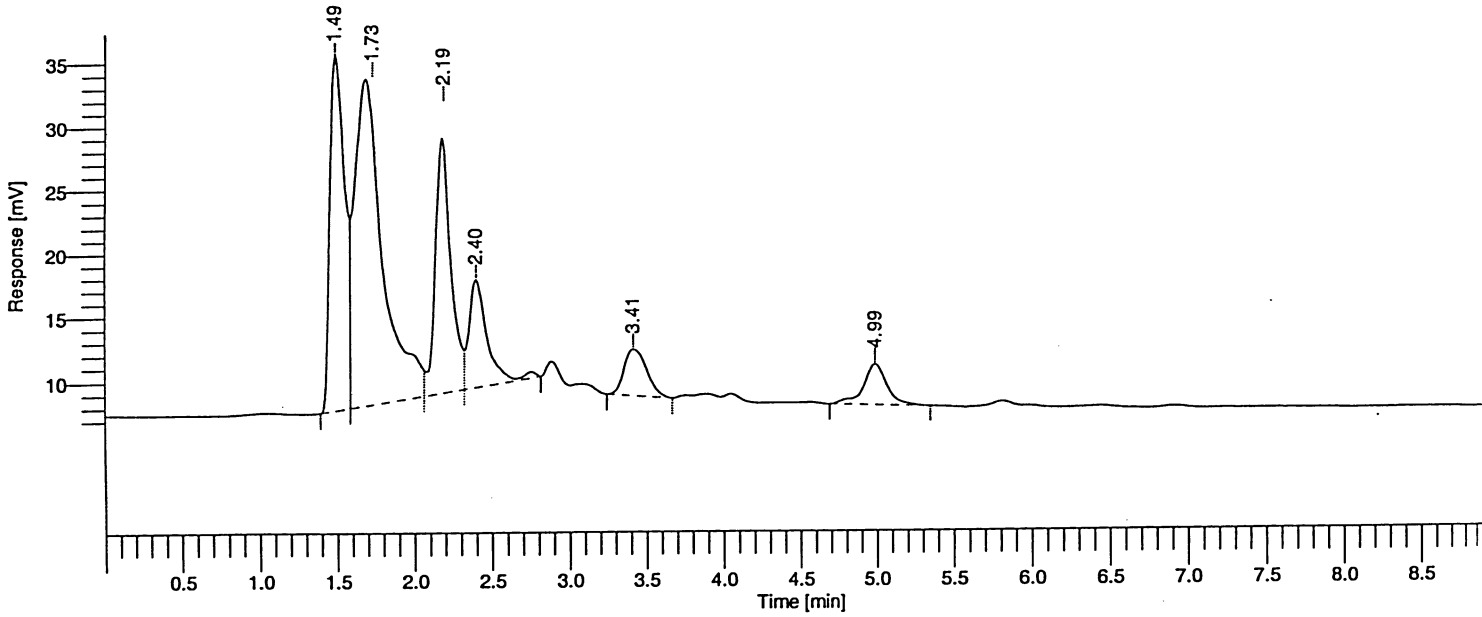
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/13/06 1:00:34 PM
Operator	: manager	Sample Name	: -VE P2 S 2
Sample Number	: 169	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/26
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/13/06 2:00:16 PM	Cycle	: 175

Raw Data File : \\Poweredge\E drive\TC\dennis\data165.raw
Result File : \\Poweredge\E drive\TC\dennis\data165.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data165.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.489	183591.52	27715.11	23.53	23.53	BV	6.6242
2	1.731	321649.03	21157.58	41.22	41.22	VV	15.2025
3	2.192	138808.79	19036.86	17.79	17.79	VV	7.2916
4	2.398	67824.66	8332.67	8.69	8.69	VB	8.1396
5	3.412	35990.00	3501.79	4.61	4.61	BB	10.2776
6	4.986	32522.00	3010.37	4.17	4.17	BB	10.8033
		780386.00	82754.38	100.00	100.00		

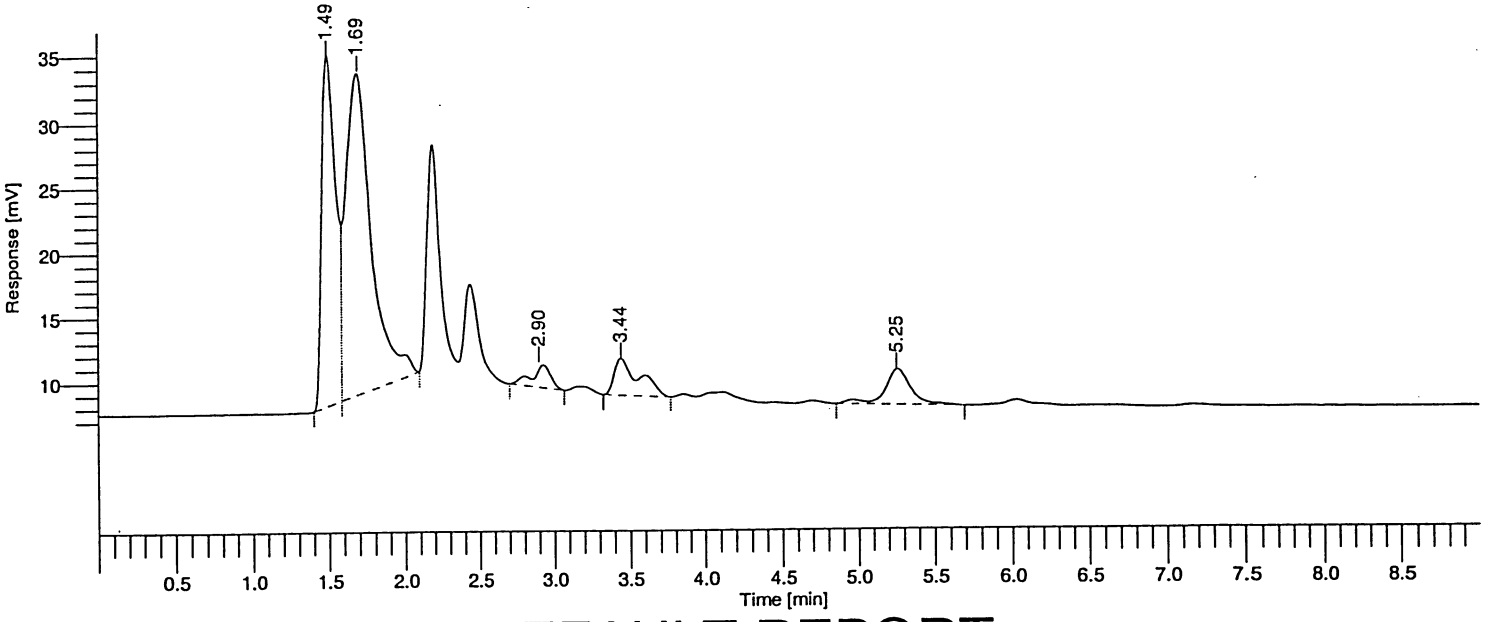
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/13/06 1:10:31 PM
Operator	: manager	Sample Name	: -VE P2 S 3
Sample Number	: 170	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/27
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/13/06 2:10:16 PM	Cycle	: 176

Raw Data File : \\Poweredge\E drive\TC\dennis\data166.raw
Result File : \\Poweredge\E drive\TC\dennis\data166.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data166.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.491	175008.02	26937.51	32.36	32.36	BV	6.4968
2	1.693	286283.78	24770.75	52.94	52.94	VB	11.5573
3	2.898	15236.80	1332.42	2.82	2.82	BB	11.4354
4	3.444	32069.60	2839.43	5.93	5.93	BB	11.2944
5	5.254	32139.80	2722.89	5.94	5.94	BB	11.8035
		540738.00	58603.01	100.00	100.00		

Missing Component Report

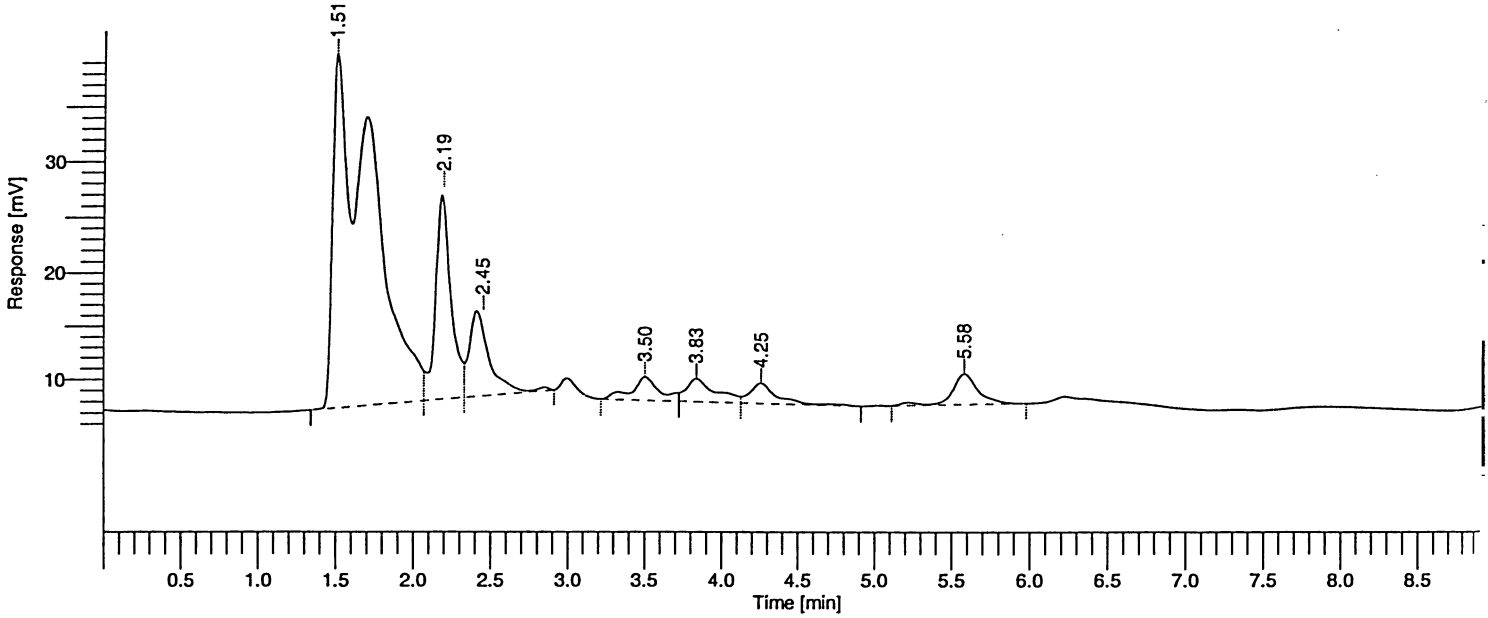
Component	Expected Retention (Calibration File)

All components were found

repeat

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 2:09:29 PM
Operator	: manager	Sample Name	: -VE P23,S
Sample Number	: 241	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/15
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 3:09:29 PM	Cycle	: 244

Raw Data File : \\Poweredge\E drive\TC\dennis\data233.raw
Result File : \\Poweredge\E drive\TC\dennis\data233.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data233.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.510	567958.02	32449.14	64.42	64.42	BV	17.5030
2	2.195	132089.13	17848.26	14.98	14.98	VV	7.4007
3	2.449	72503.25	5780.59	8.22	8.22	VB	12.5425
4	3.499	26533.48	2130.10	3.01	3.01	BV	12.4564
5	3.831	27319.72	2087.82	3.10	3.10	VV	13.0853
6	4.254	22701.00	1840.48	2.57	2.57	VB	12.3343
7	5.576	32589.00	2785.18	3.70	3.70	BB	11.7009
		881693.60	64921.57	100.00	100.00		

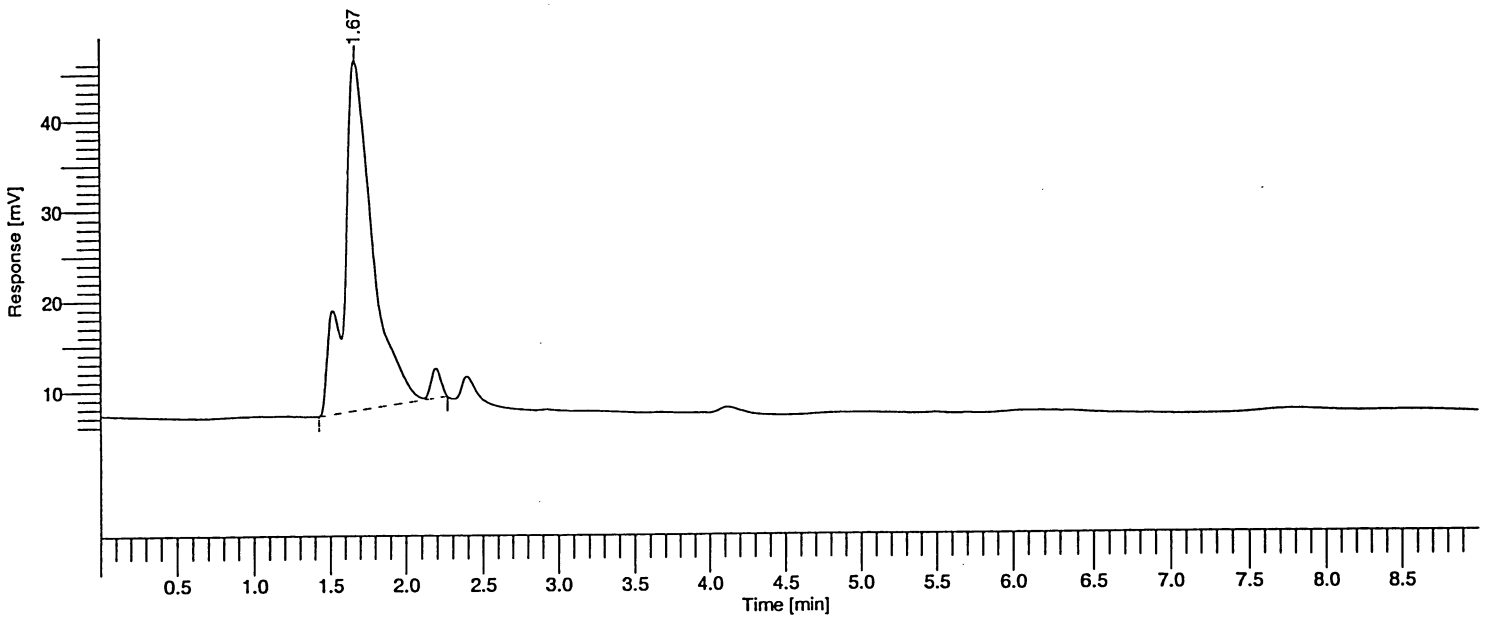
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 2:19:25 PM
Operator	: manager	Sample Name	: -VE P11 EPS
Sample Number	: 242	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/16
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 3:19:29 PM	Cycle	: 245

Raw Data File : \\Poweredge\E drive\TC\dennis\data234.raw
Result File : \\Poweredge\E drive\TC\dennis\data234.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data234.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.668	529113.80	38758.86	100.00	100.00	BB	13.6514
		529113.80	38758.86	100.00	100.00		

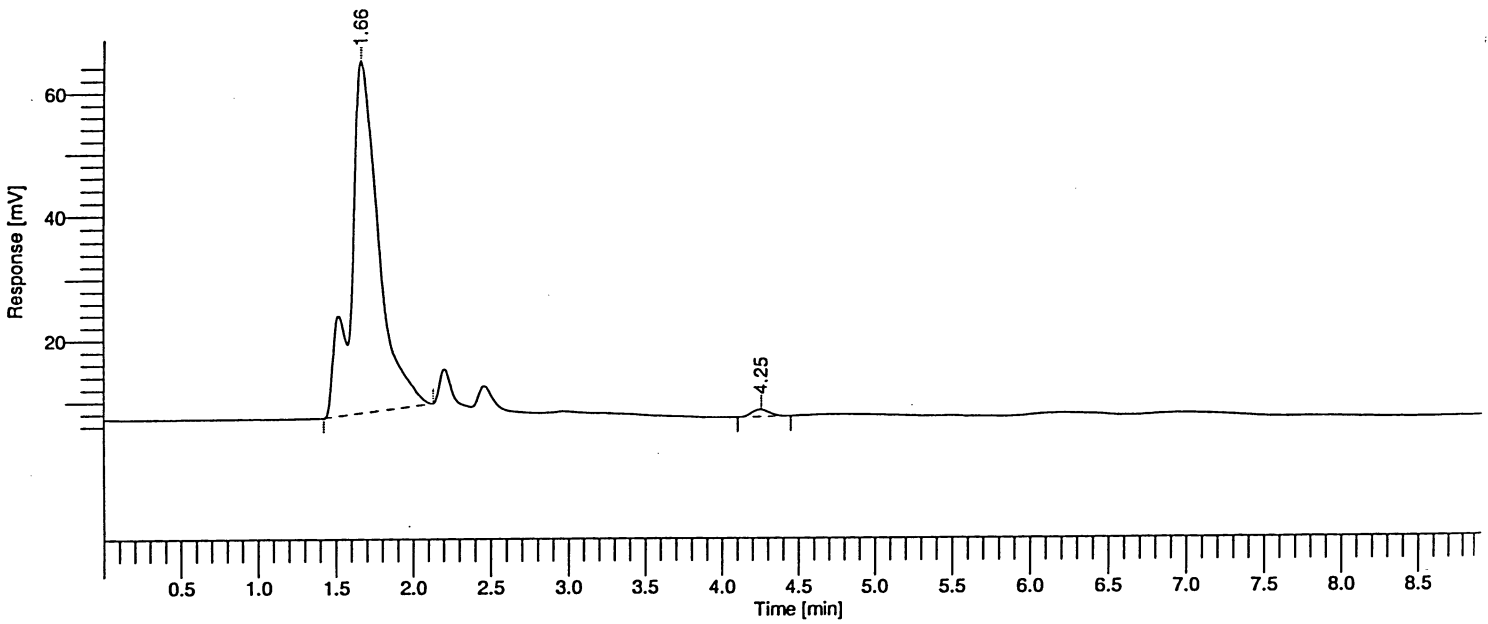
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 2:29:28 PM
Operator	: manager	Sample Name	: -VE P12 EPS
Sample Number	: 243	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/17
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 3:29:29 PM	Cycle	: 246

Raw Data File : \\Poweredge\E drive\TC\dennis\data235.raw
Result File : \\Poweredge\E drive\TC\dennis\data235.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data235.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.665	712517.20	56847.25	98.73	98.73	BB	12.5339
2	4.253	9131.20	1136.50	1.27	1.27	BB	8.0345
		721648.40	57983.75	100.00	100.00		

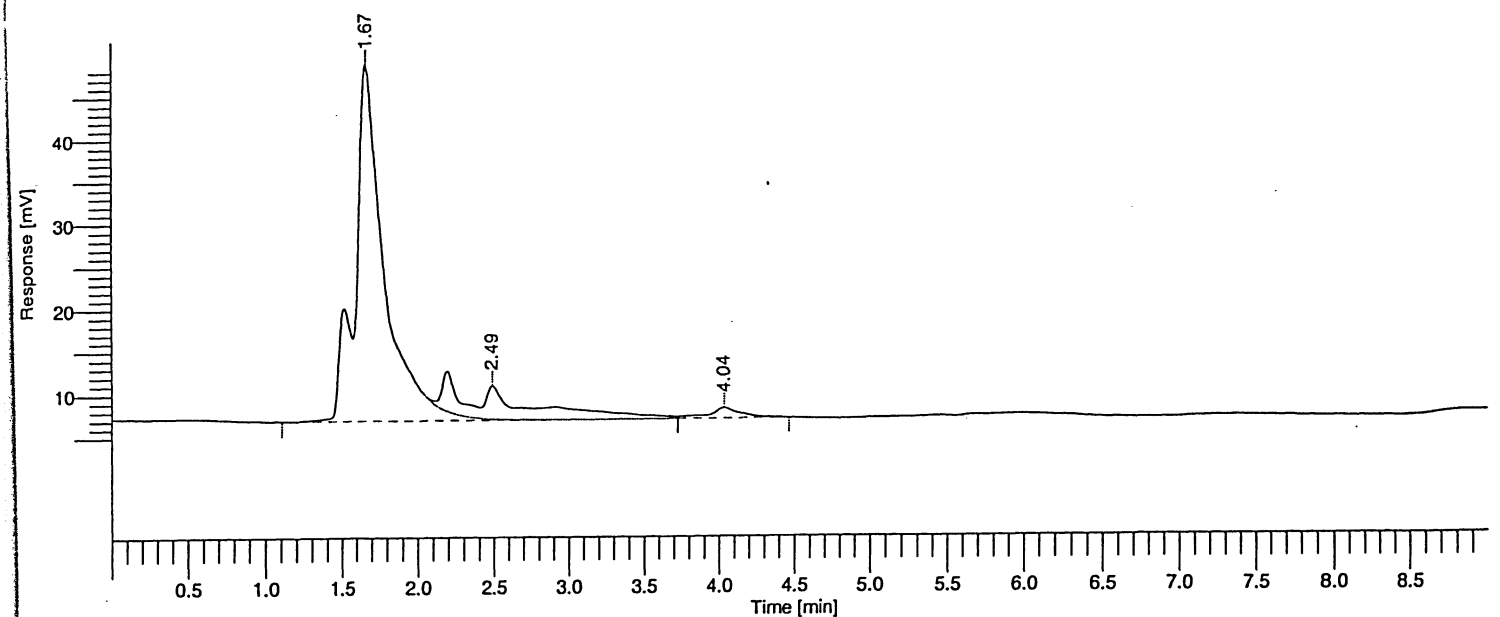
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 2:39:24 PM
Operator	: manager	Sample Name	: -VEP13 EPS
Sample Number	: 244	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/18
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 3:39:29 PM	Cycle	: 247

Raw Data File : \\Poweredge\E drive\TC\dennis\data236.raw
Result File : \\Poweredge\E drive\TC\dennis\data236.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data236.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.667	553422.97	41962.82	79.15	79.15	BE	13.1884
2	2.491	128019.20	3893.59	18.31	18.31	EV	32.8795
3	4.037	17763.50	1160.01	2.54	2.54	VB	15.3133
		699205.67	47016.41	100.00	100.00		

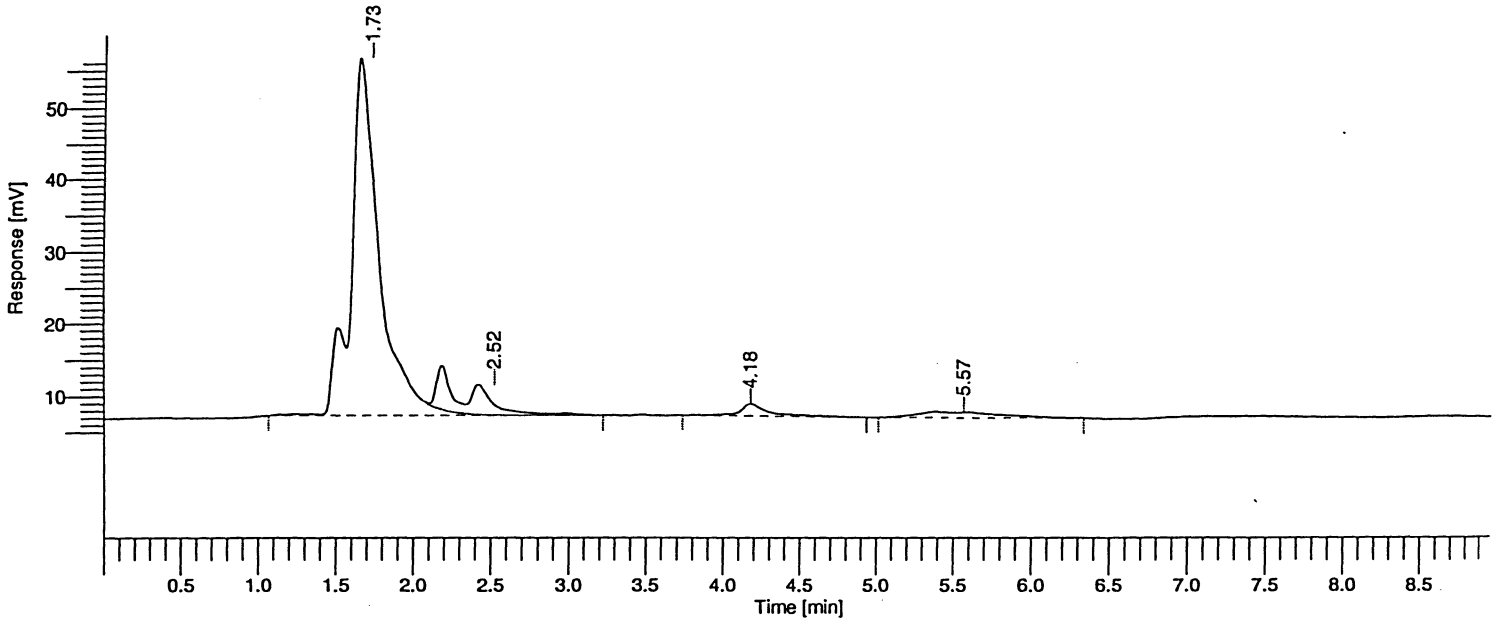
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 2:49:28 PM
Operator	: manager	Sample Name	: -VEP14 EPS
Sample Number	: 245	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/19
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 3:49:29 PM	Cycle	: 248

Raw Data File : \\Poweredge\E drive\TC\dennis\data237.raw
Result File : \\Poweredge\E drive\TC\dennis\data237.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data237.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.733	630290.74	33511.25	82.58	82.58	BE	18.8083
2	2.522	80219.55	1417.42	10.51	10.51	EB	56.5956
3	4.176	23688.00	1641.45	3.10	3.10	BB	14.4312
4	5.566	29029.60	728.42	3.80	3.80	BB	39.8528
		763227.89	37298.53	100.00	100.00		

Missing Component Report

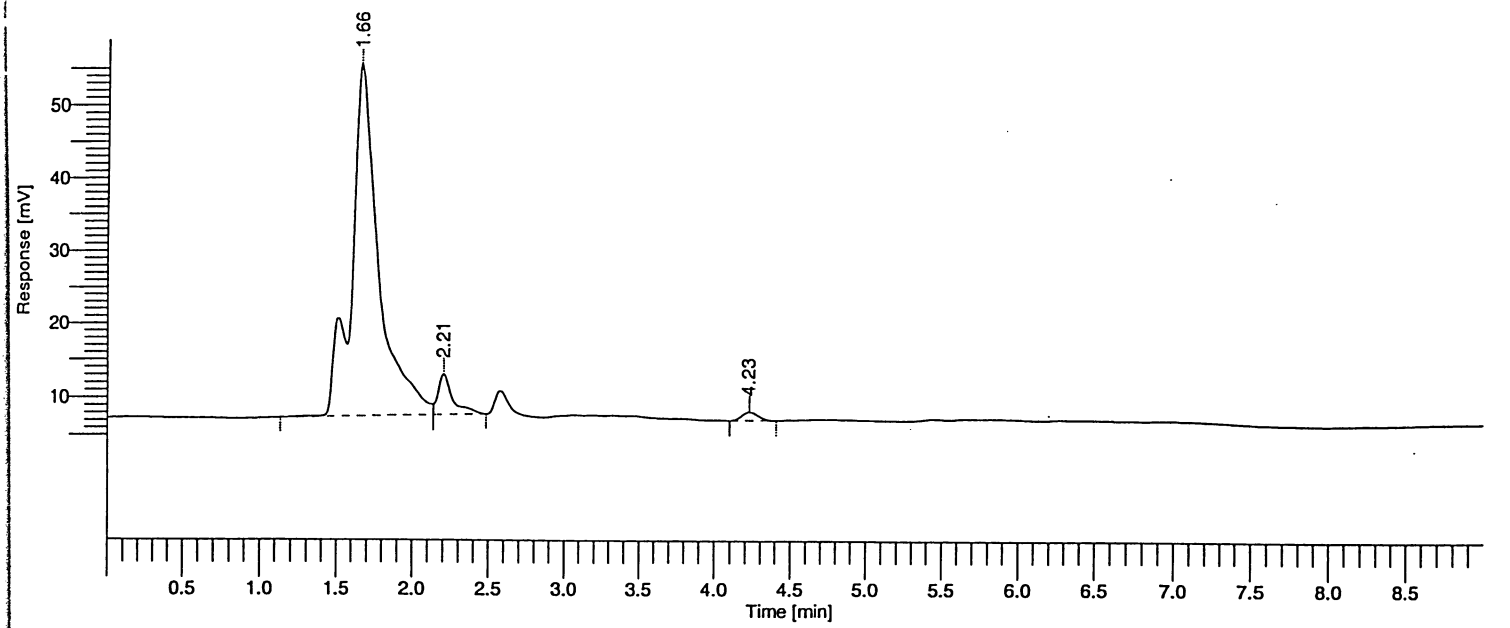
Component	Expected Retention (Calibration File)

All components were found

4000

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 2:59:24 PM
Operator	: manager	Sample Name	: -VE P15 EPS
Sample Number	: 246	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/20
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 3:59:30 PM	Cycle	: 249

Raw Data File : \\Poweredge\E drive\TC\dennis\data238.raw
Result File : \\Poweredge\E drive\TC\dennis\data238.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data238.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.663	592949.45	48168.39	93.12	93.12	BV	12.3099
2	2.206	35333.75	5332.99	5.55	5.55	VB	6.6255
3	4.227	8444.00	1145.97	1.33	1.33	BB	7.3684
		636727.20	54647.35	100.00	100.00		

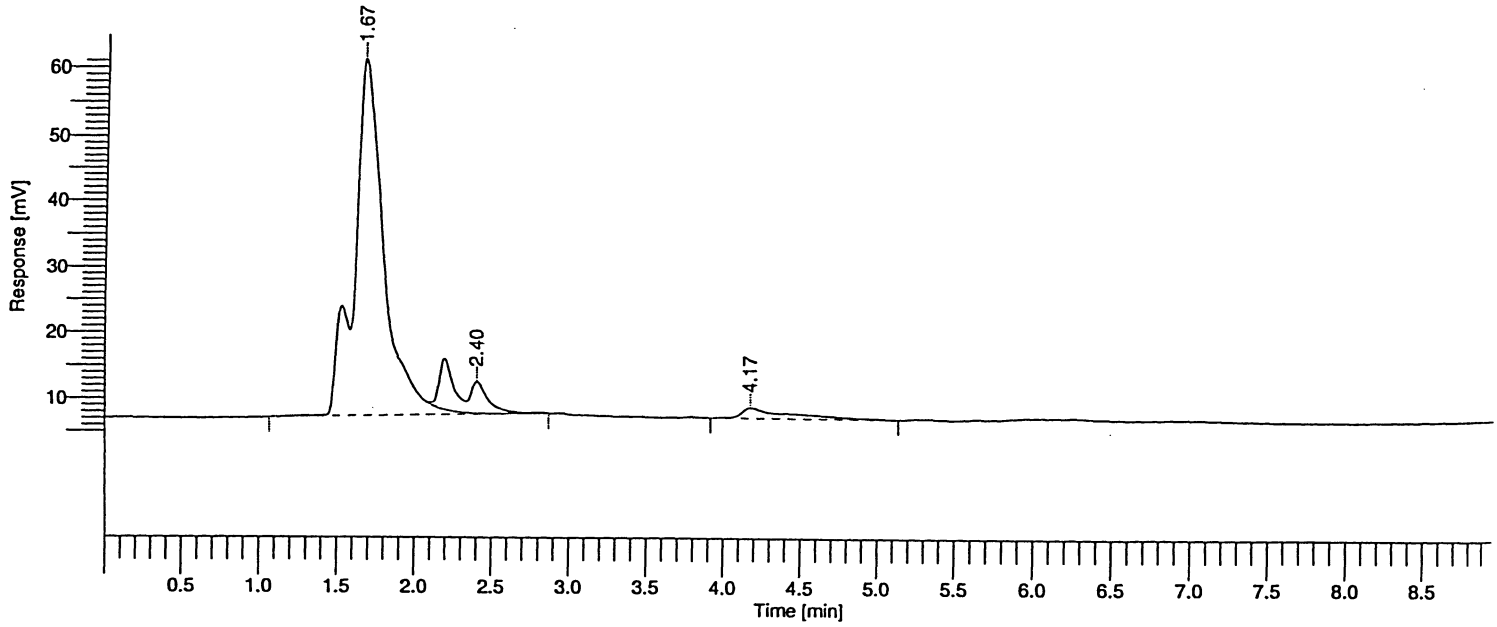
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 3:09:28 PM
Operator	: manager	Sample Name	: -VE P16EPS
Sample Number	: 247	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/21
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 4:09:32 PM	Cycle	: 250

Raw Data File : \\Poweredge\E drive\TC\dennis\data239.raw
Result File : \\Poweredge\E drive\TC\dennis\data239.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data239.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.671	713839.64	54073.00	85.12	85.12	BE	13.2014
2	2.402	89822.00	4780.17	10.71	10.71	EB	18.7905
3	4.172	34931.20	1554.69	4.17	4.17	BB	22.4683
		838592.84	60407.86	100.00	100.00		

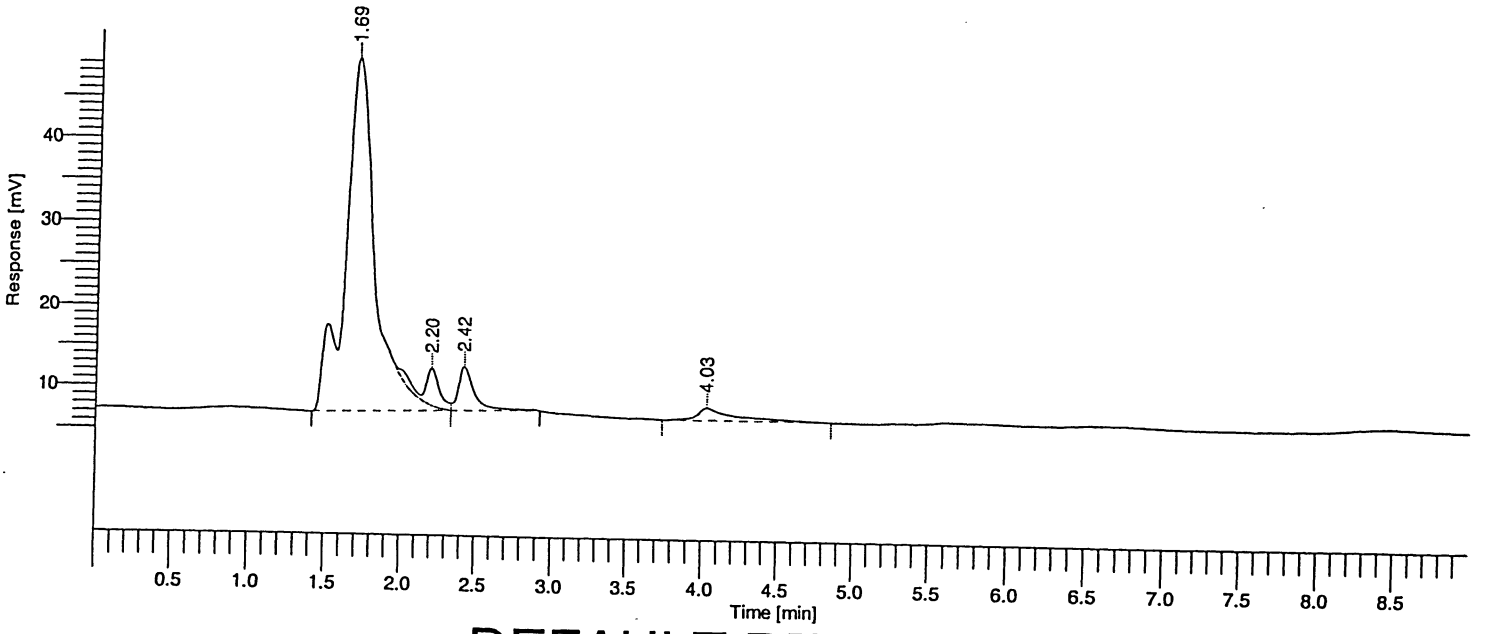
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 3:19:31 PM
Operator	: manager	Sample Name	: -VEP21 EPS
Sample Number	: 248	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/22
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 4:19:32 PM	Cycle	: 251

Raw Data File : \\Poweredge\E drive\TC\dennis\data240.raw
Result File : \\Poweredge\E drive\TC\dennis\data240.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data240.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.687	583410.69	42720.11	85.20	85.20	BE	13.6566
2	2.204	35021.20	4527.35	5.11	5.11	EV	7.7355
3	2.421	39515.11	5309.38	5.77	5.77	VB	7.4425
4	4.028	26817.20	1487.45	3.92	3.92	BB	18.0290
		684764.20	54044.29	100.00	100.00		

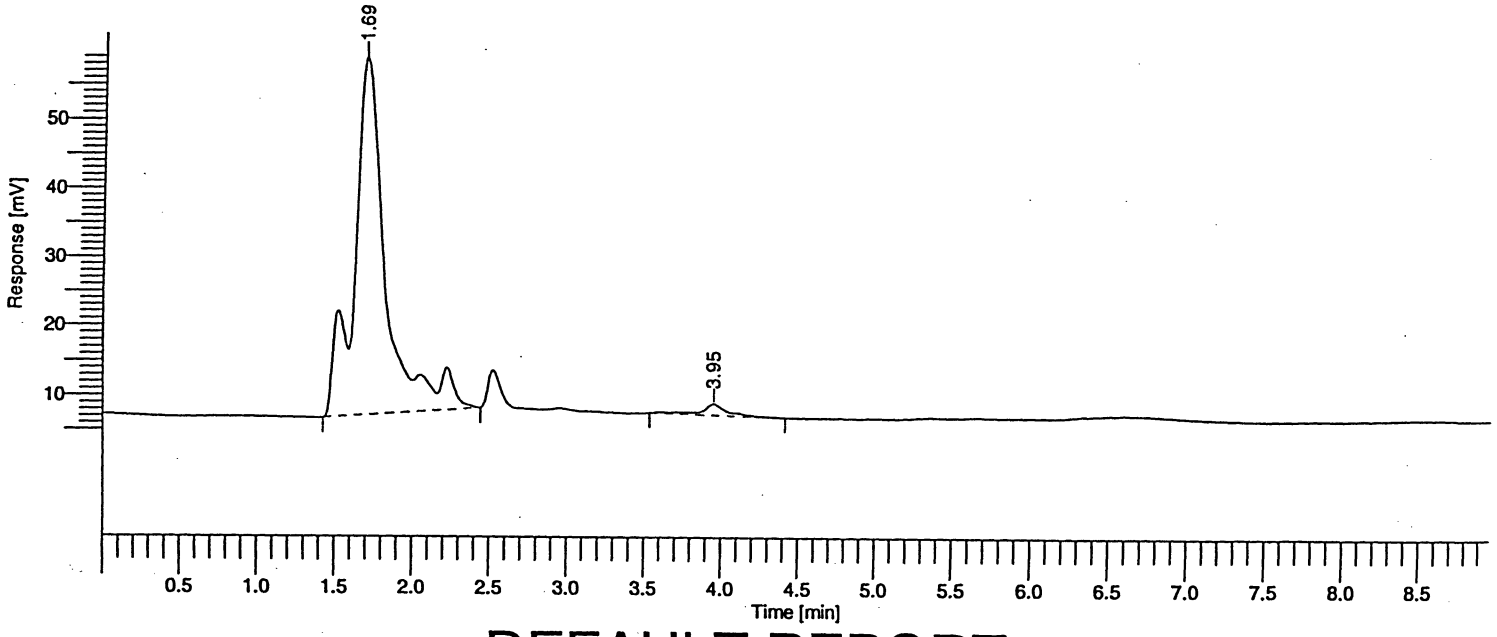
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 3:29:27 PM
Operator	: manager	Sample Name	: -VEP22 EPS
Sample Number	: 249	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/23
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 4:29:32 PM	Cycle	: 252

Raw Data File : \\Poweredge\E drive\TC\dennis\data241.raw
Result File : \\Poweredge\E drive\TC\dennis\data241.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data241.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.693	736701.00	51793.90	97.40	97.40	BB	14.2237
2	3.950	19654.60	1587.46	2.60	2.60	BB	12.3811
		756355.60	53381.37	100.00	100.00		

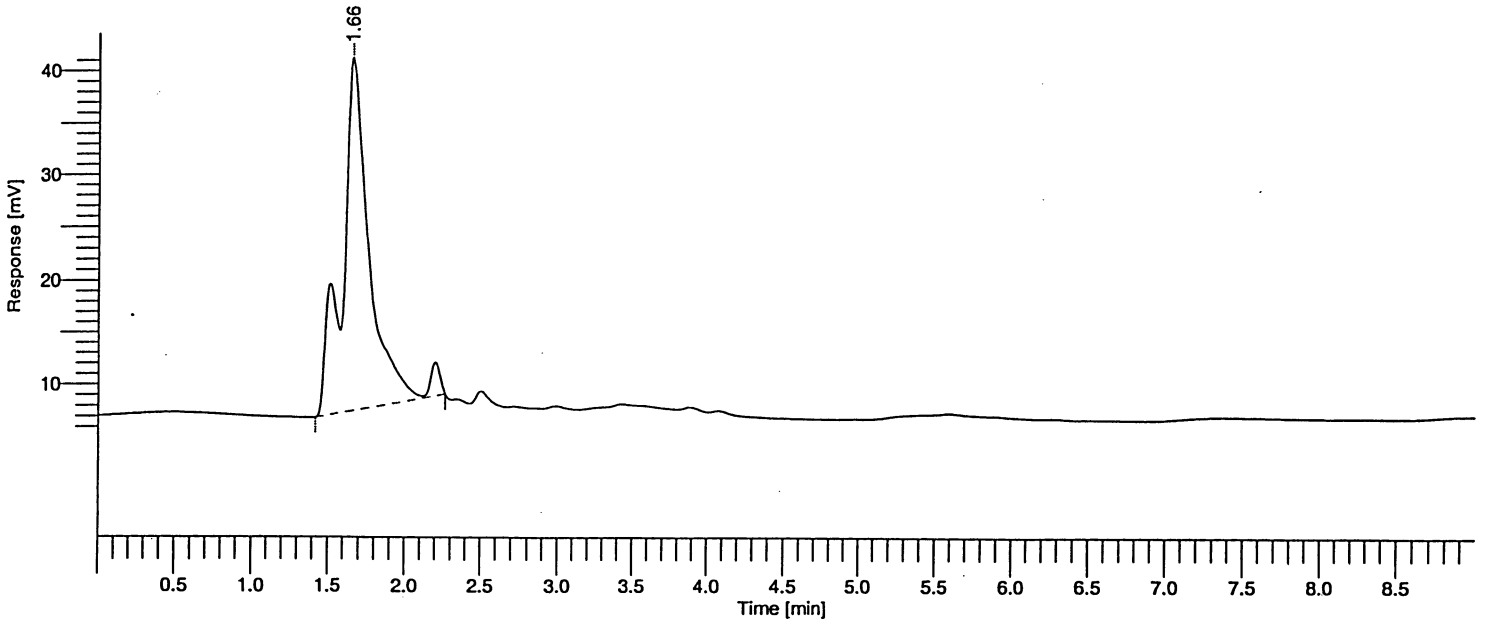
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 3:39:30 PM
Operator	: manager	Sample Name	: -VEP23 EPS
Sample Number	: 250	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/24
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 4:39:33 PM	Cycle	: 253

Raw Data File : \\Poweredge\E drive\TC\dennis\data242.raw
Result File : \\Poweredge\E drive\TC\dennis\data242.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data242.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.664	419782.80	33809.20	100.00	100.00	BB	12.4162
		419782.80	33809.20	100.00	100.00		

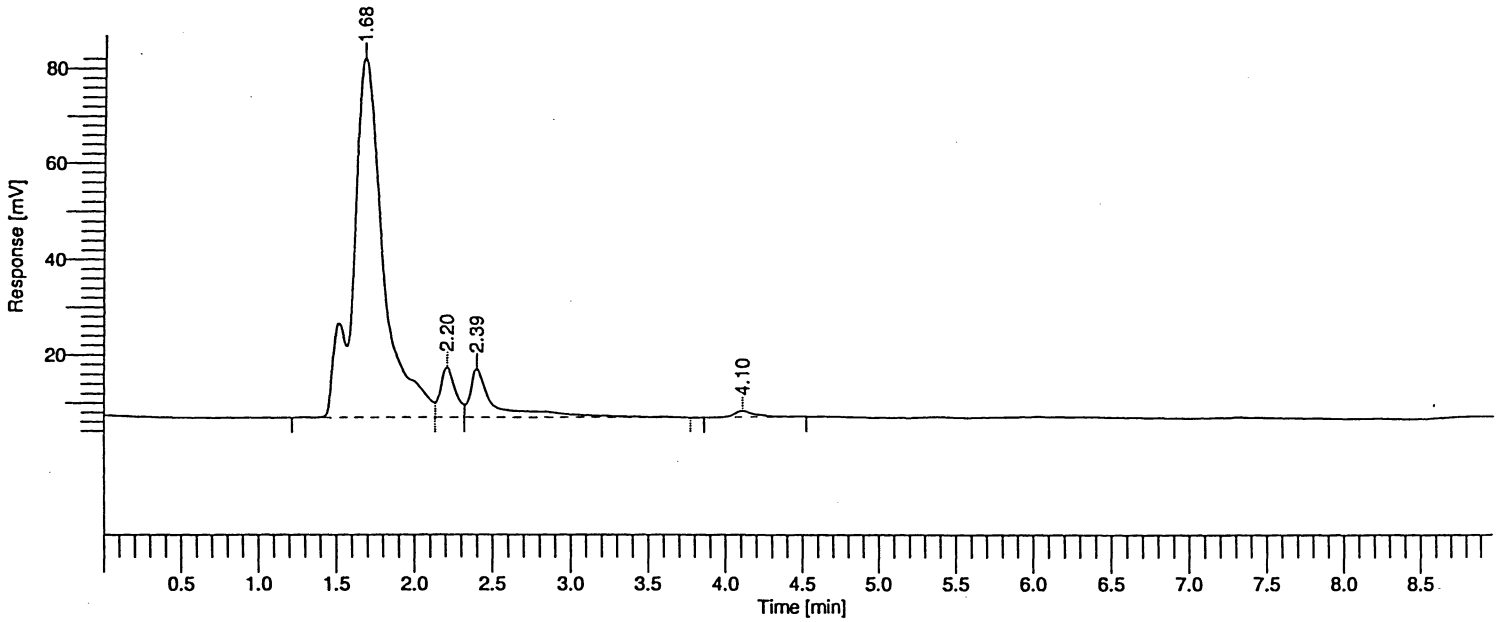
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 3:49:25 PM
Operator	: manager	Sample Name	: -VEP24 EPS
Sample Number	: 251	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/25
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 4:49:33 PM	Cycle	: 254

Raw Data File : \\Poweredge\E drive\TC\dennis\data243.raw
Result File : \\Poweredge\E drive\TC\dennis\data243.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data243.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.677	1022610.50	75335.74	83.63	83.63	BV	13.5740
2	2.202	70610.74	10467.53	5.77	5.77	VV	6.7457
3	2.393	117078.76	10183.50	9.58	9.58	VB	11.4969
4	4.104	12421.80	1297.21	1.02	1.02	BB	9.5758
		1222721.80	97283.98	100.00	100.00		

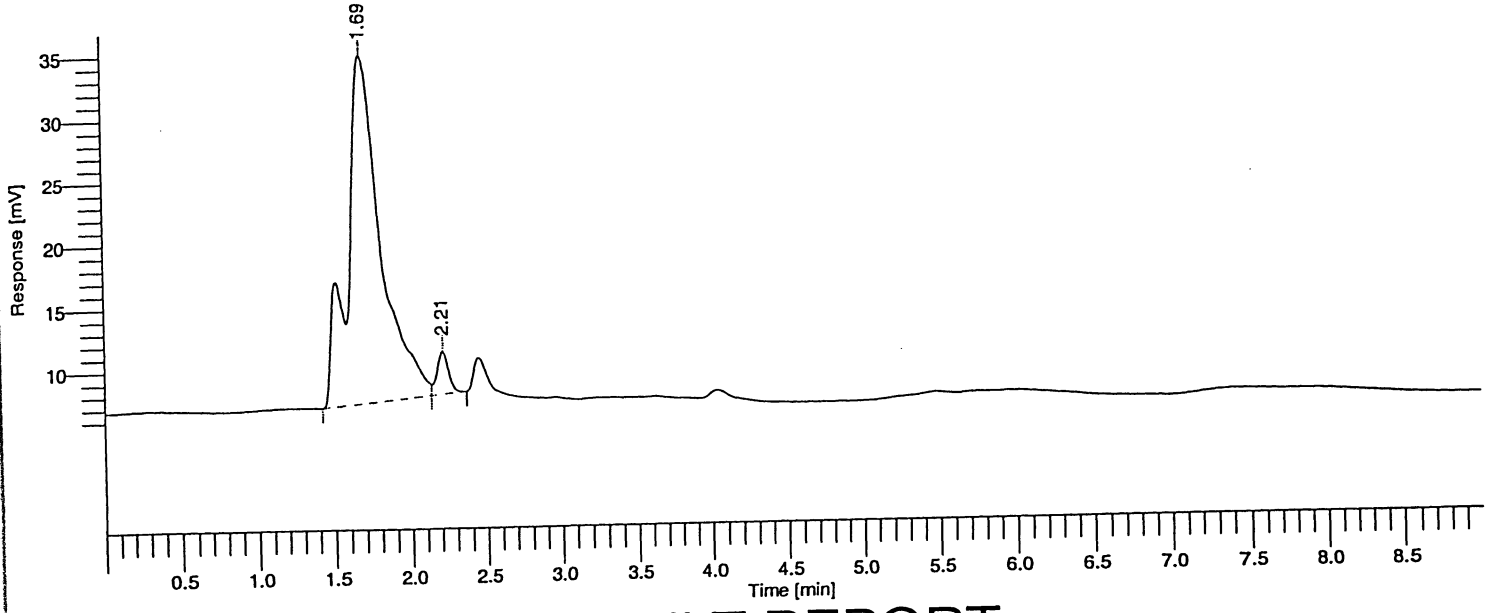
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 3:59:28 PM
Operator	: manager	Sample Name	: -VE P25 EPS
Sample Number	: 252	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/26
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 4:59:33 PM	Cycle	: 255

Raw Data File : \\Poweredge\E drive\TC\dennis\data244.raw
Result File : \\Poweredge\E drive\TC\dennis\data244.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data244.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.691	416991.97	27702.42	95.88	95.88	BV	15.0525
2	2.210	17908.43	3347.06	4.12	4.12	VB	5.3505
		434900.40	31049.49	100.00	100.00		

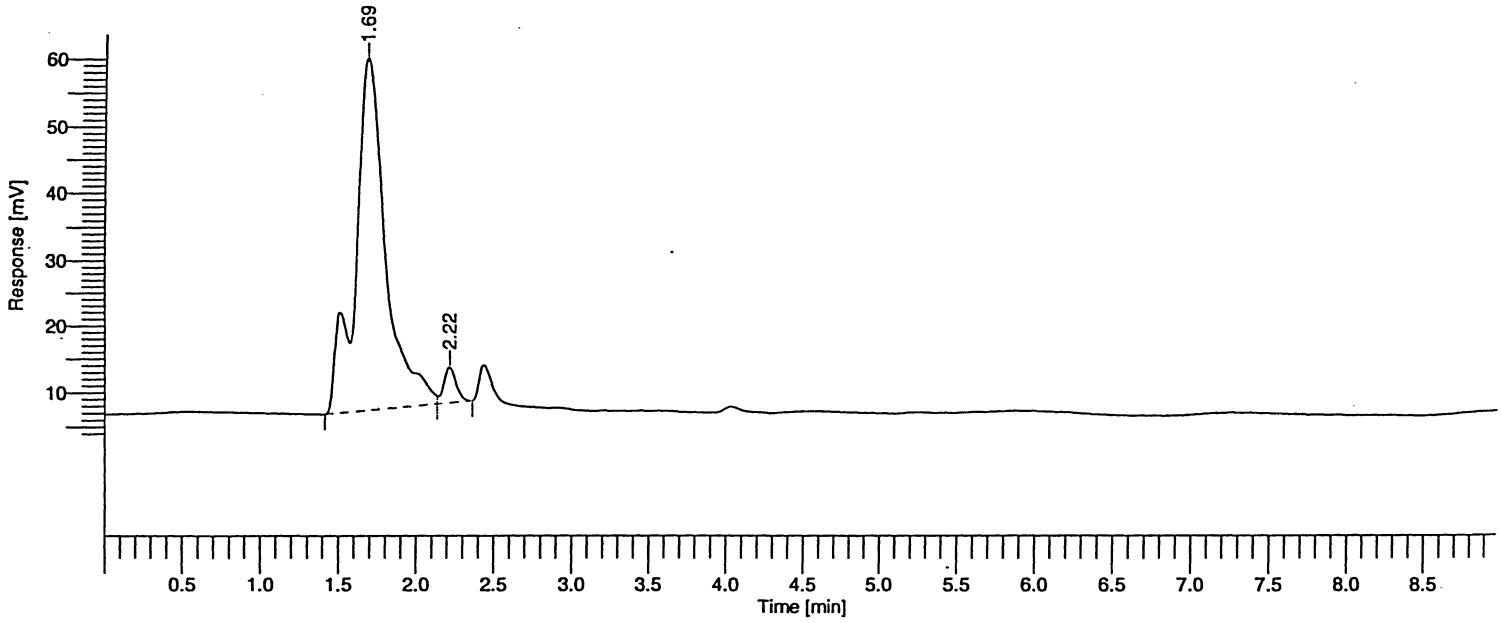
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 4:09:25 PM
Operator	: manager	Sample Name	: -VE P26 EPS
Sample Number	: 253	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/27
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 5:09:33 PM	Cycle	: 256

Raw Data File : \\Poweredge\E drive\TC\dennis\data245.raw
Result File : \\Poweredge\E drive\TC\dennis\data245.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data245.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.687	712343.03	52819.49	96.03	96.03	BV	13.4864
2	2.217	29433.77	5270.79	3.97	3.97	VB	5.5843
		741776.80	58090.28	100.00	100.00		

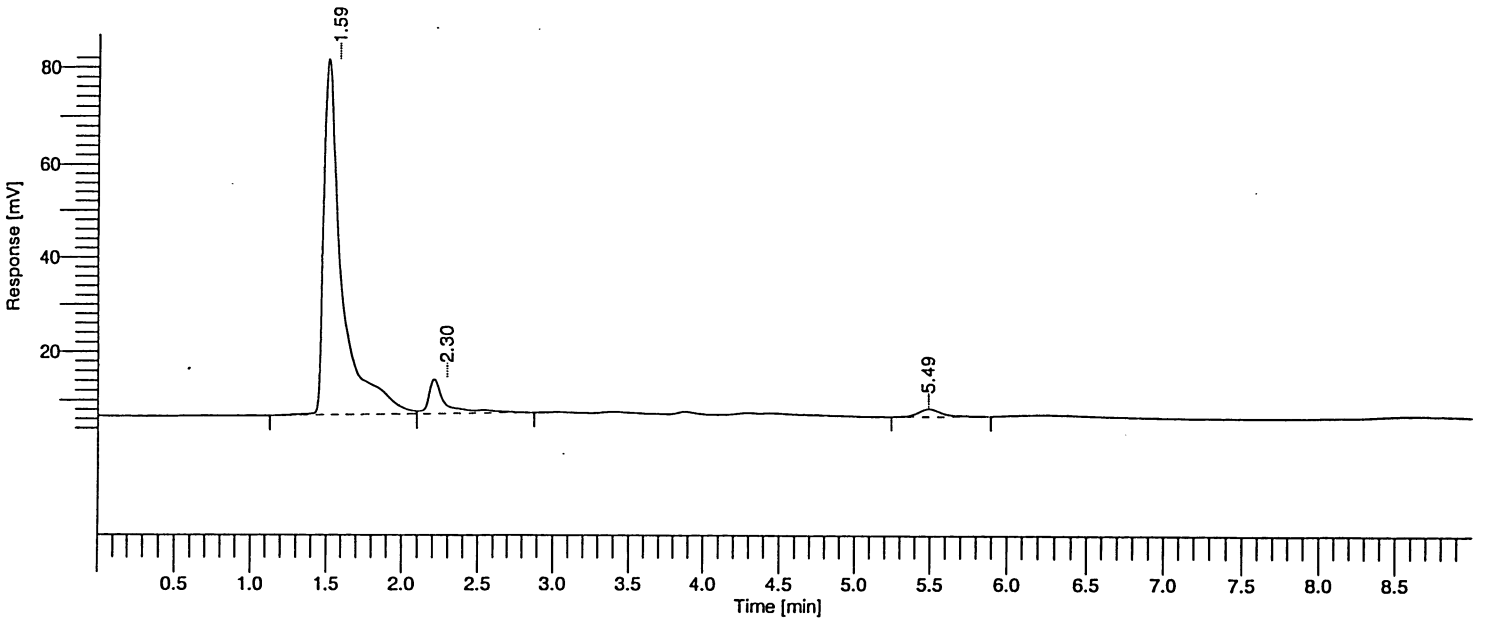
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 4:19:29 PM
Operator	: manager	Sample Name	: -VEP11 CELL
Sample Number	: 254	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/28
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 5:19:33 PM	Cycle	: 257

Raw Data File : \\Poweredge\E drive\TC\dennis\data246.raw
Result File : \\Poweredge\E drive\TC\dennis\data246.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data246.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.590	643253.48	32519.03	89.21	89.21	BV	19.7808
2	2.295	59619.72	1727.96	8.27	8.27	VB	34.5030
3	5.487	18162.00	1671.69	2.52	2.52	BB	10.8645
		721035.20	35918.67	100.00	100.00		

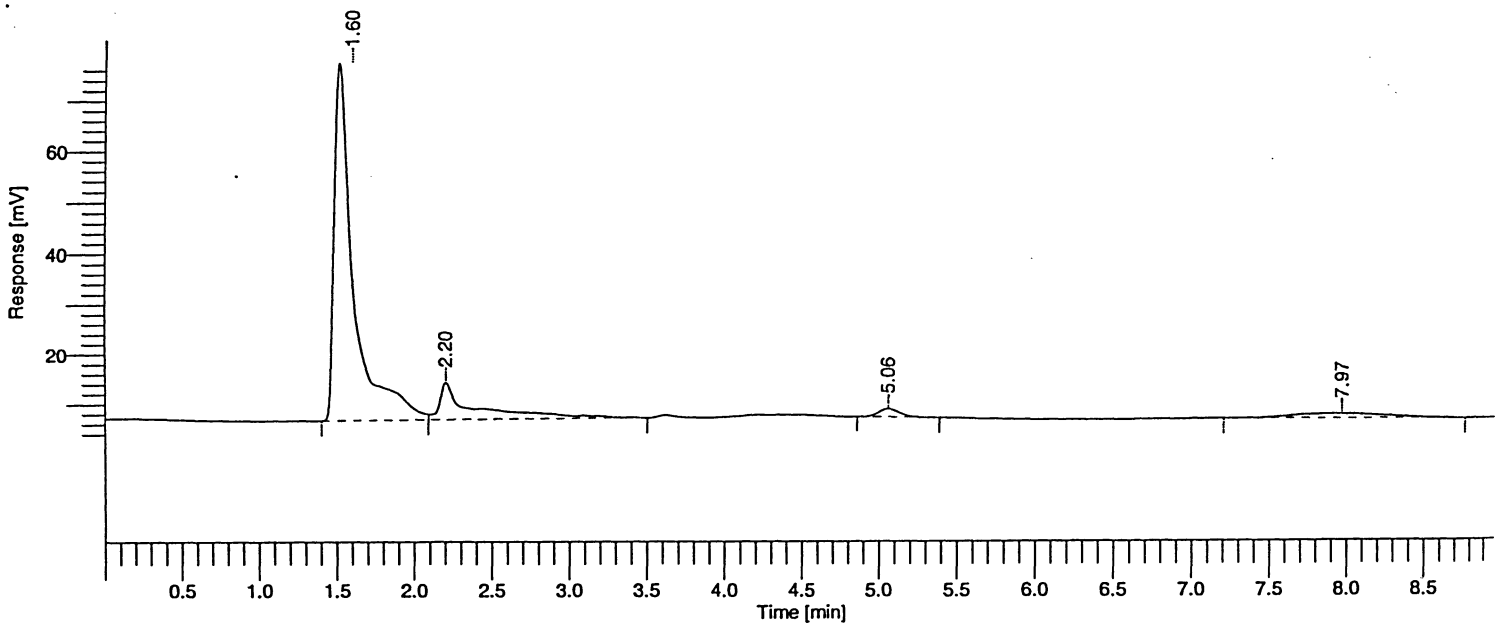
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 4:29:25 PM
Operator	: manager	Sample Name	: -VE P12 CELL
Sample Number	: 255	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/29
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 5:29:34 PM	Cycle	: 258

Raw Data File : \\Poweredge\E drive\TC\dennis\data247.raw
Result File : \\Poweredge\E drive\TC\dennis\data247.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data247.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.598	619389.22	26941.80	78.44	78.44	BV	22.9899
2	2.200	116490.78	7396.02	14.75	14.75	VB	15.7505
3	5.058	15619.20	1631.70	1.98	1.98	BB	9.5723
4	7.974	38147.20	878.19	4.83	4.83	BB	43.4385
		789646.40	36847.70	100.00	100.00		

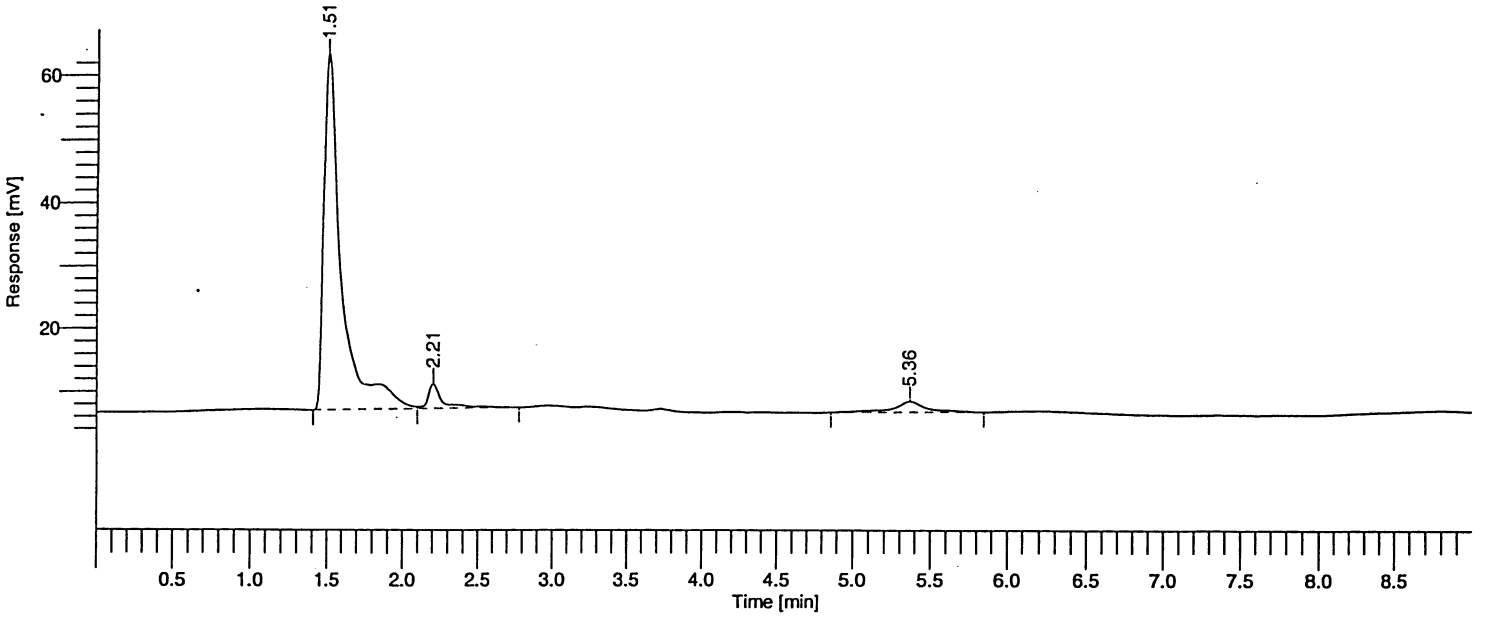
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 4:39:29 PM
Operator	: manager	Sample Name	: -VE P13 CELL
Sample Number	: 256	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/30
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 5:39:38 PM	Cycle	: 259

Raw Data File : \\Poweredge\E drive\TC\dennis\data248.raw
Result File : \\Poweredge\E drive\TC\dennis\data248.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data248.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.510	475941.77	56734.35	89.68	89.68	BV	8.3890
2	2.208	27553.83	4026.55	5.19	5.19	VB	6.8430
3	5.364	27199.20	1753.00	5.13	5.13	BB	15.5158
		530694.80	62513.90	100.00	100.00		

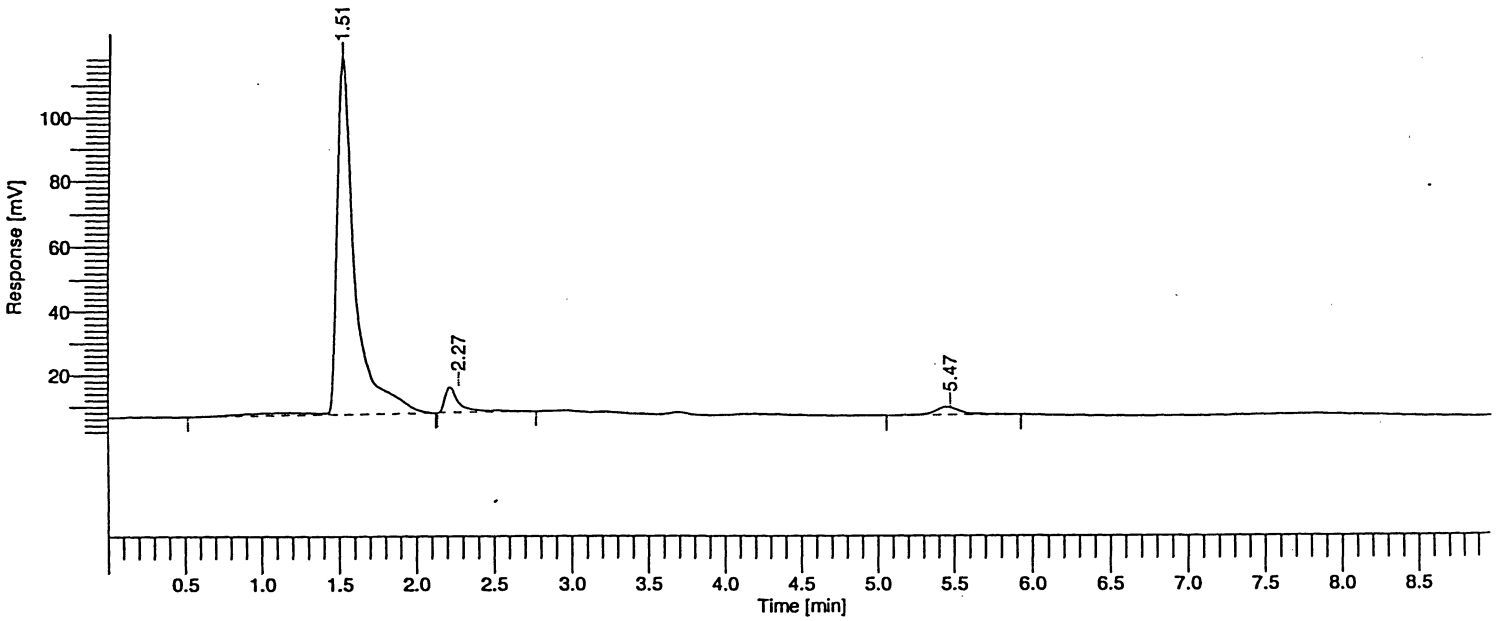
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 4:49:32 PM
Operator	: manager	Sample Name	: -VE P14 CELL
Sample Number	: 257	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/31
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 5:49:40 PM	Cycle	: 260

Raw Data File : \\Poweredge\E drive\TC\dennis\data249.raw
Result File : \\Poweredge\E drive\TC\dennis\data249.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data249.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.515	914027.40	111384.87	91.63	91.63	BB	8.2060
2	2.268	54013.60	3143.77	5.41	5.41	BB	17.1812
3	5.471	29443.80	2324.35	2.95	2.95	BB	12.6675
		997484.80	116852.99	100.00	100.00		

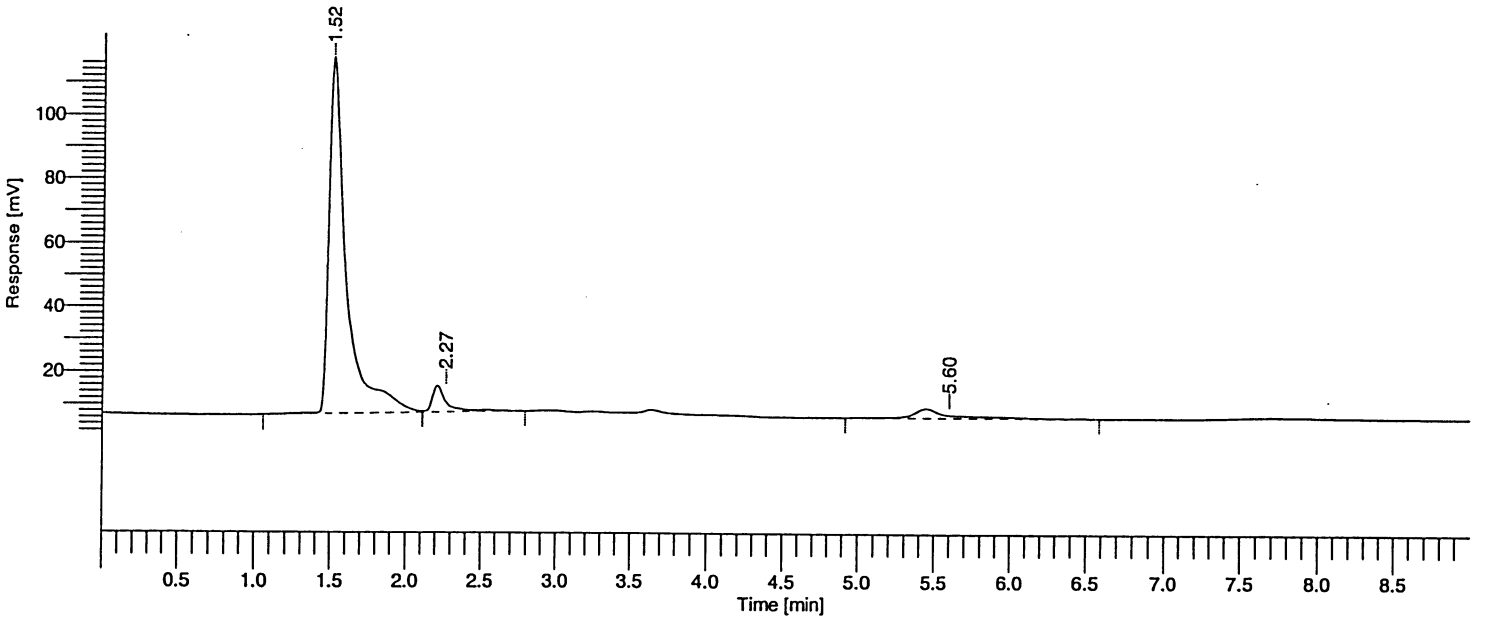
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 4:59:35 PM
Operator	: manager	Sample Name	: -VE P15 CELL
Sample Number	: 258	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/32
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 5:59:40 PM	Cycle	: 261

Raw Data File : \\Poweredge\E drive\TC\dennis\data250.raw
Result File : \\Poweredge\E drive\TC\dennis\data250.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data250.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.519	898839.19	110974.04	88.91	88.91	BV	8.0995
2	2.271	56502.61	2959.19	5.59	5.59	VB	19.0939
3	5.602	55616.40	875.37	5.50	5.50	BB	63.5349
		1010958.20	114808.60	100.00	100.00		

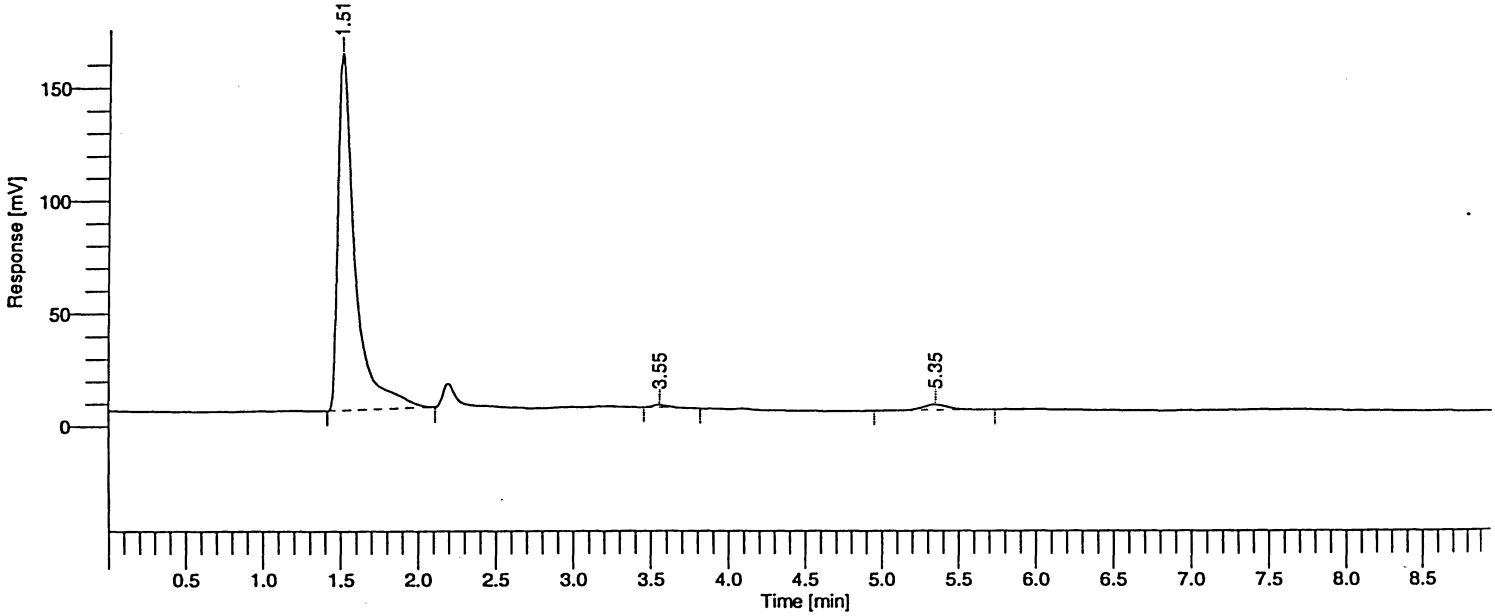
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 5:09:31 PM
Operator	: manager	Sample Name	: -VE P16 CELL
Sample Number	: 259	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/33
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 6:09:40 PM	Cycle	: 262

Raw Data File : \\Poweredge\E drive\TC\dennis\data251.raw
Result File : \\Poweredge\E drive\TC\dennis\data251.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data251.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.513	1217982.20	158893.36	97.10	97.10	BB	7.6654
2	3.553	8195.00	1185.06	0.65	0.65	BB	6.9152
3	5.346	28127.00	2445.17	2.24	2.24	BB	11.5031
		1254304.20	162523.60	100.00	100.00		

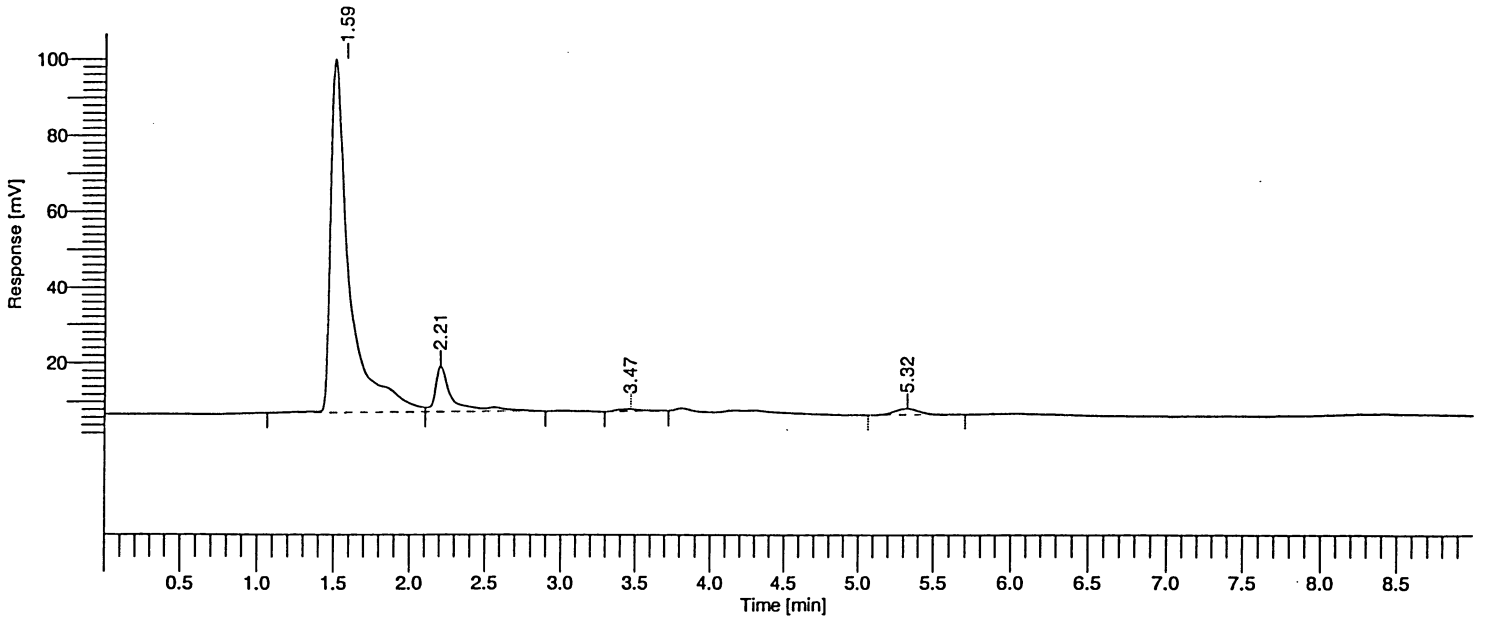
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 5:19:34 PM
Operator	: manager	Sample Name	: -VE P21 CELL
Sample Number	: 260	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/34
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 6:19:41 PM	Cycle	: 263

Raw Data File : \\Poweredge\E drive\TC\dennis\data252.raw
Result File : \\Poweredge\E drive\TC\dennis\data252.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data252.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

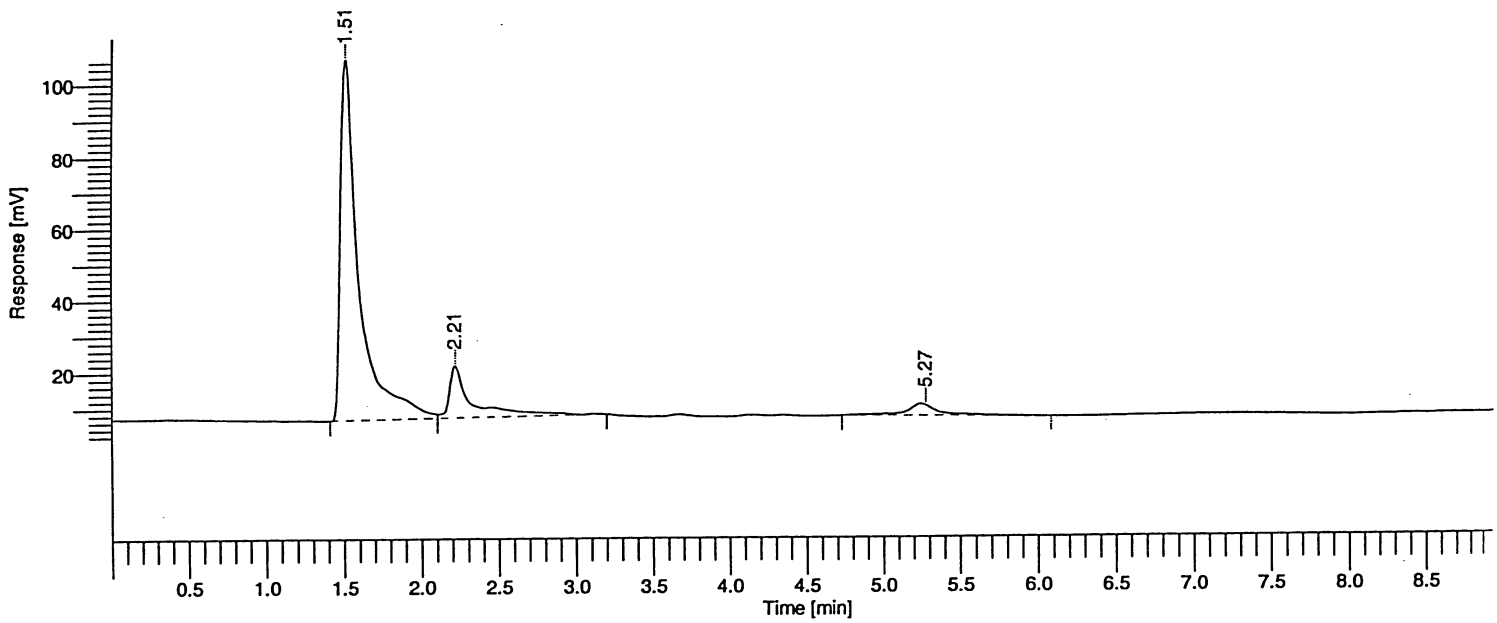
Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.589	796313.70	39541.12	87.14	87.14	BV	20.1389
2	2.208	92601.90	11823.68	10.13	10.13	VB	7.8319
3	3.469	6586.00	573.28	0.72	0.72	BB	11.4883
4	5.325	18301.20	1619.12	2.00	2.00	BB	11.3032
		913802.80	53557.20	100.00	100.00		

Missing Component Report
Component Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 5:29:37 PM
Operator	: manager	Sample Name	: -VE P22 CELL
Sample Number	: 261	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/35
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 6:29:41 PM	Cycle	: 264

Raw Data File : \\Poweredge\E drive\TC\dennis\data253.raw
Result File : \\Poweredge\E drive\TC\dennis\data253.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data253.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.510	842034.06	100301.53	80.35	80.35	BV	8.3950
2	2.211	154294.34	14641.30	14.72	14.72	VB	10.5383
3	5.271	51608.40	2991.46	4.92	4.92	BB	17.2519
		1047936.80	117934.28	100.00	100.00		

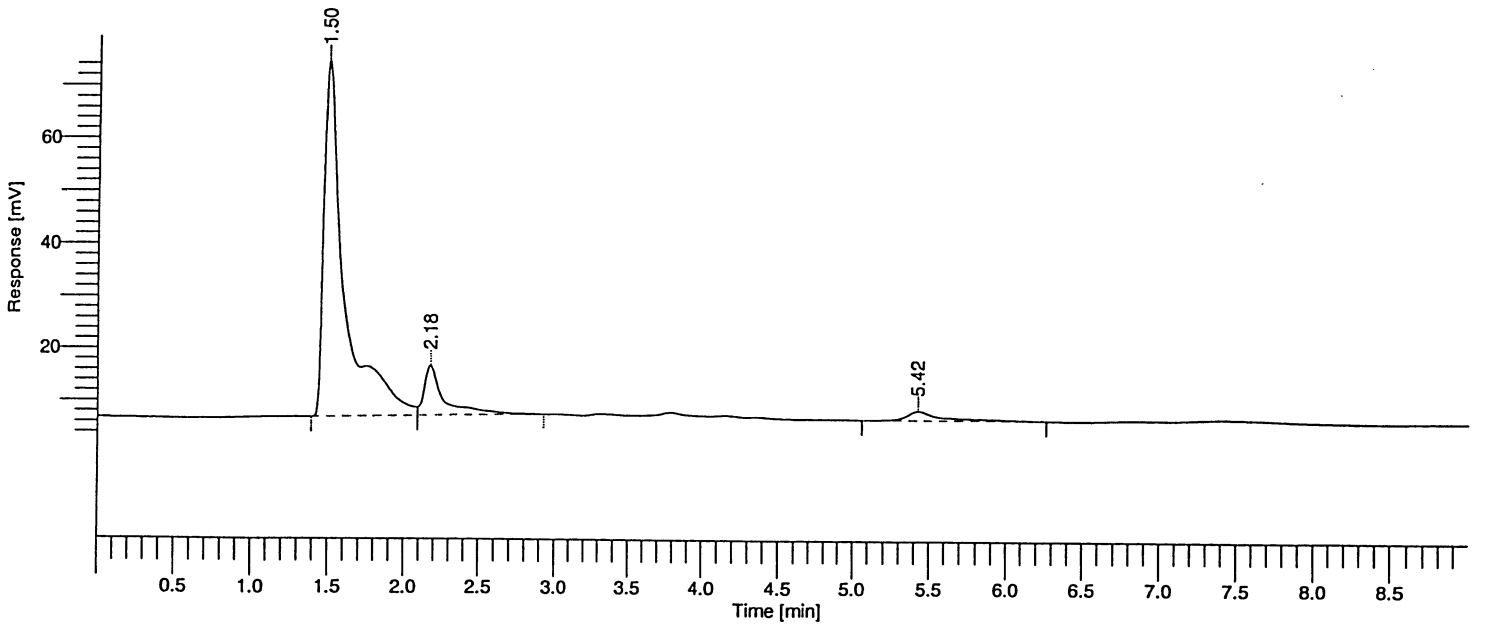
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 5:39:33 PM
Operator	: manager	Sample Name	: -VE P23 CELL
Sample Number	: 262	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/36
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 6:39:41 PM	Cycle	: 265

Raw Data File : \\Poweredge\E drive\TC\dennis\data254.raw
Result File : \\Poweredge\E drive\TC\dennis\data254.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data254.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.503	634780.89	67901.85	84.36	84.36	BV	9.3485
2	2.178	87709.71	9621.41	11.66	11.66	VB	9.1161
3	5.424	29974.00	1803.42	3.98	3.98	BB	16.6206
		752464.60	79326.68	100.00	100.00		

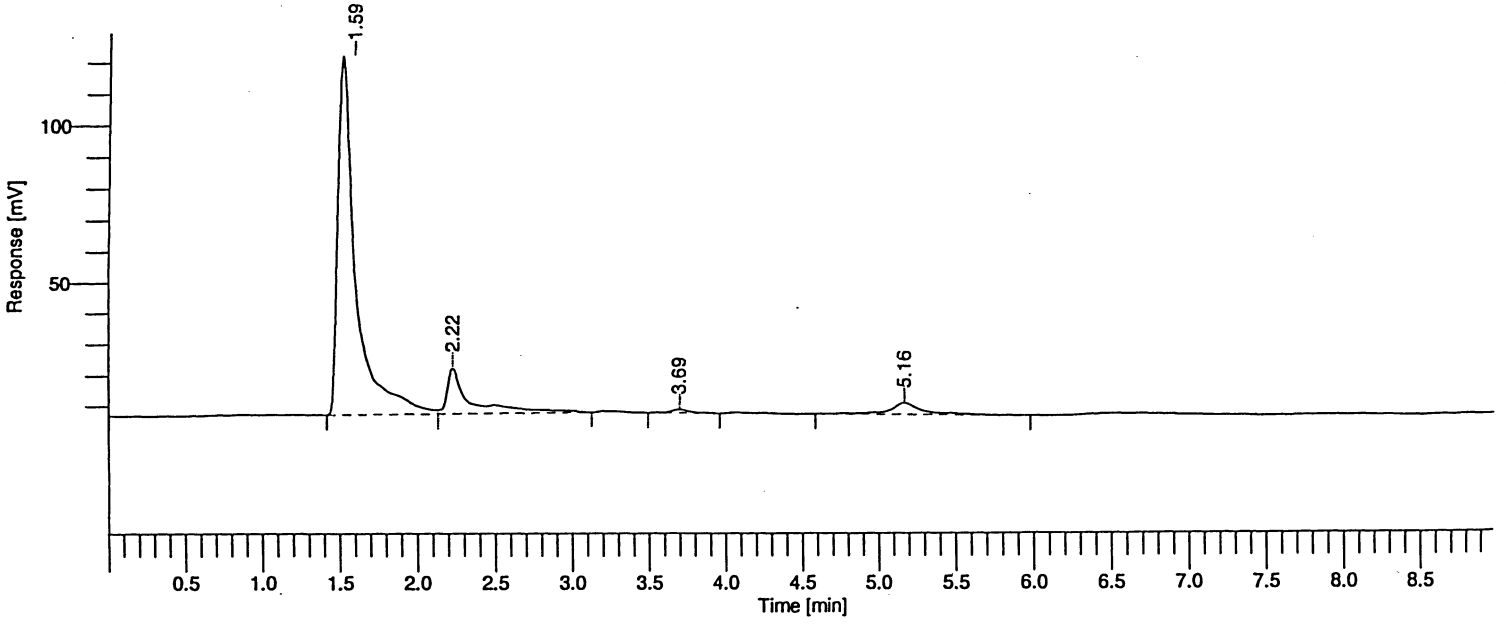
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 5:49:36 PM
Operator	: manager	Sample Name	: -VE P24 CELL
Sample Number	: 263	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/37
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 6:49:41 PM	Cycle	: 266

Raw Data File : \\Poweredge\E drive\TC\dennis\data255.raw
Result File : \\Poweredge\E drive\TC\dennis\data255.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data255.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.588	937754.25	47239.73	80.67	80.67	BV	19.8510
2	2.219	157012.95	14915.26	13.51	13.51	VB	10.5270
3	3.691	9401.20	1093.36	0.81	0.81	BB	8.5984
4	5.164	58265.20	3797.85	5.01	5.01	BB	15.3416
		1162433.60	67046.19	100.00	100.00		

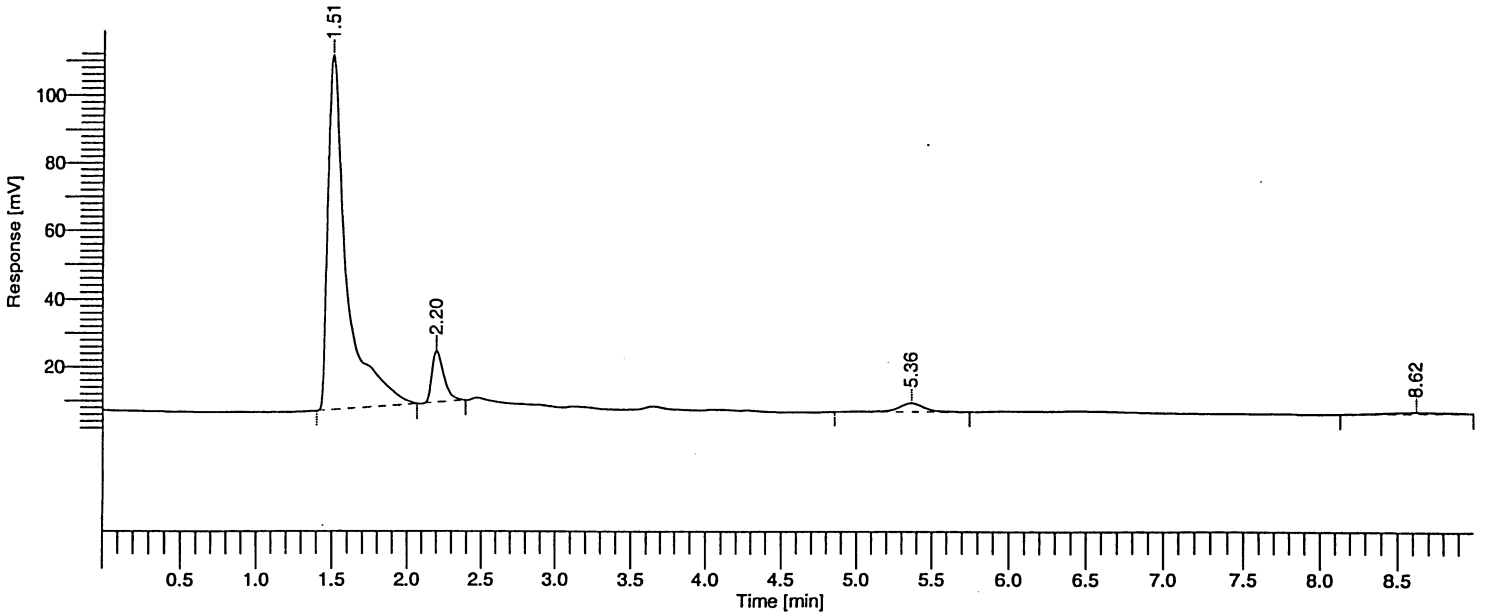
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 5:59:33 PM
Operator	: manager	Sample Name	: -V3 P25 CELL
Sample Number	: 264	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/38
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 6:59:41 PM	Cycle	: 267

Raw Data File : \\Poweredge\E drive\TC\dennis\data256.raw
Result File : \\Poweredge\E drive\TC\dennis\data256.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data256.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.507	879775.50	104680.09	87.16	87.16	BV	8.4044
2	2.200	83549.75	15415.05	8.28	8.28	VB	5.4200
3	5.361	32591.00	2652.90	3.23	3.23	BB	12.2850
4	8.621	13449.60	501.97	1.33	1.33	BB	26.7937
		1009365.86	123250.01	100.00	100.00		

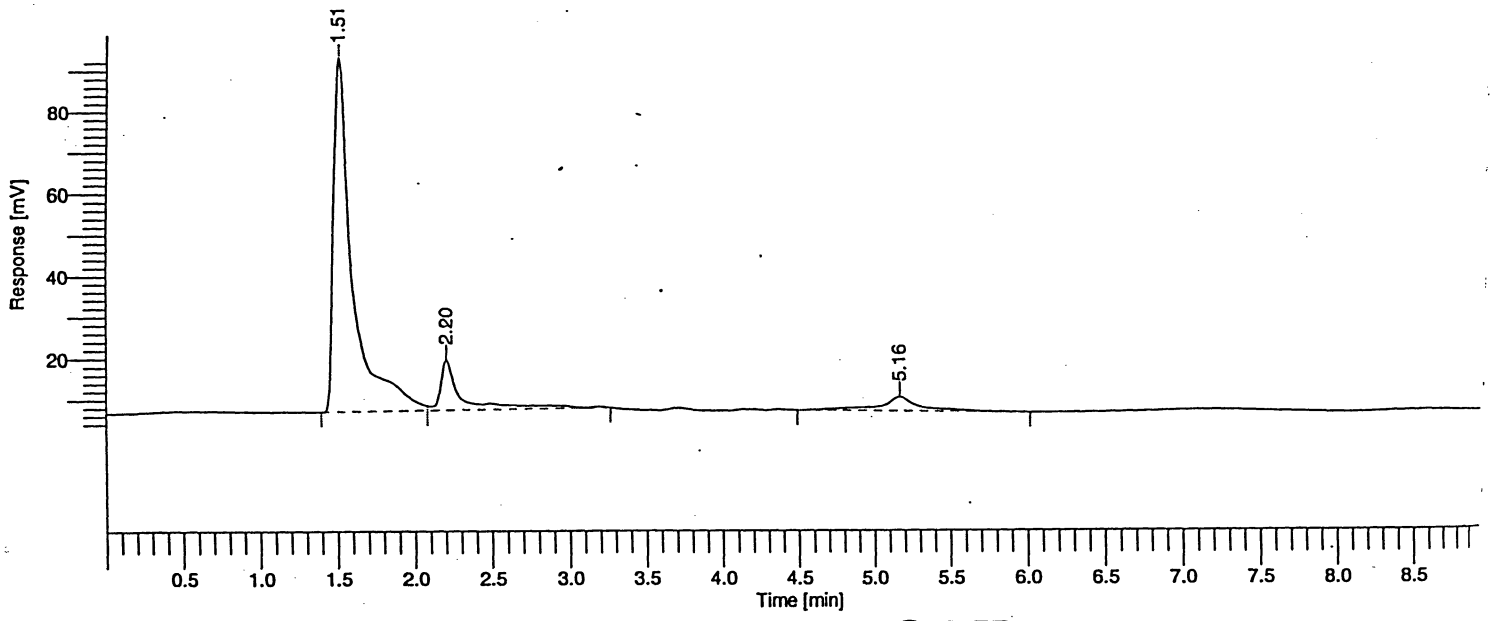
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 6:09:36 PM
Operator	: manager	Sample Name	: -VEP26 CELL
Sample Number	: 268	Study	: ANTIBIOTICS
AutoSampler	: SER200	Rack/Vial	: 1/39
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 7:09:42 PM	Cycle	: 268

Raw Data File : \\Poweredge\E drive\TC\dennis\data257.raw
Result File : \\Poweredge\E drive\TC\dennis\data257.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data257.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.507	742066.90	86128.54	80.06	80.06	BV	8.6158
2	2.199	120124.70	12088.66	12.96	12.96	VB	9.9370
3	5.160	64656.00	3426.93	6.98	6.98	BB	18.8671
		926847.60	101644.12	100.00	100.00		

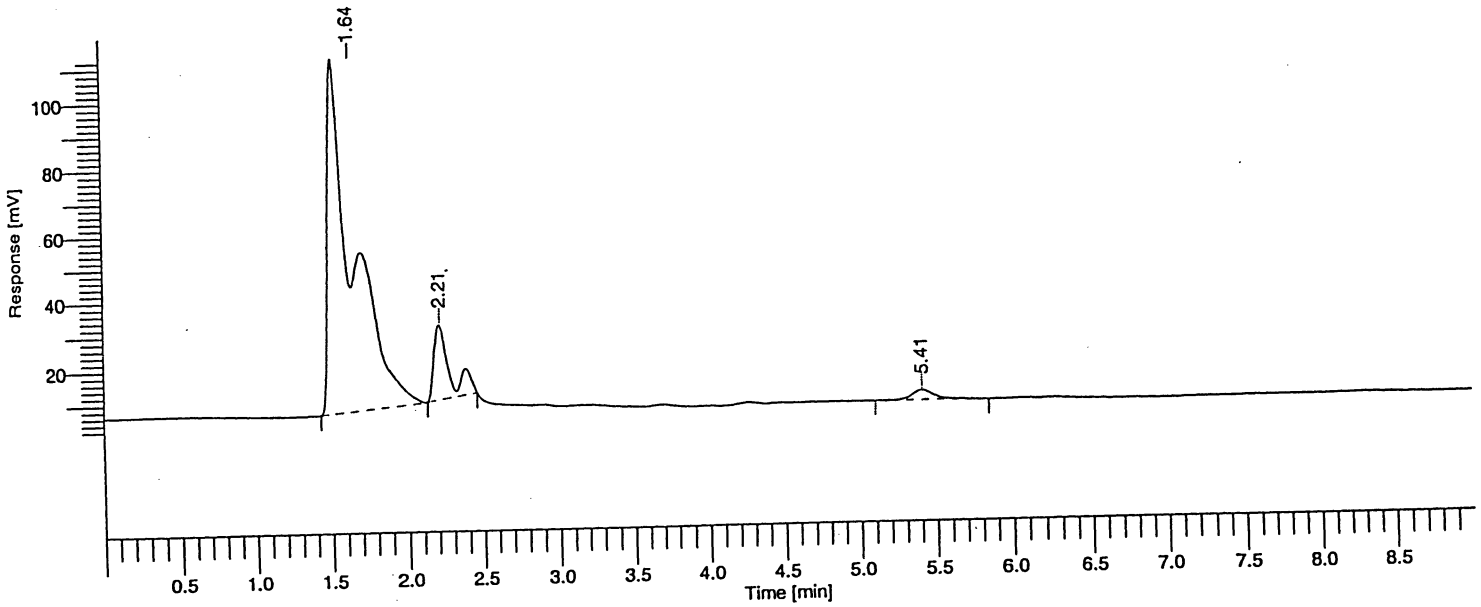
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 11:38:19 AM
Operator	: manager	Sample Name	: E11,CELL
Sample Number	: 226	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/2
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 12:38:19 PM	Cycle	: 231

Raw Data File : \\Poweredge\E drive\TC\dennis\data220.raw
Result File : \\Poweredge\E drive\TC\dennis\data220.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data220.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.640	1228221.20	36619.55	86.46	86.46	BB	33.5400
2	2.213	159526.40	22015.08	11.23	11.23	BB	7.2462
3	5.407	32757.00	3069.72	2.31	2.31	BB	10.6710
		1420504.60	61704.35	100.00	100.00		

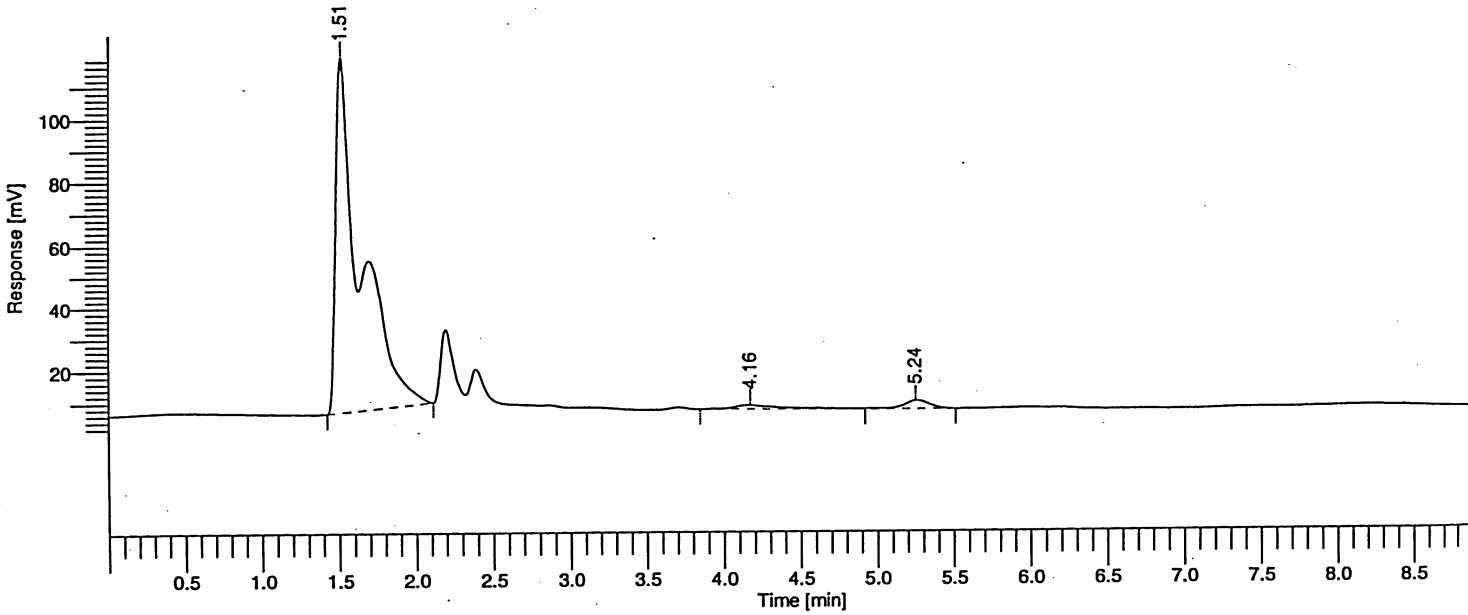
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 11:48:14 AM
Operator	: manager	Sample Name	: E12,CELL
Sample Number	: 227	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/3
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 12:48:19 PM	Cycle	: 232

Raw Data File : \\Poweredge\E drive\TC\dennis\data221.raw
Result File : \\Poweredge\E drive\TC\dennis\data221.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data221.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.513	1258802.00	112697.37	95.43	95.43	BB	11.1698
2	4.164	29667.32	1285.69	2.25	2.25	BV	23.0751
3	5.244	30587.88	2582.32	2.32	2.32	VB	11.8451
		1319057.20	116565.37	100.00	100.00		

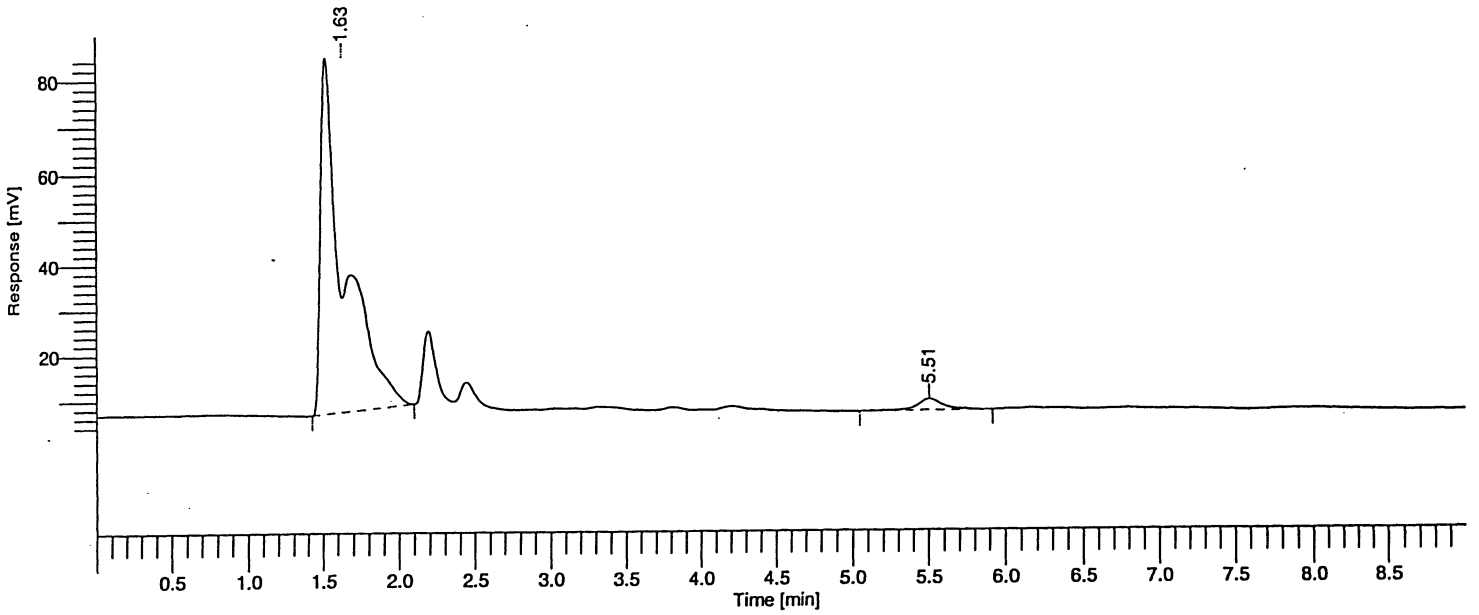
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 11:58:18 AM
Operator	: manager	Sample Name	: E13,CELL
Sample Number	: 228	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/4
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 12:58:20 PM	Cycle	: 233

Raw Data File : \\Poweredge\E drive\TC\dennis\data222.raw
Result File : \\Poweredge\E drive\TC\dennis\data222.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data222.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.632	860388.80	25213.60	96.63	96.63	BB	34.1240
2	5.506	29971.60	2457.72	3.37	3.37	BB	12.1949
		890360.40	27671.31	100.00	100.00		

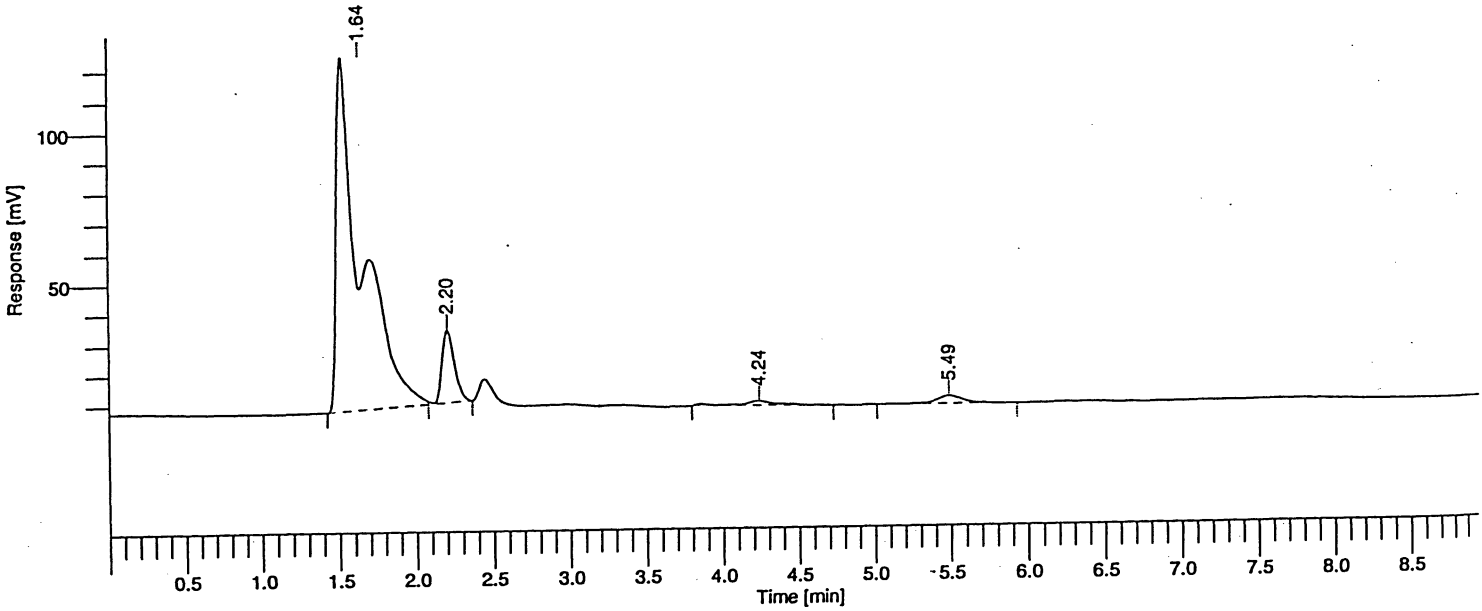
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 12:08:14 PM
Operator	: manager	Sample Name	: E14,CELL
Sample Number	: 229	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/5
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 1:08:20 PM	Cycle	: 234

Raw Data File : \\Poweredge\E drive\TC\dennis\data223.raw
Result File : \\Poweredge\E drive\TC\dennis\data223.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data223.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.635	1325562.30	40199.12	87.19	87.19	BV	32.9749
2	2.199	136808.90	24157.99	9.00	9.00	VB	5.6631
3	4.237	24185.00	1584.19	1.59	1.59	BB	15.2665
4	5.487	33812.00	2690.01	2.22	2.22	BB	12.5695
		1520368.20	68631.31	100.00	100.00		

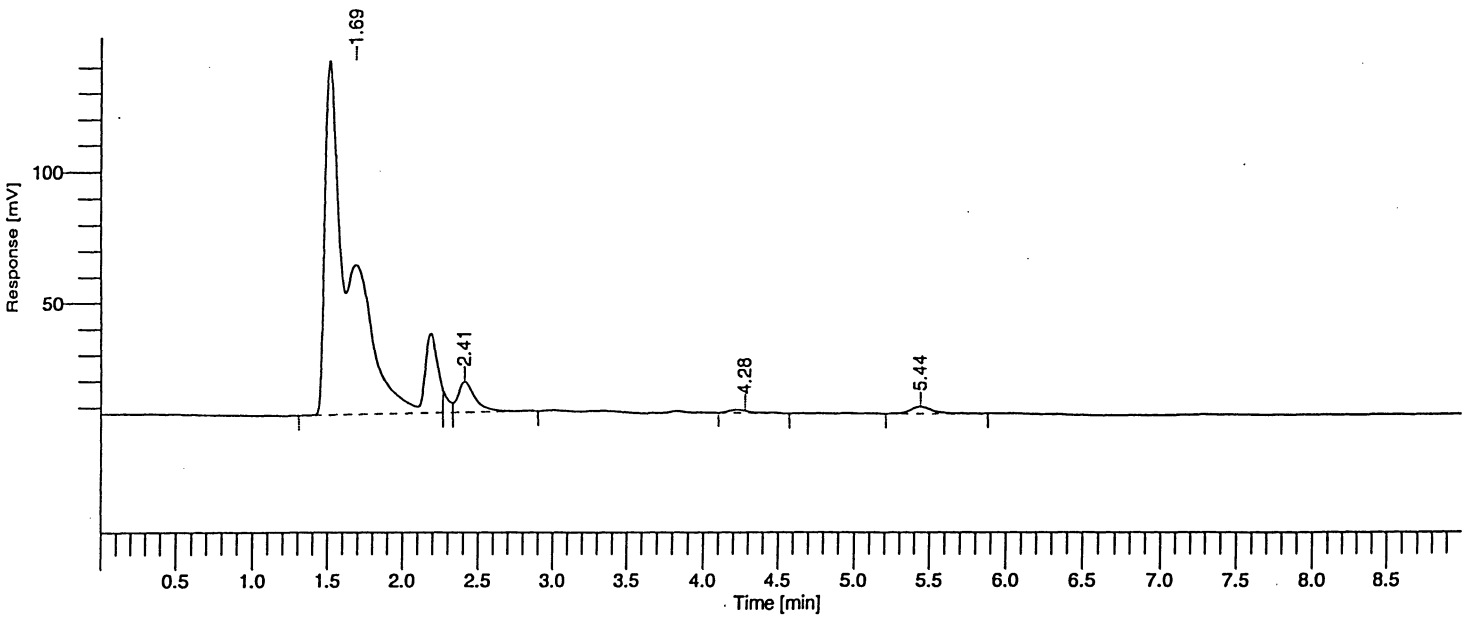
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 12:18:17 PM
Operator	: manager	Sample Name	: E15,CELL
Sample Number	: 230	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/6
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 1:18:20 PM	Cycle	: 235

Raw Data File : \\Poweredge\E drive\TC\dennis\data224.raw
Result File : \\Poweredge\E drive\TC\dennis\data224.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data224.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.689	1727241.98	57589.06	92.74	92.74	BV	29.9925
2	2.411	94495.09	11746.91	5.07	5.07	VB	8.0442
3	4.278	12896.00	846.53	0.69	0.69	BB	15.2340
4	5.440	27884.00	2720.69	1.50	1.50	BB	10.2489
		1862517.06	72903.19	100.00	100.00		

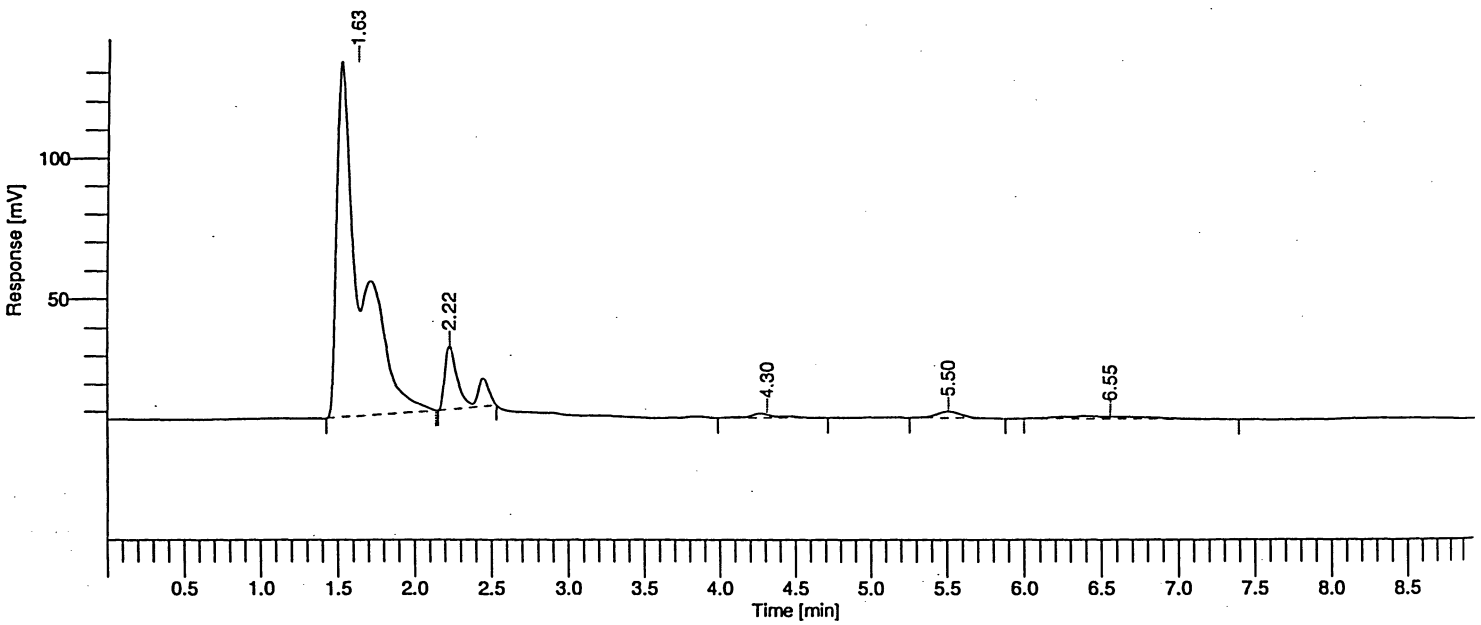
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 12:28:20 PM
Operator	: manager	Sample Name	: E16 CELL
Sample Number	: 231	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/7
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 1:28:20 PM	Cycle	: 236

Raw Data File : \\Poweredge\E drive\TC\dennis\data225.raw
Result File : \\Poweredge\E drive\TC\dennis\data225.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data225.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.628	1365799.60	38938.14	83.99	83.99	BB	35.0761
2	2.222	175787.60	22955.83	10.81	10.81	BB	7.6576
3	4.304	19230.40	1131.90	1.18	1.18	BB	16.9895
4	5.500	25982.00	2331.41	1.60	1.60	BB	11.1443
5	6.549	39312.40	806.18	2.42	2.42	BB	48.7636
		1626112.00	66163.46	100.00	100.00		

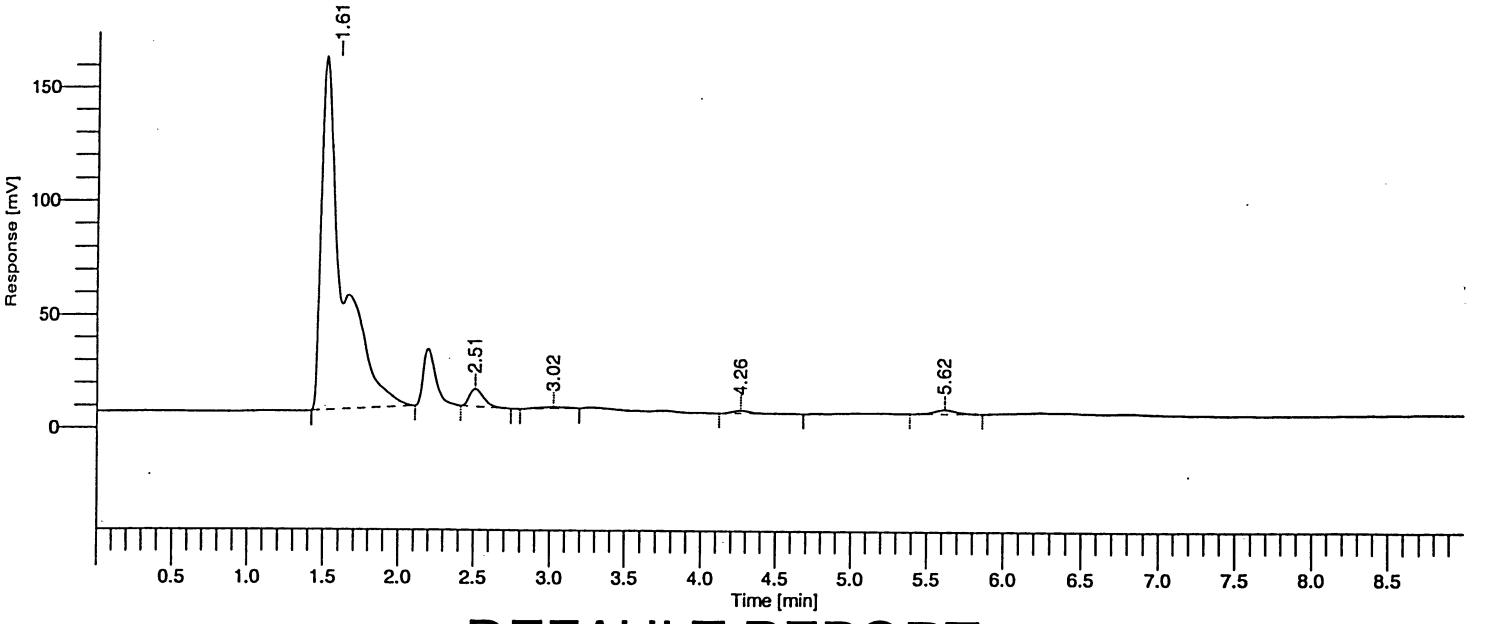
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 12:38:16 PM
Operator	: manager	Sample Name	: E21 CELL
Sample Number	: 232	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/8
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 1:38:20 PM	Cycle	: 237

Raw Data File : \\Poweredge\E drive\TC\dennis\data226.raw
Result File : \\Poweredge\E drive\TC\dennis\data226.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data226.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.606	1535749.20	51653.10	94.44	94.44	BB	29.7320
2	2.510	49844.40	7804.35	3.07	3.07	BB	6:3867
3	3.023	10223.20	747.38	0.63	0.63	BB	13.6787
4	4.264	11738.80	1316.64	0.72	0.72	BB	8.9158
5	5.617	18663.60	1839.05	1.15	1.15	BB	10.1485
		1626219.20	63360.51	100.00	100.00		

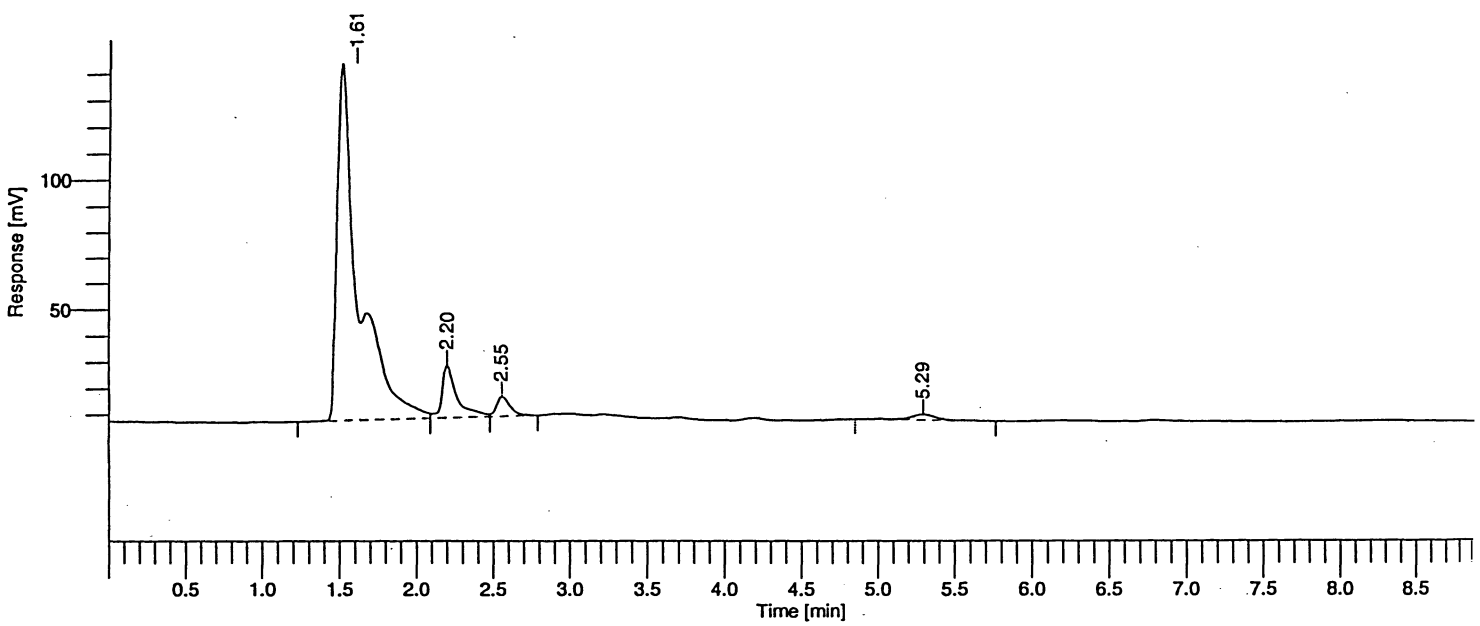
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 12:48:15 PM
Operator	: manager	Sample Name	: E22 CELL
Sample Number	: 233	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/9
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 1:48:20 PM	Cycle	: 238

Raw Data File : \\Poweredge\E drive\TC\dennis\data227.raw
Result File : \\Poweredge\E drive\TC\dennis\data227.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data227.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.608	1315481.87	43605.57	86.25	86.25	BV	30.1677
2	2.198	135447.45	20069.39	8.88	8.88	VV	6.7490
3	2.555	47388.08	7579.25	3.11	3.11	VB	6.2523
4	5.289	26951.60	2204.09	1.77	1.77	BB	12.2280
		1525269.00	73458.30	100.00	100.00		

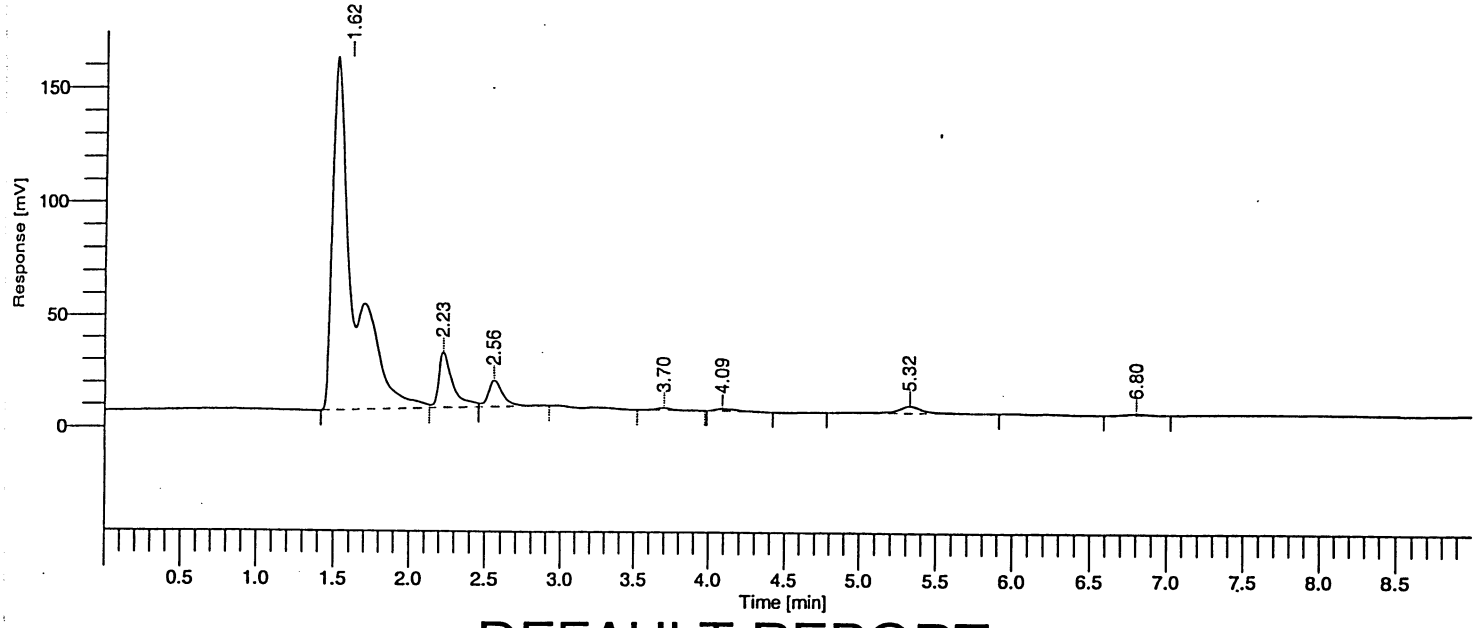
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 12:58:18 PM
Operator	: manager	Sample Name	: E23 CELL
Sample Number	: 234	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/10
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 1:58:21 PM	Cycle	: 239

Raw Data File : \\Poweredge\E drive\TC\dennis\data228.raw
Result File : \\Poweredge\E drive\TC\dennis\data228.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data228.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.619	1525085.75	44707.15	83.30	83.30	BV	34.1128
2	2.227	162062.94	24885.09	8.85	8.85	VV	6.5125
3	2.558	78621.71	11825.92	4.29	4.29	VB	6.6483
4	3.697	8487.20	1059.84	0.46	0.46	BB	8.0080
5	4.089	11402.80	1007.39	0.62	0.62	BB	11.3191
6	5.323	39256.80	3179.19	2.14	2.14	BB	12.3481
7	6.803	5877.60	599.45	0.32	0.32	BB	9.8050
		1830794.80	87264.03	100.00	100.00		

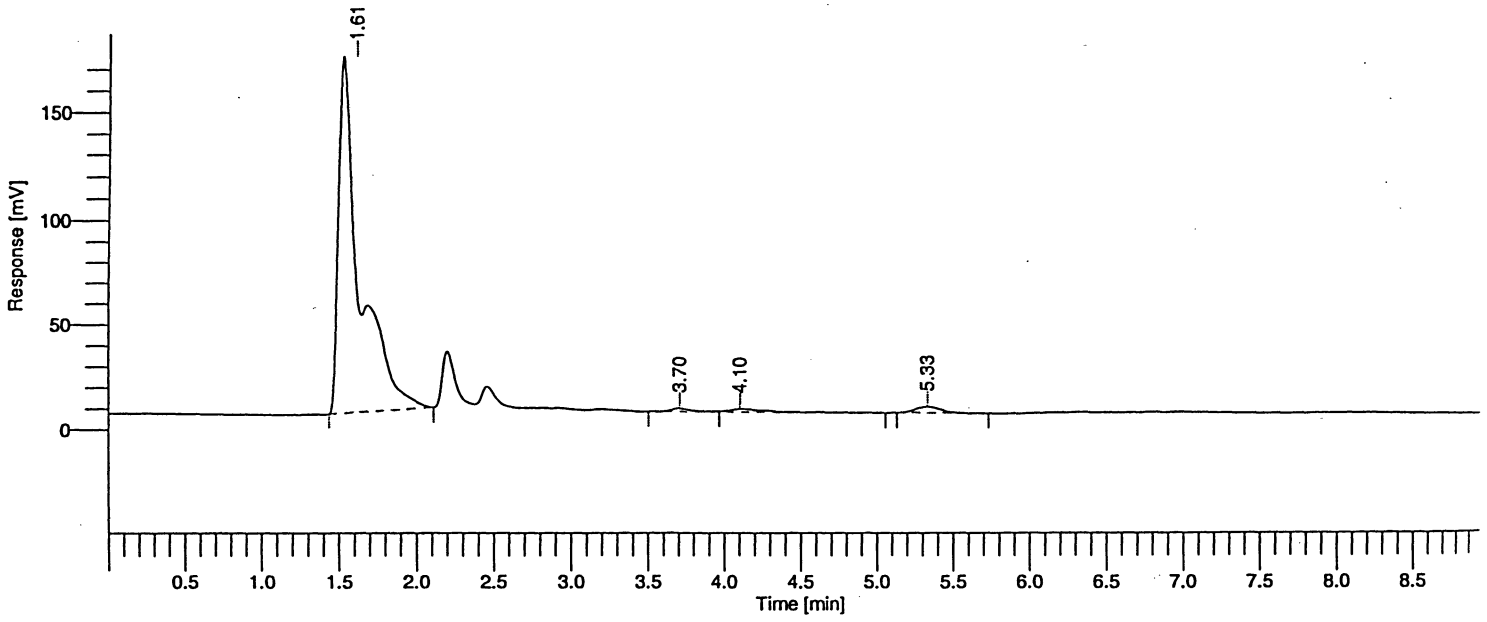
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 1:08:21 PM
Operator	: manager	Sample Name	: E24 CELL
Sample Number	: 235	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/11
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 2:08:23 PM	Cycle	: 240

Raw Data File : \\Poweredge\E drive\TC\dennis\data229.raw
Result File : \\Poweredge\E drive\TC\dennis\data229.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data229.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.615	1611714.40	56616.40	95.33	95.33	BB	28.4673
2	3.704	16645.41	1653.76	0.98	0.98	BV	10.0652
3	4.101	27746.19	1463.68	1.64	1.64	VB	18.9565
4	5.328	34495.20	2962.09	2.04	2.04	BB	11.6455
		1690601.20	62695.93	100.00	100.00		

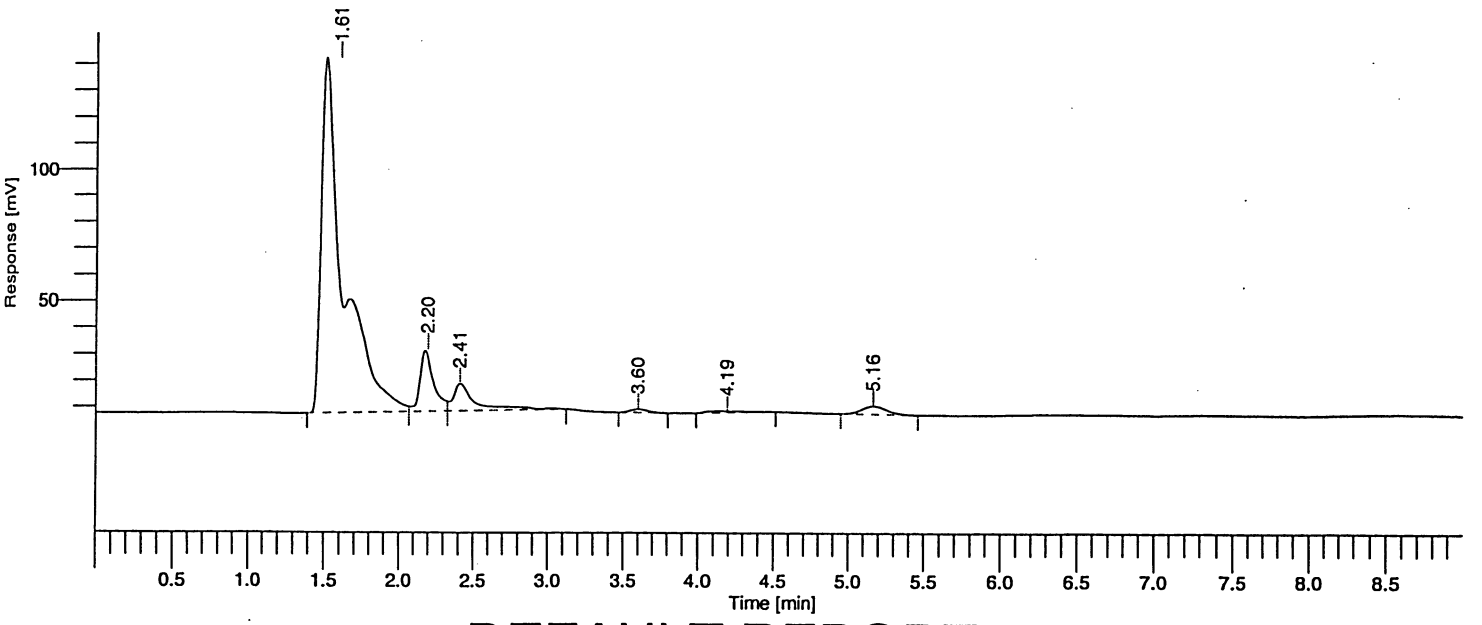
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 1:18:17 PM
Operator	: manager	Sample Name	: E25 CELL
Sample Number	: 236	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/12
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 2:18:23 PM	Cycle	: 241

Raw Data File : \\Poweredge\E drive\TC\dennis\data230.raw
Result File : \\Poweredge\E drive\TC\dennis\data230.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data230.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.610	1357723.90	43907.53	81.03	81.03	BV	30.9223
2	2.197	149901.43	20058.85	8.95	8.95	VV	7.4731
3	2.412	108587.87	10631.38	6.48	6.48	VB	10.2139
4	3.600	10917.40	1377.84	0.65	0.65	BB	7.9236
5	4.190	11660.80	551.75	0.70	0.70	BB	21.1342
6	5.163	36820.80	3220.22	2.20	2.20	BB	11.4342
		1675612.20	79747.57	100.00	100.00		

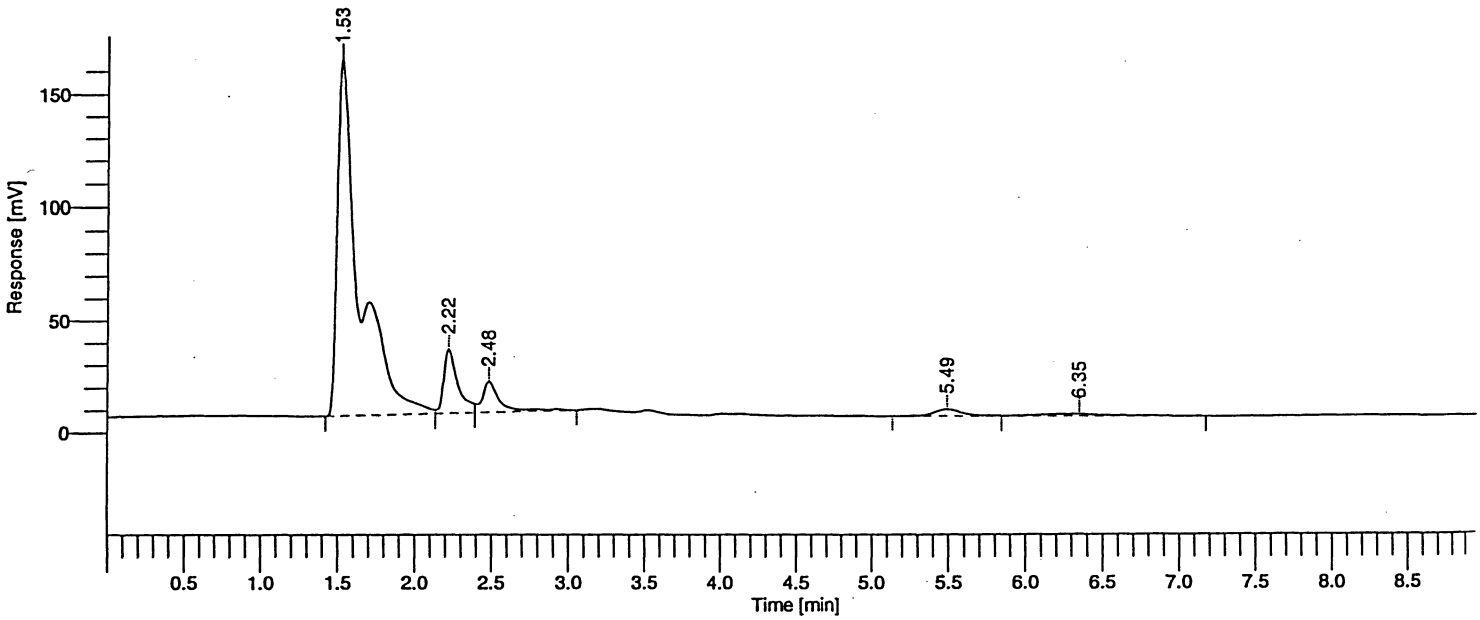
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/21/06 1:28:20 PM
Operator	: manager	Sample Name	: E26 CELL
Sample Number	: 237	Study	: antibiotic
AutoSampler	: SER200	Rack/Vial	: 1/13
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/21/06 2:28:23 PM	Cycle	: 242

Raw Data File : \\Poweredge\E drive\TC\dennis\data231.raw
Result File : \\Poweredge\E drive\TC\dennis\data231.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data231.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.530	1586945.29	157869.63	80.98	80.98	BV	10.0523
2	2.220	184021.07	28476.56	9.39	9.39	VV	6.4622
3	2.484	111473.24	13518.03	5.69	5.69	VB	8.2463
4	5.489	40458.42	3032.29	2.06	2.06	BV	13.3425
5	6.347	36825.98	1020.18	1.88	1.88	VB	36.0976
		1959724.00	203916.68	100.00	100.00		

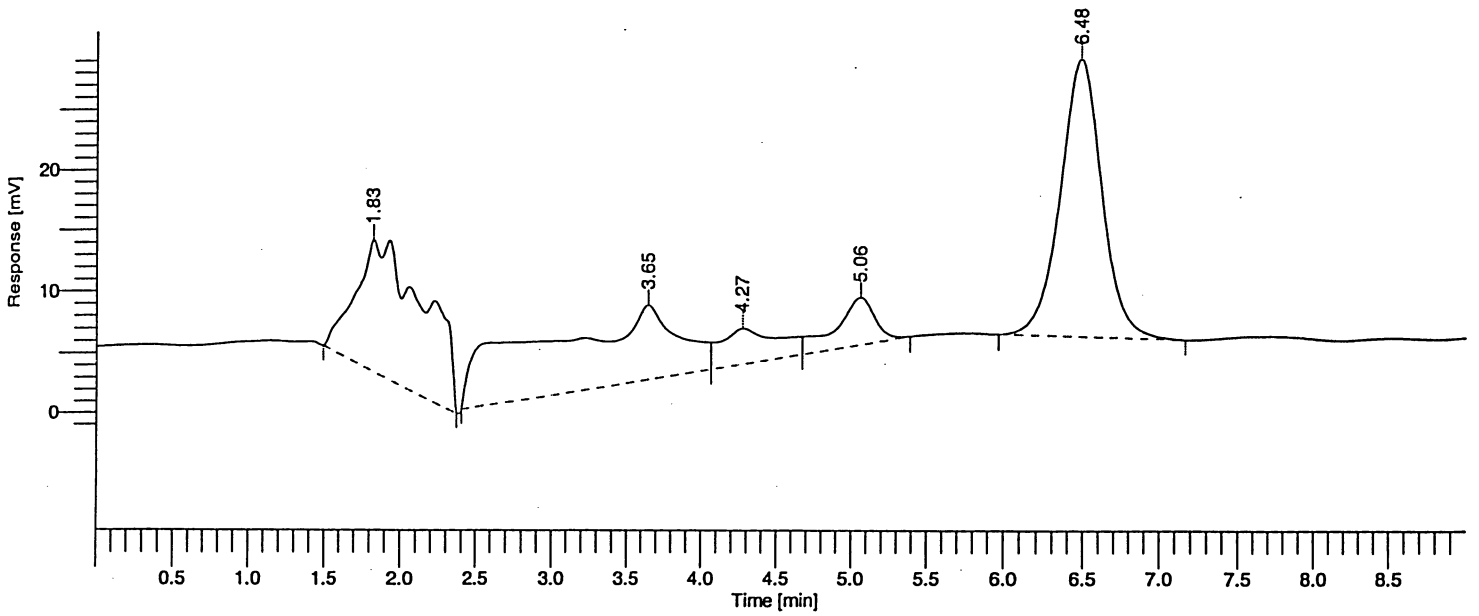
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 10:52:53 AM
Operator	: manager	Sample Name	: ctc 20ppm, meoh
Sample Number	: 269	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/40
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 11:53:00 AM	Cycle	: 269

Raw Data File : \\Poweredge\E drive\TC\dennis\data258.raw
Result File : \\Poweredge\E drive\TC\dennis\data258.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data258.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.827	351786.40	10766.92	26.25	26.25	BB	32.6729
2	3.646	414700.25	6033.90	30.95	30.95	BV	68.7284
3	4.272	75494.29	2873.63	5.63	5.63	VV	26.2714
4	5.059	69258.45	3793.40	5.17	5.17	VB	18.2576
5	6.484	428825.00	23051.61	32.00	32.00	BB	18.6028
		1340064.40	46519.46	100.00	100.00		

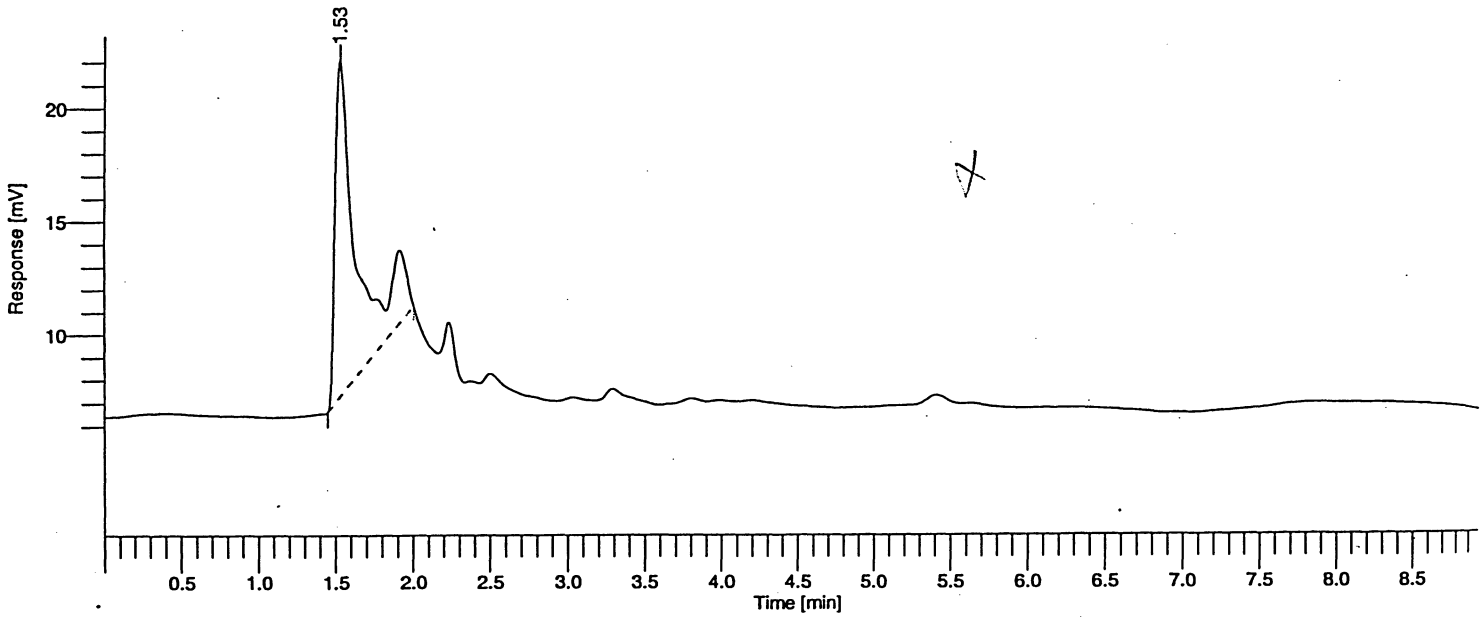
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 11:02:56 AM
Operator	: manager	Sample Name	: EDTA1,S1
Sample Number	: 270	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/41
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 12:03:02 PM	Cycle	: 270

Raw Data File : \\Poweredge\E drive\TC\dennis\data259.raw
Result File : \\Poweredge\E drive\TC\dennis\data259.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data259.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.533	146096.00	14773.16	100.00	100.00	BB	9.8893
		146096.00	14773.16	100.00	100.00		

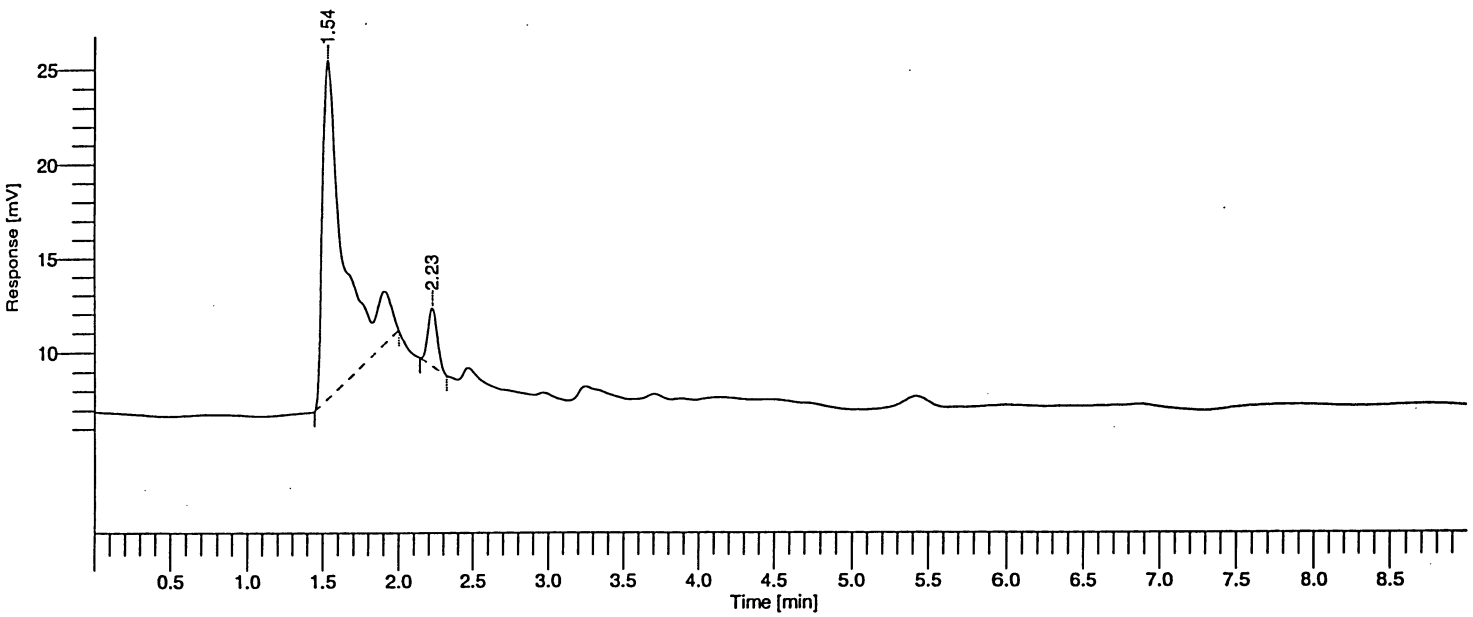
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 11:12:59 AM
Operator	: manager	Sample Name	: EDTA1,S2
Sample Number	: 271	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/42
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 12:13:02 PM	Cycle	: 271

Raw Data File : \\Poweredge\E drive\TC\dennis\data260.raw
Result File : \\Poweredge\E drive\TC\dennis\data260.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data260.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.536	177856.00	17949.83	92.99	92.99	BB	9.9085
2	2.227	13410.00	3031.01	7.01	7.01	BB	4.4243
		191266.00	20980.84	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

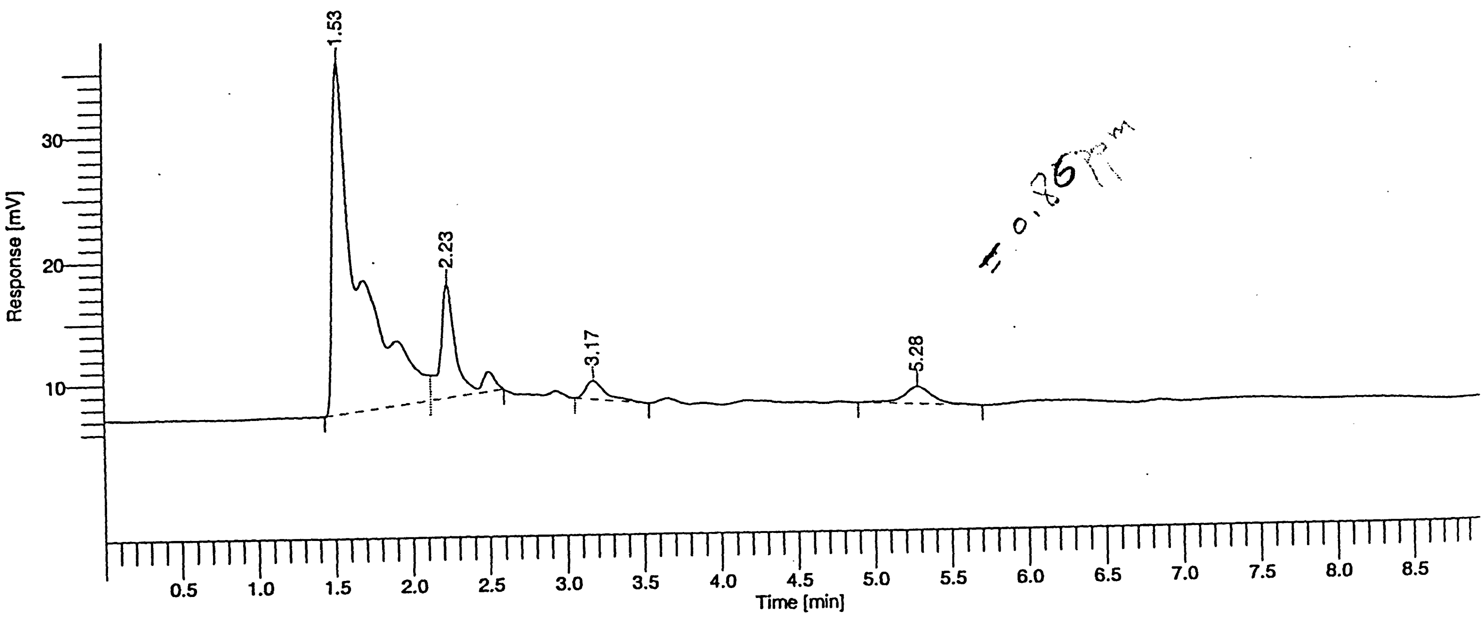
All components were found

Software Version : 6.1.2.0.1:D19
Operator : manager
Sample Number : 272
AutoSampler : SER200
Instrument Name : LC
Instrument Serial # : None
Delay Time : 0.00 min
Sampling Rate : 2.5000 pts/s
Volume Injected : 1.000000 ul
Sample Amount : 1.0000
Data Acquisition Time : 6/22/06 12:23:02 PM

Date : 6/22/06 11:22:54 AM
Sample Name : EDTA1S 3
Study : ANTIBIOTIC
Rack/Vial : 1/43
Channel : A
A/D mV Range : 1000
End Time : 8.99 min

Area Reject : 0.000000
Dilution Factor : 1.00
Cycle : 272

Raw Data File : \\Poweredge\E drive\TC\dennis\data261.raw
Result File : \\Poweredge\E drive\TC\dennis\data261.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data261.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.531	349255.73	28323.19	77.45	77.45	BV	12.3311
2	2.235	69948.07	9280.50	15.51	15.51	VB	7.5371
3	3.172	13333.00	1496.68	2.96	2.96	BB	8.9084
4	5.278	18396.80	1388.77	4.08	4.08	BB	13.2468
		450933.60	40489.14	100.00	100.00		

1 18396.8 × 20 / 42.8825

3.6 = 18000 × 20 / 430000
43

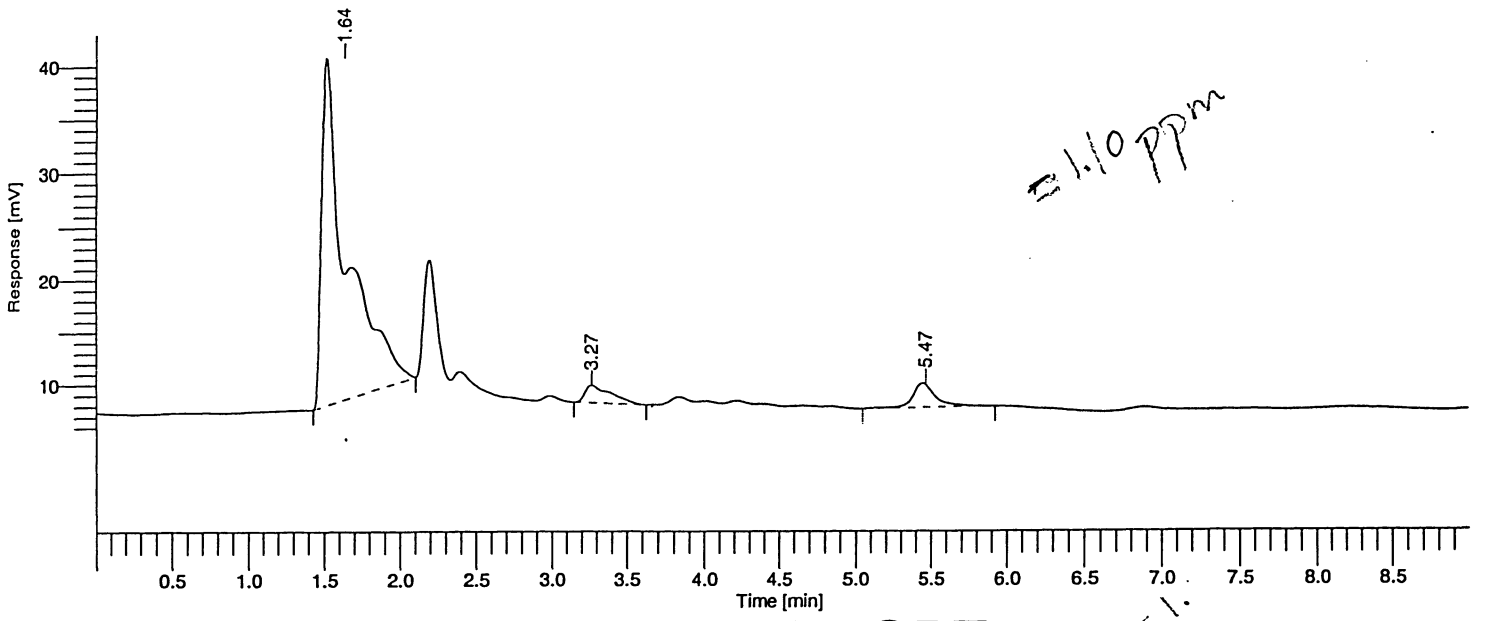
Missing Component Report

Component Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 11:32:58 AM
Operator	: manager	Sample Name	: EDTA1S4
Sample Number	: 273	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/44
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 12:33:03 PM	Cycle	: 273

Raw Data File : \\Poweredge\E drive\TC\dennis\data262.raw
Result File : \\Poweredge\E drive\TC\dennis\data262.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data262.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



= 1.10 ppm

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.644	382812.40	12042.09	89.94	89.94	BB	31.7895
2	3.267	19216.60	1629.38	4.51	4.51	BB	11.7938
3	5.469	23601.40	2117.15	5.55	5.55	BB	11.1477
		425630.40	15788.62	100.00	100.00		

= 48/43 = 1.

2400 / 2360 * 20 / 43000

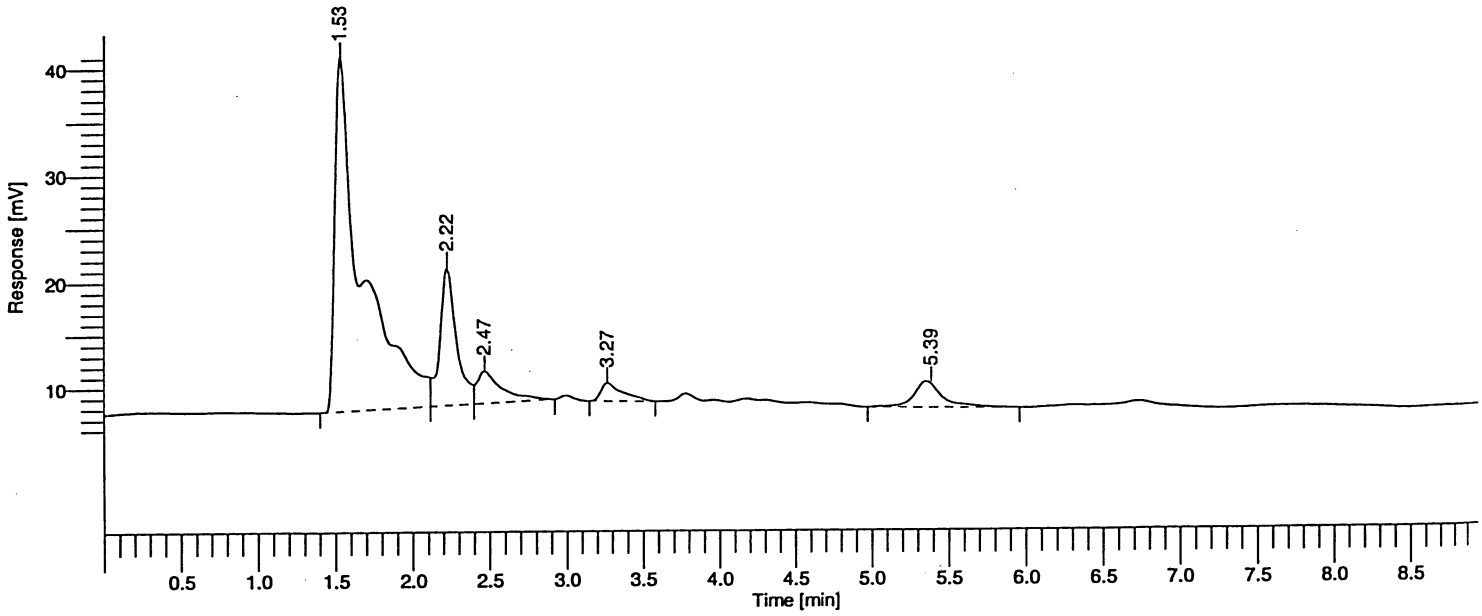
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 11:42:54 AM
Operator	: manager	Sample Name	: EDTA1S5
Sample Number	: 274	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/45
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 12:43:03 PM	Cycle	: 274

Raw Data File : \\Poweredge\E drive\TC\dennis\data263.raw
Result File : \\Poweredge\E drive\TC\dennis\data263.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data263.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.531	415574.72	33328.96	69.39	69.39	BV	12.4689
2	2.223	98950.91	13026.56	16.52	16.52	VV	7.5961
3	2.467	35181.77	3120.75	5.87	5.87	VB	11.2735
4	3.268	17379.20	1769.74	2.90	2.90	BB	9.8202
5	5.387	31797.40	2250.01	5.31	5.31	BB	14.1321
		598884.00	53496.02	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

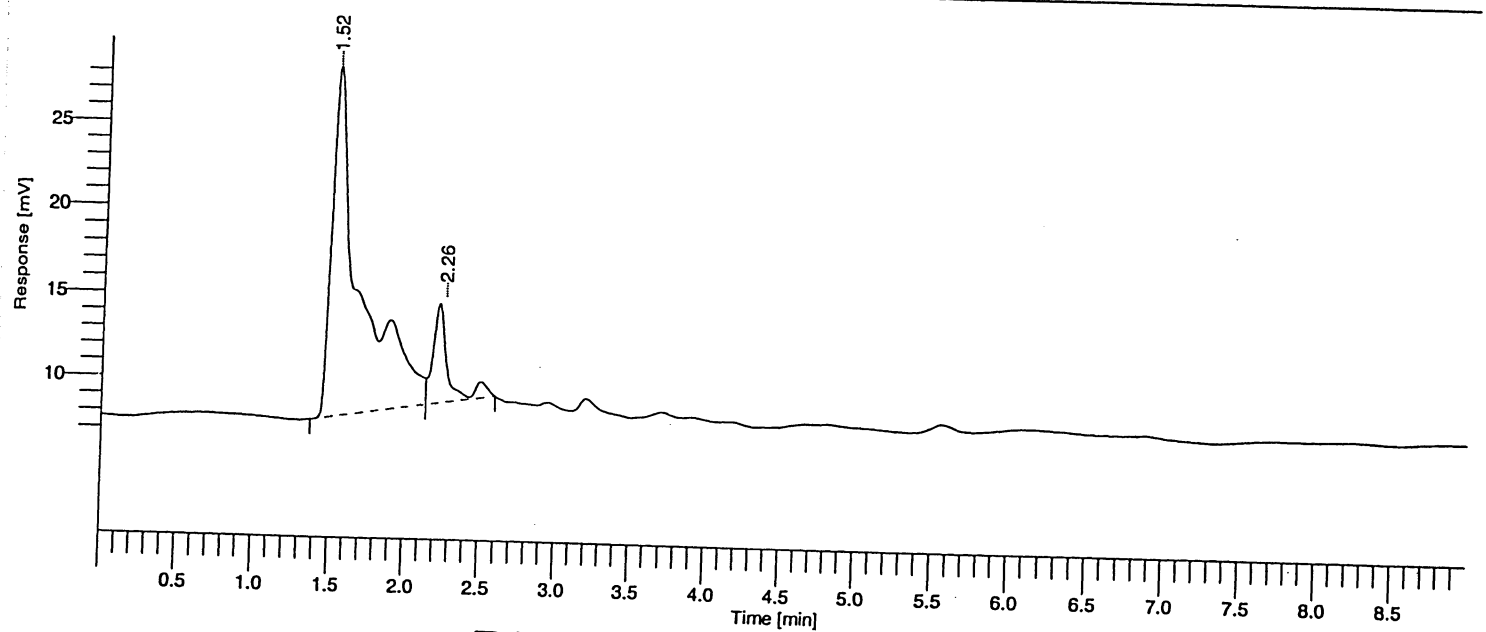
All components were found

32.000
31797.40 x 20
43.0000

1.48 ≅ 64/43 =

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 11:52:57 AM
Operator	: manager	Sample Name	: EDTA1S6
Sample Number	: 275	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/46
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 12:53:03 PM	Cycle	: 275

Raw Data File : \\Poweredge\E drive\TC\dennis\data264.raw
Result File : \\Poweredge\E drive\TC\dennis\data264.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data264.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.519	272972.09	20640.17	86.92	86.92	BV	13.2253
2	2.262	41074.31	2858.10	13.08	13.08	VB	14.3712
		314046.40	23498.27	100.00	100.00		

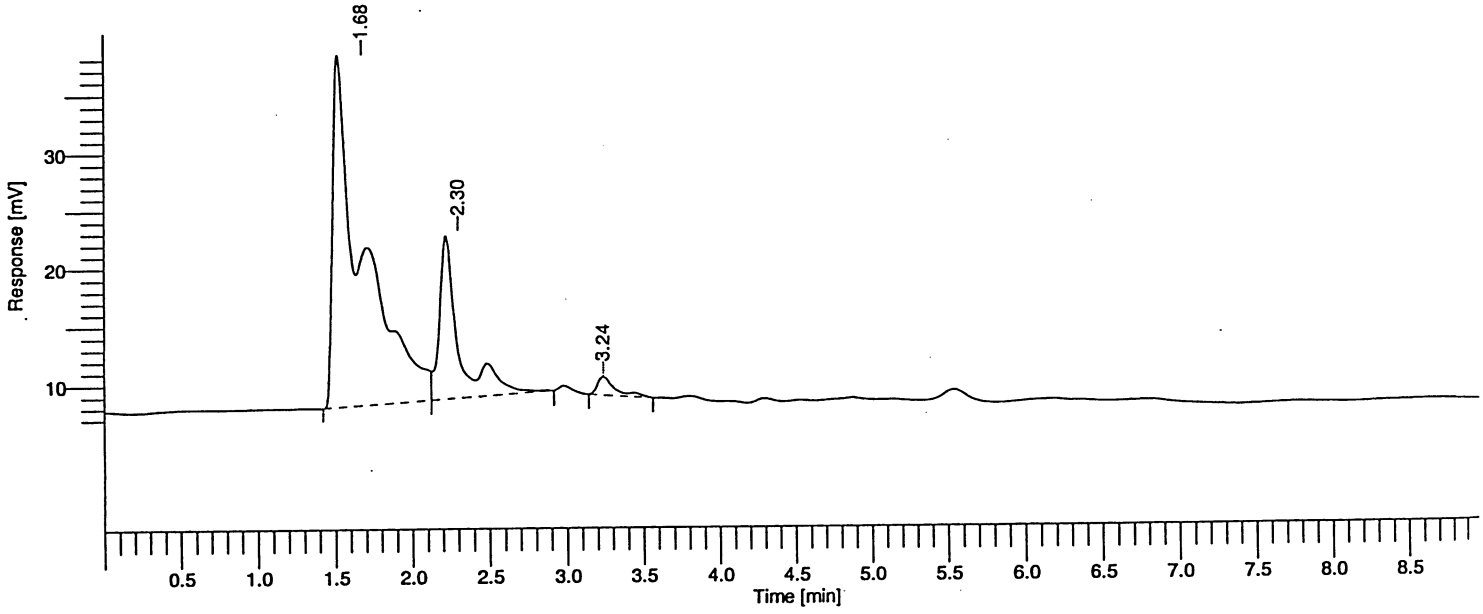
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 12:03:00 PM
Operator	: manager	Sample Name	: EDTA2S1
Sample Number	: 276	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/47
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 1:03:03 PM	Cycle	: 276

Raw Data File : \\Poweredge\E drive\TC\dennis\data265.raw
Result File : \\Poweredge\E drive\TC\dennis\data265.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data265.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.678	409927.20	13014.35	74.90	74.90	BV	31.4981
2	2.300	124528.00	3894.18	22.75	22.75	VB	31.9780
3	3.237	12813.20	1555.87	2.34	2.34	BB	8.2354
		547268.40	18464.40	100.00	100.00		

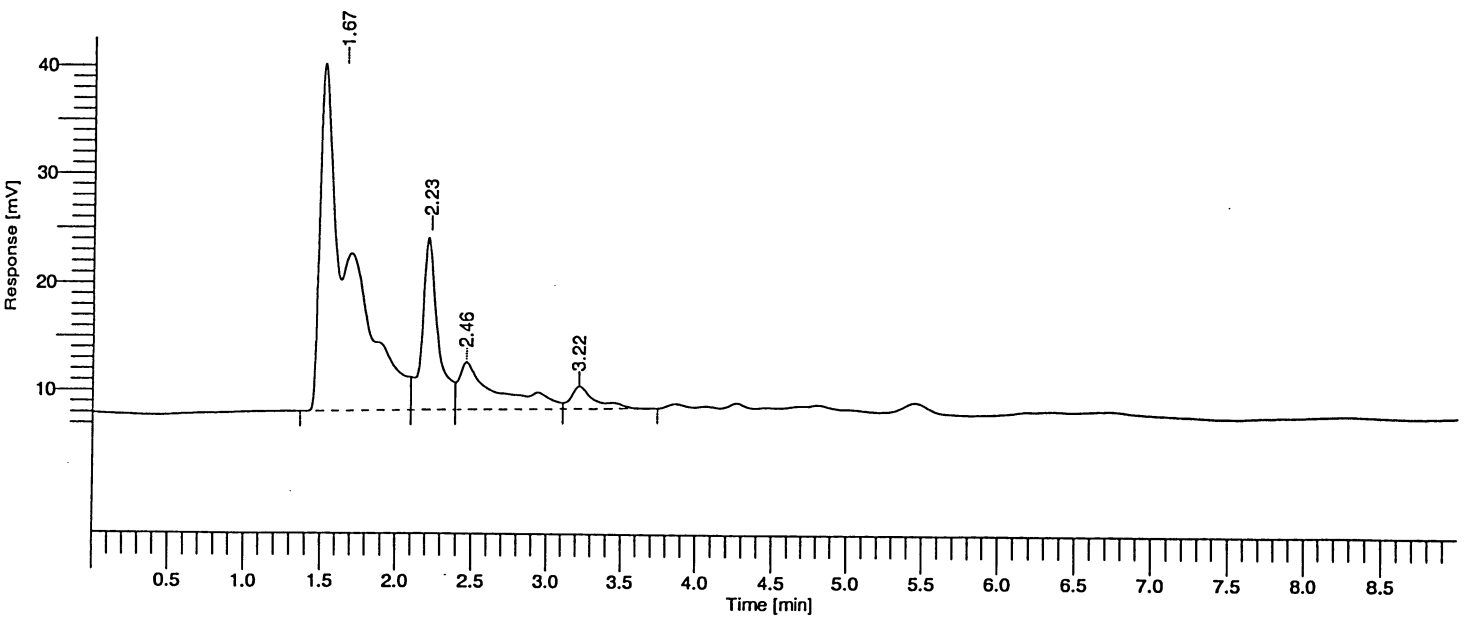
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 12:12:56 PM
Operator	: manager	Sample Name	: EDTA2S2
Sample Number	: 277	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/48
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 1:13:03 PM	Cycle	: 277

Raw Data File : \\Poweredge\E drive\TC\dennis\data266.raw
Result File : \\Poweredge\E drive\TC\dennis\data266.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data266.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.671	434631.00	13731.35	66.61	66.61	BV	31.6525
2	2.229	116570.62	14373.95	17.86	17.86	VV	8.1099
3	2.463	77585.42	4362.90	11.89	11.89	VV	17.7830
4	3.218	23744.36	2067.87	3.64	3.64	VB	11.4825
		652531.40	34536.07	100.00	100.00		

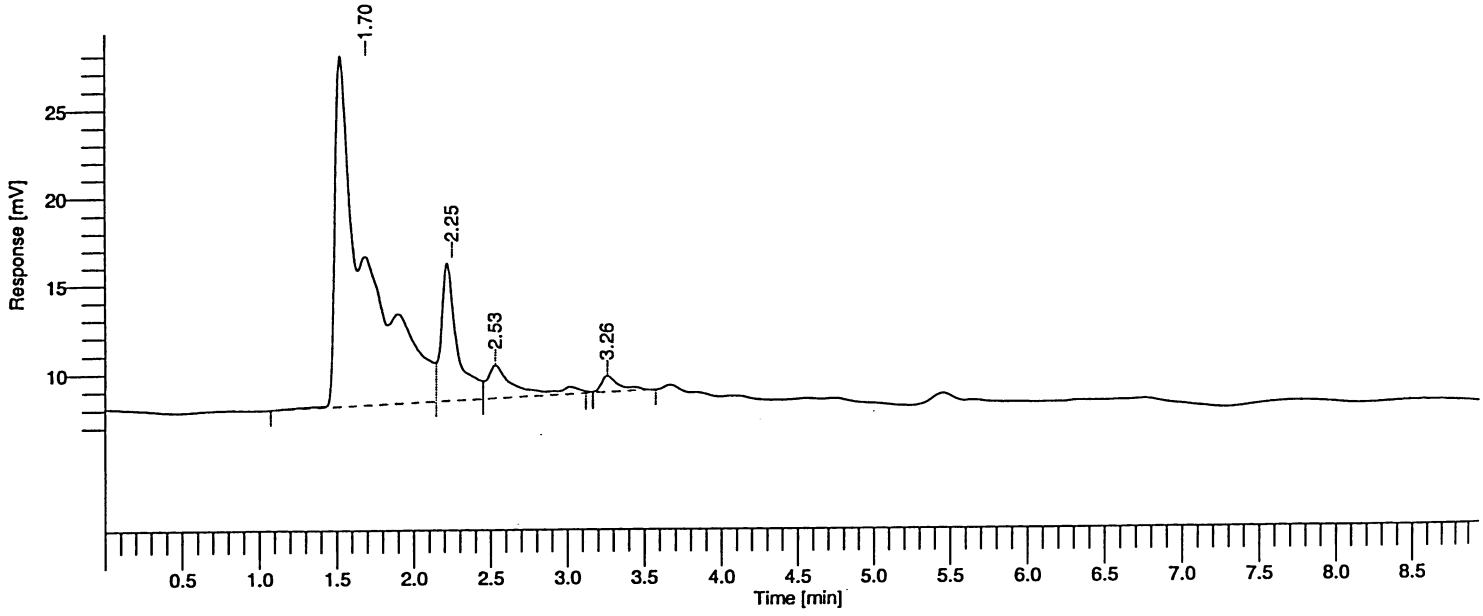
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 12:22:59 PM
Operator	: manager	Sample Name	: EDTA2S3
Sample Number	: 278	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/49
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 1:23:04 PM	Cycle	: 278

Raw Data File : \\Poweredge\E drive\TC\dennis\data267.raw
Result File : \\Poweredge\E drive\TC\dennis\data267.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data267.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.696	279970.98	8339.61	76.10	76.10	BV	33.5712
2	2.251	56201.82	5287.43	15.28	15.28	VV	10.6293
3	2.532	24324.29	1862.84	6.61	6.61	VV	13.0576
4	3.260	7407.67	913.76	2.01	2.01	VB	8.1068
		367904.75	16403.65	100.00	100.00		

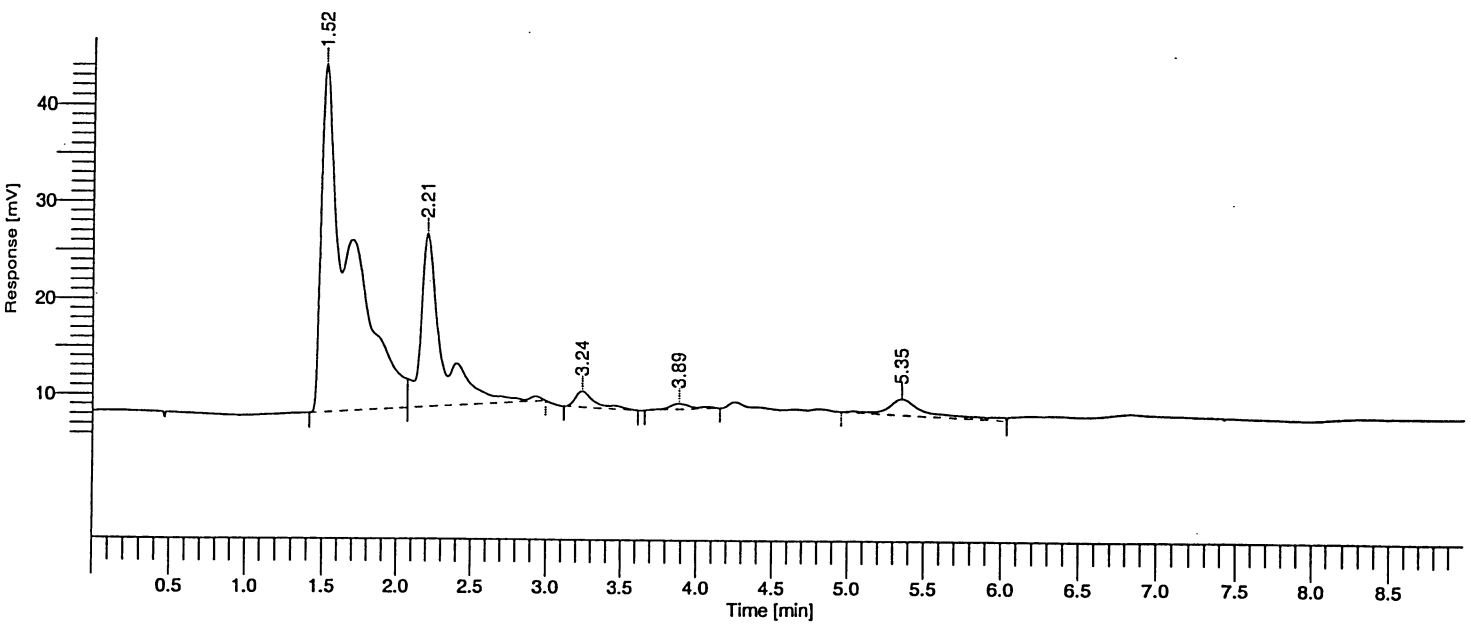
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 12:33:02 PM
Operator	: manager	Sample Name	: EDTA2S4
Sample Number	: 279	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/50
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 1:33:08 PM	Cycle	: 279

Raw Data File : \\Poweredge\E drive\TC\dennis\data268.raw
Result File : \\Poweredge\E drive\TC\dennis\data268.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data268.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.520	488032.42	36214.20	67.18	67.18	BV	13.4763
2	2.206	184429.98	18027.65	25.39	25.39	VB	10.2304
3	3.238	15492.40	1649.17	2.13	2.13	BB	9.3941
4	3.886	6575.80	602.86	0.91	0.91	BB	10.9077
5	5.352	31936.20	1701.97	4.40	4.40	BB	18.7643
		726466.80	58195.84	100.00	100.00		

$\frac{32\,000 + 20}{43\,000} \approx 1.49$ ppm

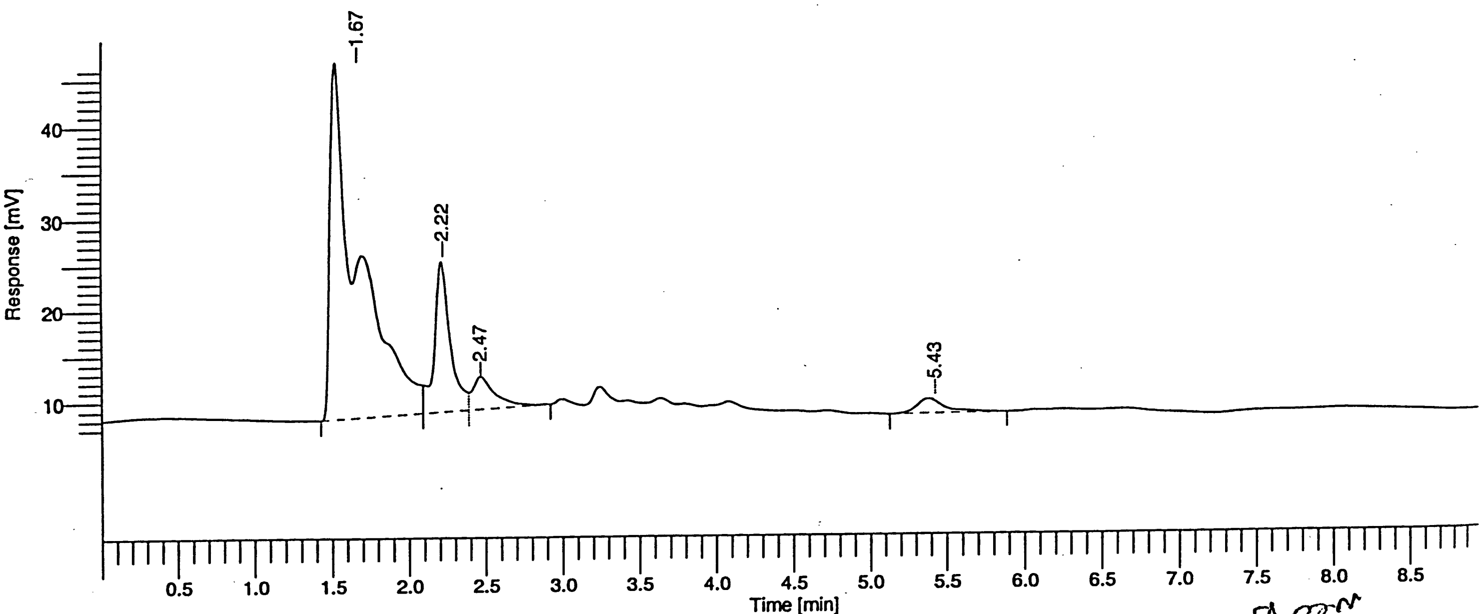
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version : 6.1.2.0.1:D19		Date	: 6/22/06 12:43:05 PM
Operator : manager		Sample Name	: EDTA2S5
Sample Number : 280		Study	: ANTIBIOTIC
AutoSampler : SER200		Rack/Vial	: 1/51
Instrument Name : LC		Channel	: A
Instrument Serial # : None		A/D mV Range	: 1000
Delay Time : 0.00 min		End Time	: 8.99 min
Sampling Rate : 2.5000 pts/s			
Volume Injected : 1.000000 ul		Area Reject	: 0.000000
Sample Amount : 1.0000		Dilution Factor	: 1.00
Data Acquisition Time : 6/22/06 1:43:10 PM		Cycle	: 280

Raw Data File : \\Poweredge\E drive\TC\dennis\data269.raw
Result File : \\Poweredge\E drive\TC\dennis\data269.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data269.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

40/43 ≈ 0.9 ppm

~~20 ppb × 20 / 43 ppb~~

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.670	504814.52	16682.47	74.17	74.17	BV	30.2602
2	2.224	119820.41	15742.03	17.60	17.60	VV	7.6115
3	2.467	35852.07	3530.69	5.27	5.27	VB	10.1544
4	5.425	20147.80	1285.28	2.96	2.96	BB	15.6758
		680634.80	37240.47	100.00	100.00		

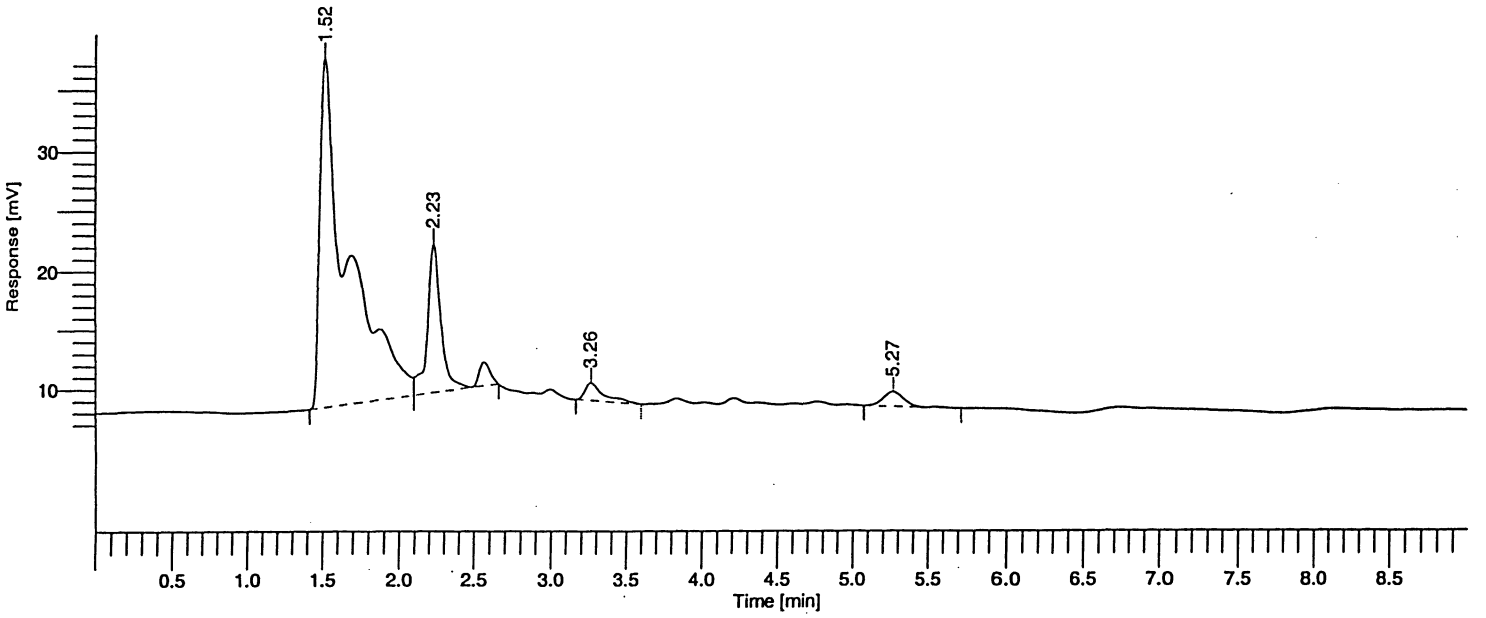
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 12:53:01 PM
Operator	: manager	Sample Name	: EDTA2 S6
Sample Number	: 281	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/52
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 1:53:10 PM	Cycle	: 281

Raw Data File : \\Poweredge\E drive\TC\dennis\data270.raw
Result File : \\Poweredge\E drive\TC\dennis\data270.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data270.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.518	375265.42	29266.13	76.66	76.66	BV	12.8225
2	2.232	89256.98	12526.16	18.23	18.23	VB	7.1256
3	3.265	13138.80	1490.69	2.68	2.68	BB	8.8139
4	5.267	11880.40	1243.87	2.43	2.43	BB	9.5512
		489541.60	44526.85	100.00	100.00		

$\frac{11880.40 \times 20}{43600} = \frac{24}{43} \approx 0.6$

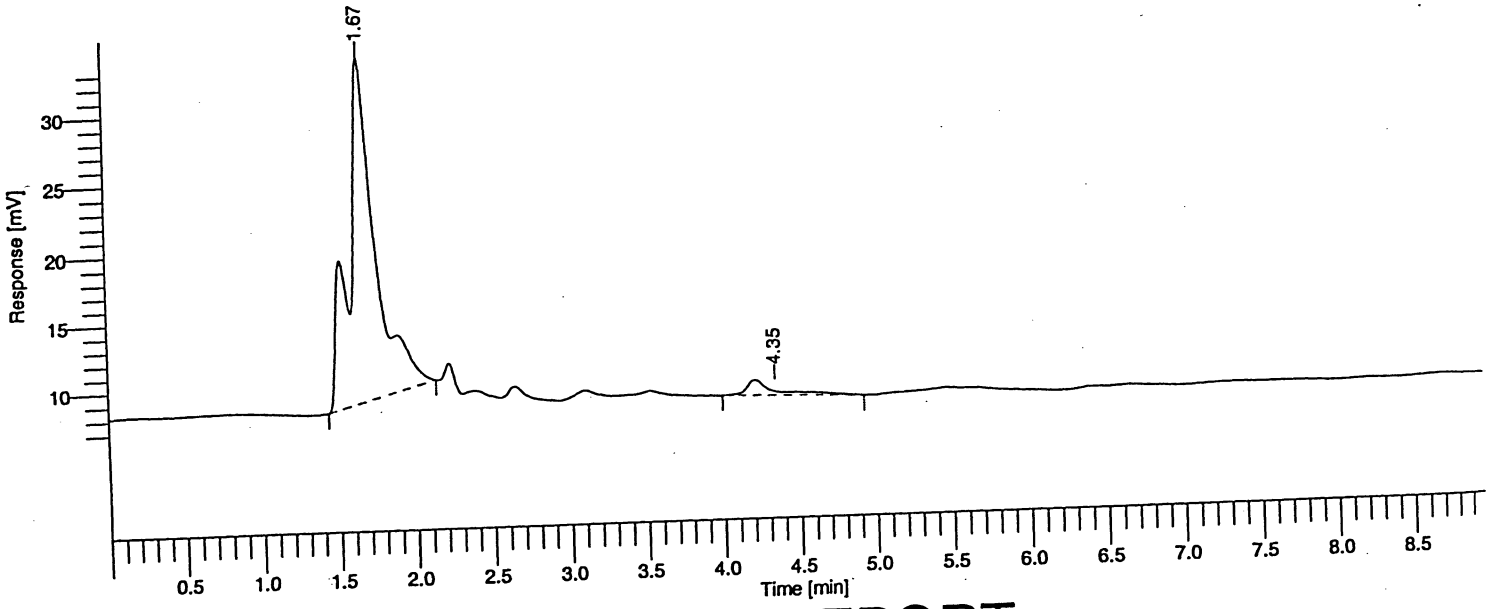
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 1:03:04 PM
Operator	: manager	Sample Name	: EDTA1,EPS1
Sample Number	: 282	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/53
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 2:03:10 PM	Cycle	: 282

Raw Data File : \\Poweredge\E drive\TC\dennis\data271.raw
Result File : \\Poweredge\E drive\TC\dennis\data271.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data271.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.670	304313.60	25108.64	95.31	95.31	BB	12.1199
2	4.346	14963.20	285.59	4.69	4.69	BB	52.3947
		319276.80	25394.22	100.00	100.00		

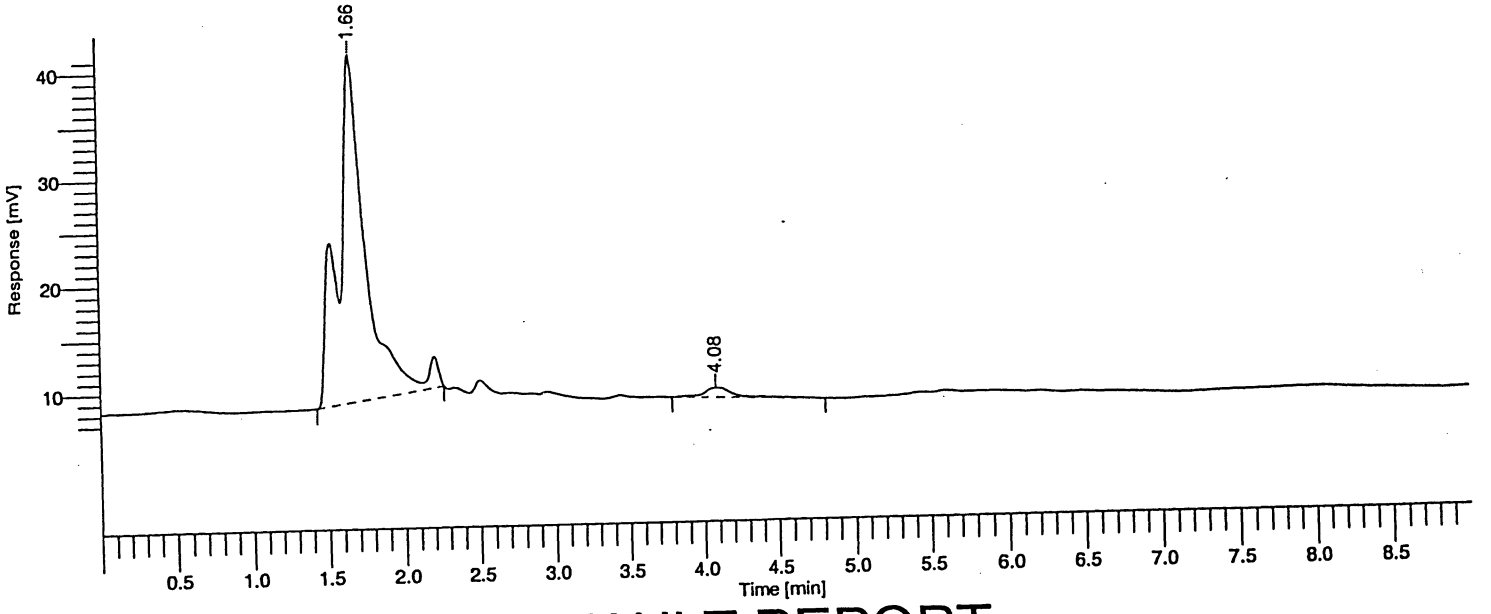
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 1:13:01 PM
Operator	: manager	Sample Name	: EDTA1,EPS2
Sample Number	: 283	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/54
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 2:13:11 PM	Cycle	: 283

Raw Data File : \\Poweredge\E drive\TC\dennis\data272.raw
Result File : \\Poweredge\E drive\TC\dennis\data272.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data272.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.665	410700.60	32546.47	96.83	96.83	BB	12.6189
2	4.082	13430.00	920.57	3.17	3.17	BB	14.5887
		424130.60	33467.04	100.00	100.00		

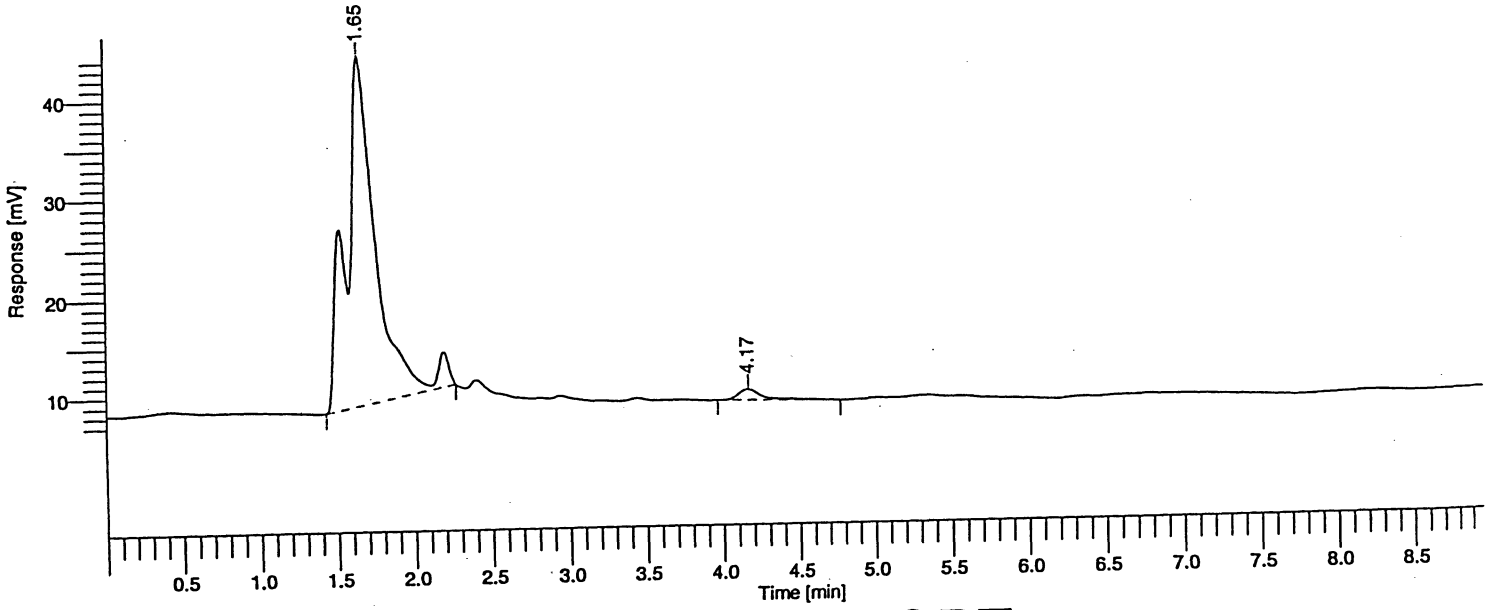
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 1:23:04 PM
Operator	: manager	Sample Name	: EDTA1,EPS3
Sample Number	: 284	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/55
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 2:23:11 PM	Cycle	: 284

Raw Data File : \\Poweredge\E drive\TC\dennis\data273.raw
Result File : \\Poweredge\E drive\TC\dennis\data273.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data273.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.651	478382.80	35364.77	97.49	97.49	BB	13.5271
2	4.165	12337.20	1086.39	2.51	2.51	BB	11.3562
		490720.00	36451.16	100.00	100.00		

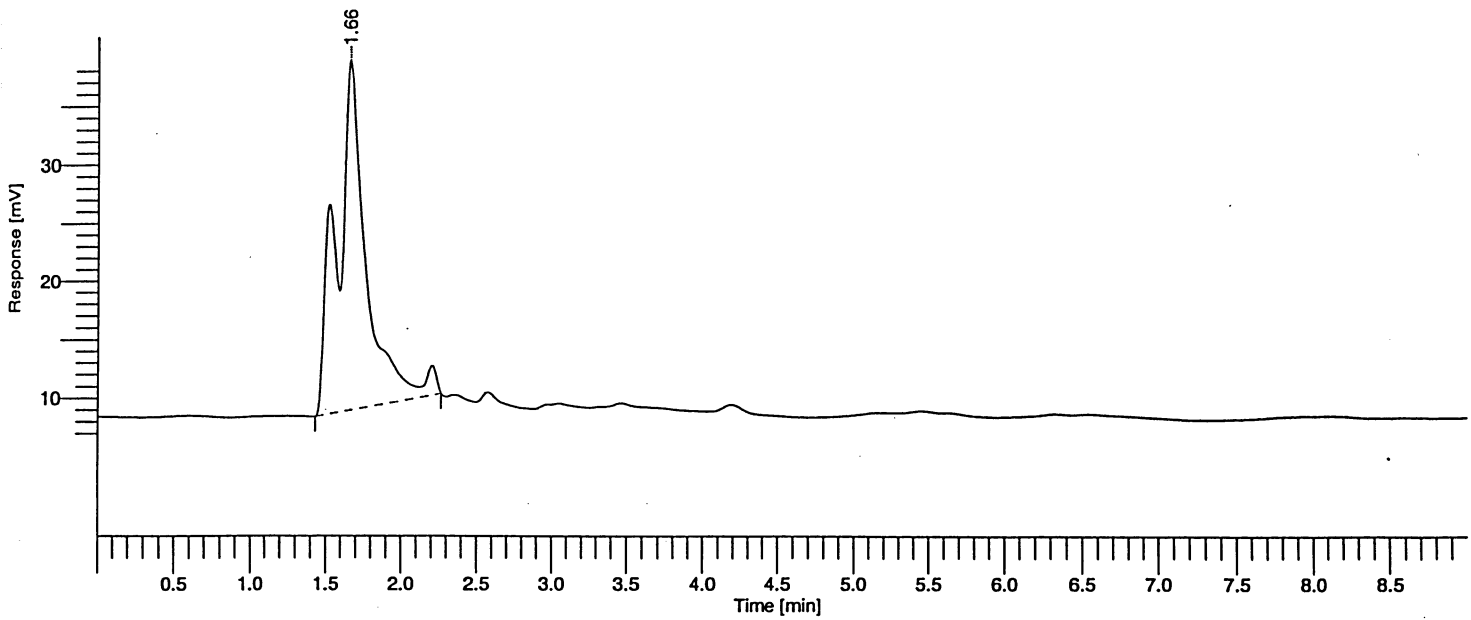
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 1:33:01 PM
Operator	: manager	Sample Name	: EDAT1,EPS4
Sample Number	: 285	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/56
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 2:33:11 PM	Cycle	: 285

Raw Data File : \\Poweredge\E drive\TC\dennis\data274.raw
Result File : \\Poweredge\E drive\TC\dennis\data274.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data274.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.665	405816.00	30021.77	100.00	100.00	BB	13.5174
		405816.00	30021.77	100.00	100.00		

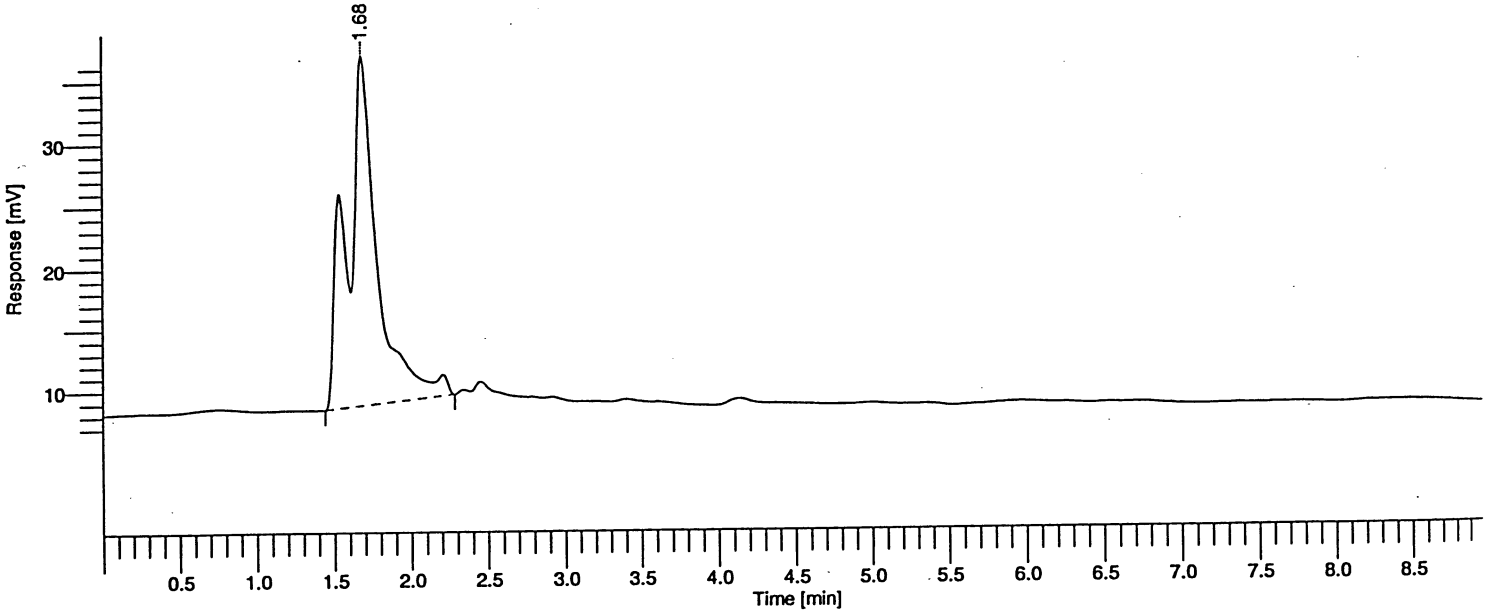
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 1:43:04 PM
Operator	: manager	Sample Name	: EDAT1,EPS5
Sample Number	: 286	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/57
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 2:43:11 PM	Cycle	: 286

Raw Data File : \\Poweredge\E drive\TC\dennis\data275.raw
Result File : \\Poweredge\E drive\TC\dennis\data275.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data275.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.682	388038.00	28142.72	100.00	100.00	BB	13.7882
		388038.00	28142.72	100.00	100.00		

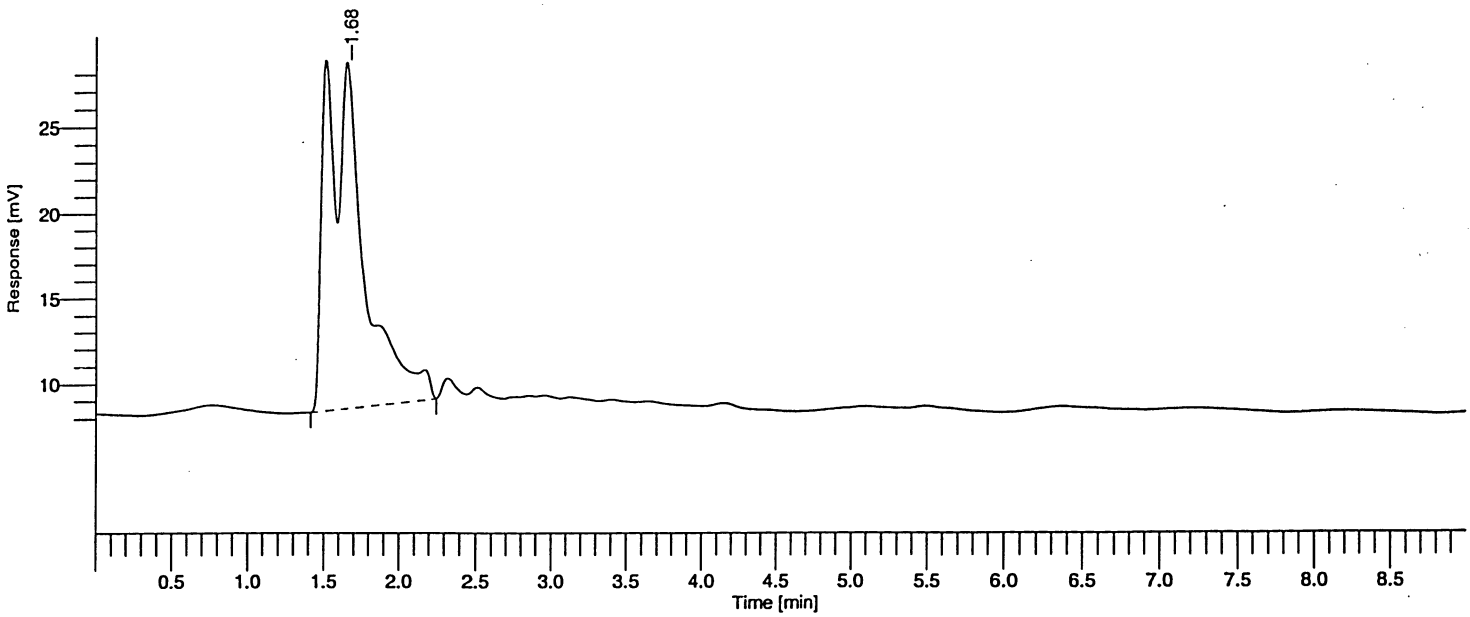
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 1:53:01 PM
Operator	: manager	Sample Name	: EDTA1,EPS6
Sample Number	: 287	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/58
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 2:53:11 PM	Cycle	: 287

Raw Data File : \\Poweredge\E drive\TC\dennis\data276.raw
Result File : \\Poweredge\E drive\TC\dennis\data276.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data276.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.684	356310.00	17872.37	100.00	100.00	BB	19.9364
		356310.00	17872.37	100.00	100.00		

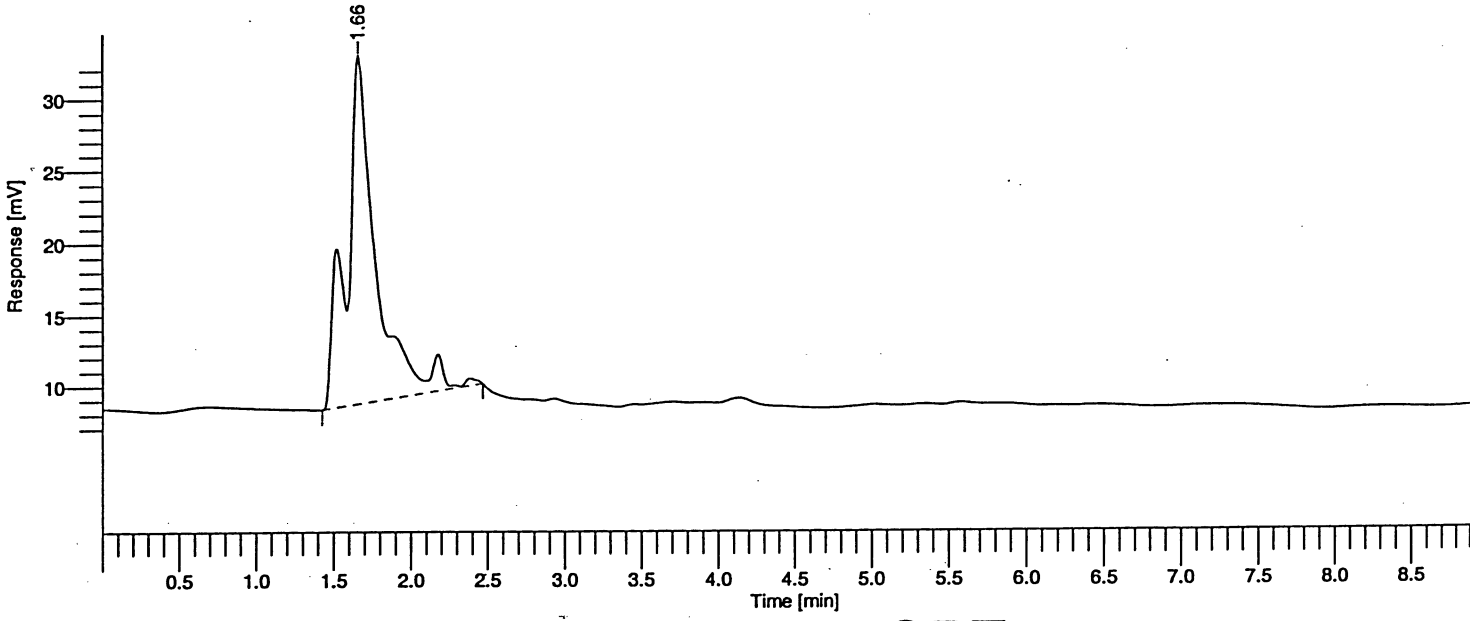
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 2:03:04 PM
Operator	: manager	Sample Name	: EDTA2,EPS1
Sample Number	: 288	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/59
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 3:03:12 PM	Cycle	: 288

Raw Data File : \\Poweredge\E drive\TC\dennis\data277.raw
Result File : \\Poweredge\E drive\TC\dennis\data277.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data277.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.663	326865.60	24200.65	100.00	100.00	BB	13.5065
		326865.60	24200.65	100.00	100.00		

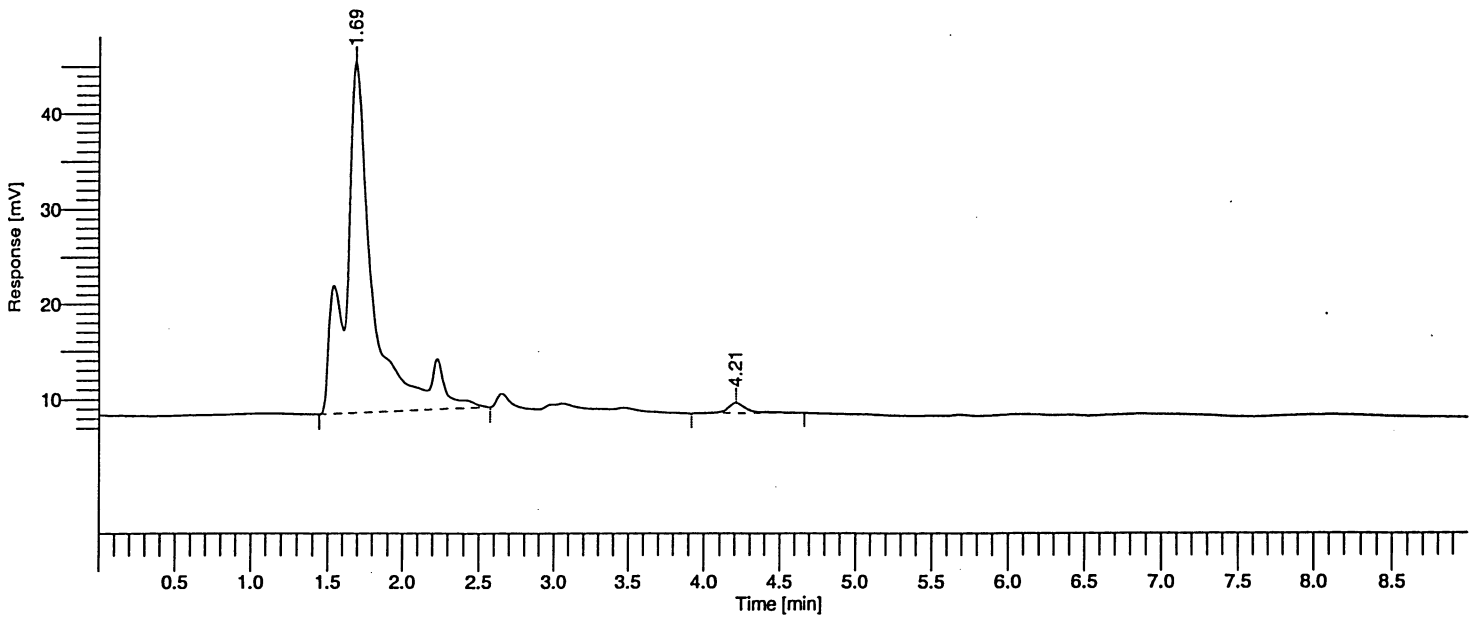
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 2:13:08 PM
Operator	: manager	Sample Name	: EDTA2,EPS2
Sample Number	: 289	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/60
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 3:13:16 PM	Cycle	: 289

Raw Data File : \\Poweredge\E drive\TC\dennis\data278.raw
Result File : \\Poweredge\E drive\TC\dennis\data278.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data278.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.693	476610.40	37087.08	97.97	97.97	BB	12.8511
2	4.208	9862.20	1069.31	2.03	2.03	BB	9.2230
		486472.60	38156.39	100.00	100.00		

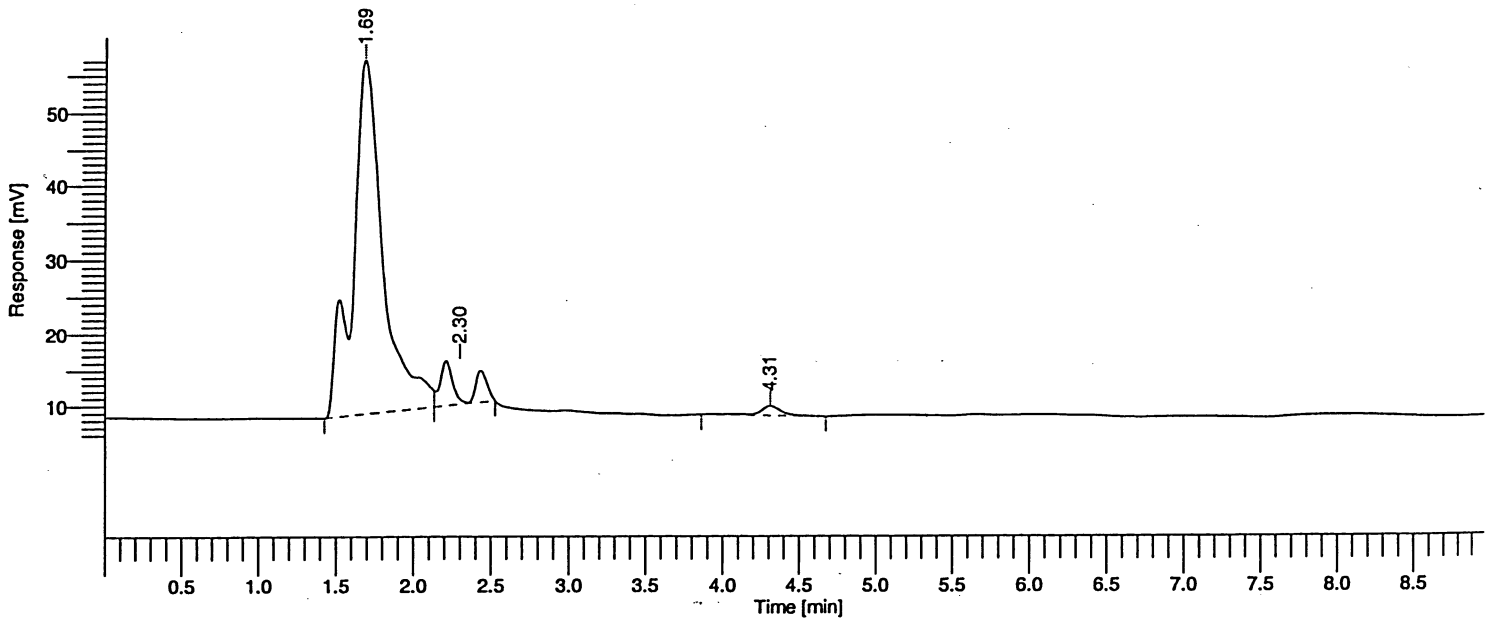
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 2:23:12 PM
Operator	: manager	Sample Name	: EDAT2,EPS3
Sample Number	: 290	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/61
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 3:23:18 PM	Cycle	: 290

Raw Data File : \\Poweredge\E drive\TC\dennis\data279.raw
Result File : \\Poweredge\E drive\TC\dennis\data279.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data279.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.688	705227.20	48274.52	95.70	95.70	BV	14.6087
2	2.300	18070.80	-869.38	2.45	2.45	VB	-20.7858
3	4.312	13628.20	1331.61	1.85	1.85	BB	10.2344
		736926.20	48736.75	100.00	100.00		

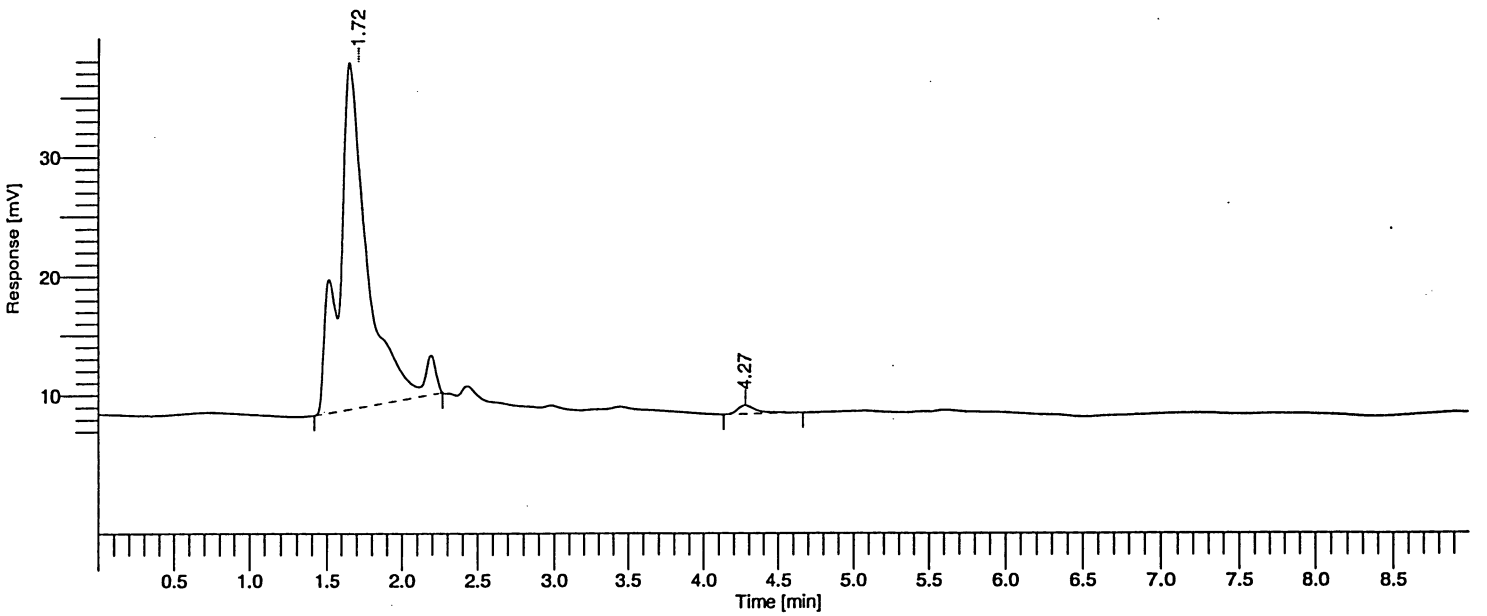
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 2:33:08 PM
Operator	: manager	Sample Name	: EDTA2,EPS4
Sample Number	: 291	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/62
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 3:33:18 PM	Cycle	: 291

Raw Data File : \\Poweredge\E drive\TC\dennis\data280.raw
Result File : \\Poweredge\E drive\TC\dennis\data280.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data280.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.715	391620.40	20517.63	98.37	98.37	BB	19.0870
2	4.275	6480.00	722.73	1.63	1.63	BB	8.9661
		398100.40	21240.35	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 2:43:11 PM
Operator	: manager	Sample Name	: EDAT2,EPS5
Sample Number	: 292	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/63
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 3:43:18 PM	Cycle	: 292

Raw Data File : \\Poweredge\E drive\TC\dennis\data281.raw
Result File : \\Poweredge\E drive\TC\dennis\data281.rst
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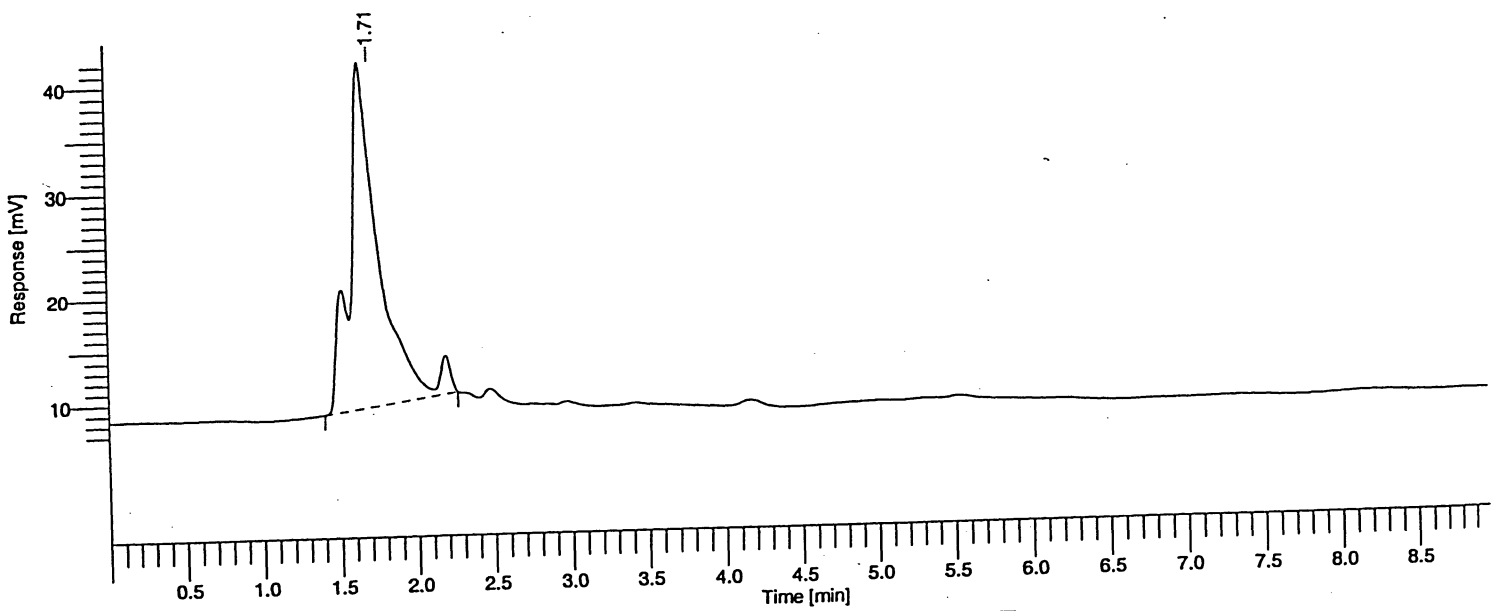
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.714	440694.00	22116.63	100.00	100.00	BB	19.9259
		440694.00	22116.63	100.00	100.00		

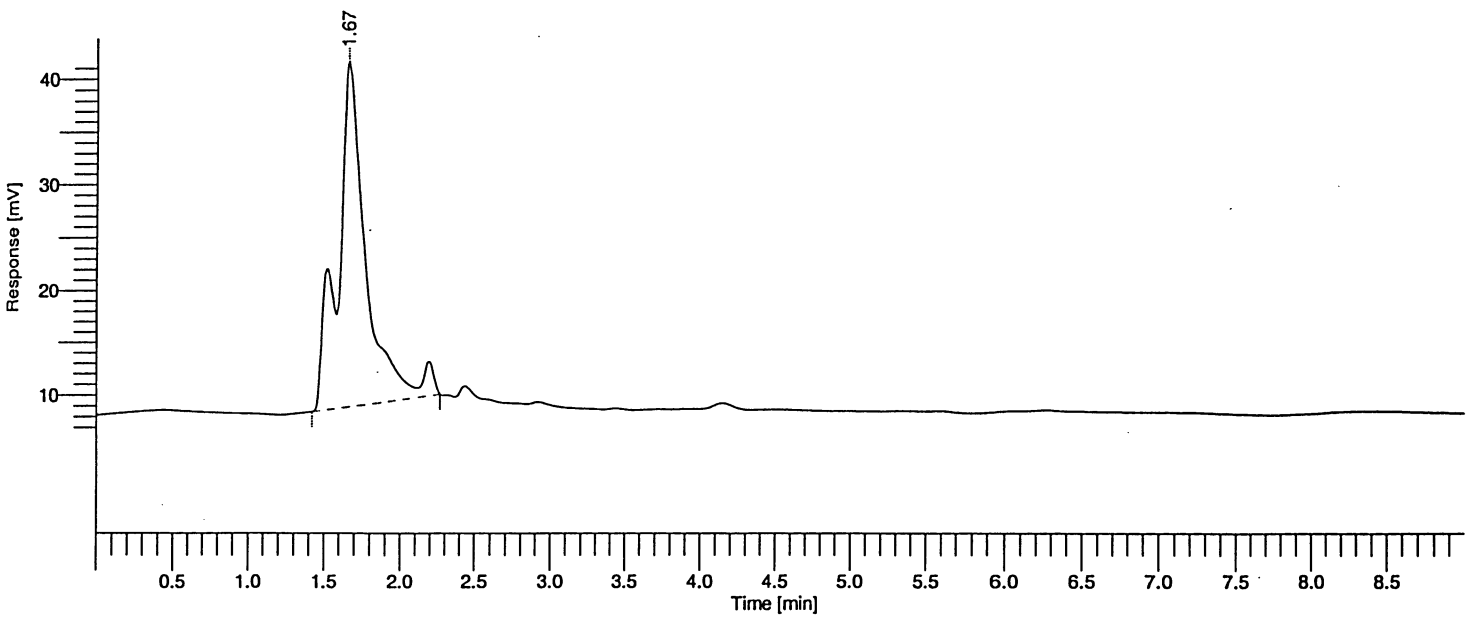
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Software Version	: 6.1.2.0.1:D19	Date	: 6/22/06 2:53:06 PM
Operator	: manager	Sample Name	: EDTA2,EPS6
Sample Number	: 293	Study	: ANTIBIOTIC
AutoSampler	: SER200	Rack/Vial	: 1/64
Instrument Name	: LC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 8.99 min
Sampling Rate	: 2.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/22/06 3:53:19 PM	Cycle	: 293

Raw Data File : \\Poweredge\E drive\TC\dennis\data282.raw
Result File : \\Poweredge\E drive\TC\dennis\data282.rst
Inst Method : \\poweredge\E drive\TC\dennis\antibiotics from \\Poweredge\E drive\TC\dennis\data282.rst
Proc Method : \\Poweredge\E drive\TC\dennis\antibiotics
Calib Method : \\Poweredge\E drive\TC\dennis\antibiotics
Sequence File : \\poweredge\E drive\TC\dennis\antibiotics.seq



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.666	426149.60	32807.12	100.00	100.00	BB	12.9895
		426149.60	32807.12	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found