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Characterization of Almiros river (North eastern Crete) by using chromatography and optical spectroscopy

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Title

‘Characterization of Almiros river (North eastern Crete) by using chromatography and optical spectroscopy’

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PREFACE

This thesis has been taken place in the Laboratory of Water and soil quality control, Department of Environmental and Natural Resources Engineering, Chania, Technological Educational Institute of Crete, Greece.

The thesis has been included in the Master of "Environmental Resources and Risks". This thesis would not have been possible without the support of many people:

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ABSTRACT

This thesis studies the physicochemical status and the pollution in the surface water of Almiros river in Heraklion. The analysis was based on regular samplings throughout an entire water cycle period. Nine samplings were performed from four points among the river during nine different months. The physicochemical parameters were determined by the standard methods of analysis. The thesis has been focused on the presence of Polycyclic Aromatic Hydrocarbons (PAH) and Sodium Dodecyl Benzene Sulfonate (SDBS) in the surface water. The simultaneous determination of these pollutants has been achieved by high performance liquid chromatography and diode array and fluorescence detectors. Additionally, the fluorescence properties of the water constituents have been studied and have shown useful information about the river quality.

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CHAPTER 1

INTRODUCTION

In this chapter, brief information about the study area, the pollution of surface water and the influence of the power plants in the pollution of surface water are presented. Moreover, a discussion is presented about the basic quality parameters of surface water and the humic substances.

1.1 Almiros River

Almiros river is located at the western part of the northern coast of Crete, prefecture of Heraklion. There are also other ecosystems with the same name in Crete, but they are located at other prefectures. Almiros means 'salty' and has been named as such because of its water saltiness. It has attracted the attention of researchers many years ago because the mechanism of its salinity is unknown.

Although it is only 1,8 km long and 5-20 m wide, it has a mean annual discharge of 235×10^6 m^3. This could solve the solution for potable water in Heraklion, but unfortunately it has brackish water.

It consists of a karstic spring at the edge of the mountain Psiloritis and a dam from where the river water begins to flow. Moreover, it includes a sandy beach zone associated with a low relief dune field, in front of an alluvial (Holocene in age) plain, consisted by alluvial, marl and riverine deposits; the latter is an agricultural area with vineyards, olive trees and graze land [1]. The river flows continuously and at the mouth of the river a delta is formed. At the middle of the river an artificial channel 800 m long and 5-10 m wide has been constructed. The channel is used to divert part of the river into the local power plant for cooling its machines.

Figure 1: Picture of Almiros river.

1.2 Water pollution

Water pollution affects not only the water of the lakes, rivers and oceans but also the living species and the ecosystems that exist in the waters. Generally, surface water can be polluted by inorganic compounds, hydrocarbons, wastes, radioactive materials and by heat.

Moreover, industrial production usually uses the water in various ways. Power plants often include pollutants which can affect the quality of the water. Industrial wastewater contains hazardous elements which pollute the water or in some cases, the pollution caused by intrusion of sea water due to over-pumping of groundwater. In addition, wastewater and sewage, septic tanks, underground storage, ocean and marine dumping, when all these are deposited into the sea, pollute the water. All these wastes take time to decompose and when they are into the water they can cause pollution and harm the organisms that live inside [2]. Electricity power plants can affect the quality of water. These power plants that produce electricity by boiler burning, by cooling processes, by heat or by coal pile run-off, impact to the contamination of water with their wastes. Water quality can be downgraded, influencing to public health and many times to the extinction of aquatic life [3].

1.3 Physicochemical parameters of water

Water can be analyzed and characterized by a series of attributes and measures in order to recognize the quality of water. Some of the most prevalent with regards to the suitability of water for human consumption or other uses are:

Potential of Hydrogen (pH): measure of the concentration of the hydrogen ion (H^+) in the solution according to the relationship $pH = -\log[H^+]$. pH indicates how acidic or alkaline a solution is. The higher the concentration of H^+ in the water, the lower its pH, and the greater its acidity.

Electrical Conductivity (EC): the ability of an aqueous solution to conduct electricity. The electrical conductivity is proportional to the concentration of dissolved salts in the water, which means that the higher the salt concentration the greater the electrical conductivity.

Salinity (S): the grams of solid dissolved in 1 kg seawater when all the carbonate has been converted to CO_2, all the iodine (I^-) and bromide ions have been replaced by chloride (Cl^-) and all the organic matter has been oxidized.

Total Dissolved Solids (TDS): all the solutes that can be found in the water with size less than 2 μm.

Hardness: measures the concentration of all calcium and magnesium ions with chlorine, sulphates, carbonates and hydrogen carbonates. Carbonates and biocarbonates of calcium and magnesium constitute the temporary hardness. Chlorides, nitrates, sulfates, phosphates, silicates and humic salts of calcium and magnesium are the permanent hardness. Permanent hardness cannot be removed by boiling. The total hardness is the sum of temporary and permanent hardness. The permanent (carbonate) hardness is equal with the alkalinity.

Chlorides (Cl^-): Chloride ions are mostly attached to sodium, potassium and calcium ions. Increased concentration of chlorides in water is usually attributed to pollution of mixing with sea water. Chlorides and Salinity are connected with the following empirical equation: S ‰= 1,80655 Cl ‰.

Alkalinity: the ability of water to neutralize certain amount of hydrogen cations. Alkalinity is a measure of water's ability to resist changes in pH. Water with low alkalinity is very susceptible to changes in pH. Generally, alkalinity measures the bicarbonate (HCO_3^{-1}), carbonate (CO_3^{-2}) and hydroxide (OH^-) ions in natural water. In hydroxide alkalinity, pH values are usually close to of higher than 10. Carbonate alkalinity, which is the total alkalinity, pH values are more than 8,5 and in bicarbonate alkalinity pH values are smaller than 8,3 [4].The bicarbonate ions are the main alkaline factor in almost all waters.

1.4 Natural Organic Matter - Humic substances

Natural organic matter (NOM) is a complex mixture of organic compounds which are present in soil and in natural surface water sources, affecting the color and the taste of the water. These compounds are humic substances, fatty acids, amino acids and proteins, carbohydrates, nucleic acids, hydrocarbons. The mostly studied compounds are humic substances and proteins. The amino acids are produced by microbial activities and are the result of protein breakdown.

Humic substances are produced by break-down of plant material by biological and chemical processes. These substances give the characteristic brown color to soils and also to surface waters. When humic substances are present in high concentrations, they give a dark color to water. Almost 50% of the dissolved organic materials in water are humic substances. These substances affect pH and alkalinity and are created as the final result of decaying organic matter [5,6].

There are three types of humic substances, which differ slightly in acidity and chemical composition:

Humic acid, is a solid substance, not soluble in water under acid conditions, which means that at pH values below 2 it is precipitated. But at higher pH values is completely soluble in water. The color of the humic acid can be dark brown or grey black. Humic acid contains a wide array of naturally occurring bio-chemicals, such as natural antibiotics.
Fulvic acid, is a natural carrier of nutrients, completely soluble in the water under all pH conditions. It has usually light yellow or yellow brown color. Fulvic acid dissolves and transports vitamins, coenzymes, hormones, and natural antibiotics.
Humin is what remains from humus, after the humic acid and the fulvic acid. It is considered a waste product and it has black color. Humin is not soluble in water at any pH values [7]. Humus is the top layer of the soil, looks like a compost and consists of organic digested plant and animal materials. Humic acid and fulvic acid coming from humus [8].

Humic and fulvic acid have effects on humans, animals and plants. Fulvic acid in humans and animals transports the heavy metals out of the body and improve the absorption of the nutrients. As for the effects on plants, humic and fulvic acid help the plants not only to grow up faster but also to be more resistant in diseases.

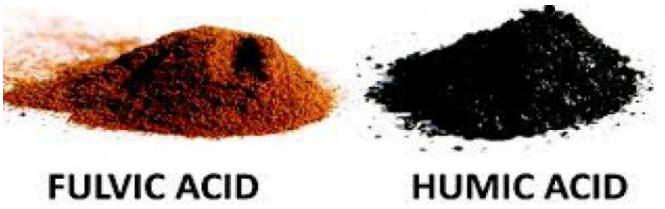


Figure 2: Appearance of the fulvic and humic acid.

The presence of humic substances in soil and water can affect both by increasing in intensity of color, in degree polymerization, in molecular weight and in carbon content. On the other hand, humic substances can also decrease in soil and water in oxygen content, in exchange acidity and in degree of solubility.

CHAPTER 2

BACKGROUND

In chapter 2, polycyclic aromatic hydrocarbons and surfactants are described. Also, information is presented about chromatography, spectroscopy and solid phase extraction. Specifically, the detection in liquid chromatography and the detectors are discussed.

2.1 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic Aromatic Hydrocarbons consist of hundreds of separate chemicals that occur together as mixtures. PAHs are a diverse group of organic compounds which consist of two or more fused aromatic rings made up of carbon and hydrogen atoms which they create multiple rings.

PAHs are nonpolar and do not dissolve in water. They can be found in the environment by the burning of fossil fuels or by the incomplete burning of organic substances such as coal, oil, natural gas and wood. PAHs also could be found by burning processes in wastes using for heating and incineration. PAHs are not dissolved easily in the water, so these compounds could stay for long in the bottom of lakes or rivers. PAHs have complex chemical structures, as a result they cannot break down easily and they are resistant in the environment [1]. There are sixteen (16) compounds of polycyclic aromatic hydrocarbons that have been characterized as high priority pollutants by the United States Environmental Protection Agency. Below, in figure 3, their names and their chemical structures are presented.

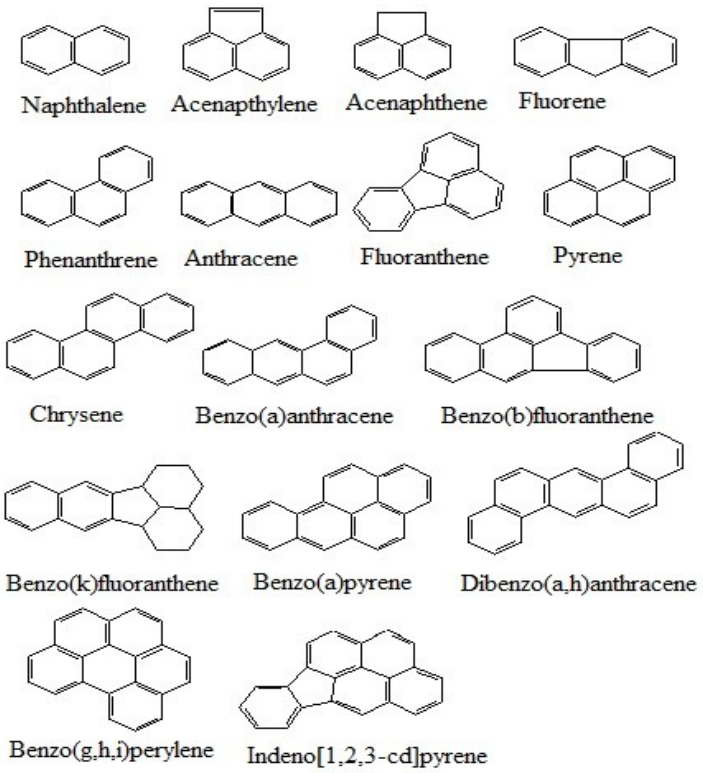


Figure 3: The sixteen PAHs depending on the number of the aromatic ring that they constitute

Each of the above types of PAHs have different aromatic rings, some of them are much more complicated than others. For example, the simplest PAH is naphthalene that has two aromatics rings and phenanthrene that has three aromatic rings in contrast to benzo(g,h,i)perylene that has six aromatic rings. PAHs consisting up to six aromatic rings are called small PAHs and those consisting more than six aromatic rings called large PAHs [9].

Eight PAHs have been listed by the Directive of European Union [10] and in the following table their Maximum Allowable Concentrations (MAC) in ppb are presented for inlet and other surface water. Five of them (presented in bold letters) are recognized as highest priority pollutants:

Table 1: PAHs with Maximum Allowable Concentrations.

PAH	MAC inland surface waters*	MAC other surface waters
Anthracene	0,1	0,1
Naphthalene	130	130
Fluoranthene	0,12	0,12
Benzo(a) pyrene	0,27	0,027
Benzo (b) fluoranthene	0,017	0,017
Benzo (k) fluoranthene	0,017	0,017
Benzo (g,h,i) perylene	0,0082	0,00082
Indeno[1,2,3-cd]pyrene	-	-

*Rivers and lakes and related artificial or heavily modified water bodies.

Generally, most surface waters contain individual PAHs at levels up to 0,05 μg/L (ppb), but highly polluted rivers can have concentrations of up to 6 μg/L (ppb) [13].

2.1.1 Physical and chemical properties of polycyclic aromatic hydrocarbons

In their purest form, PAHs are solid with high molecular weight and have different appearance, some of them are white or pale yellow green and sometimes they might be colorless. The most important physical and chemicals properties of PAHs are the following:

Molecular weight
Melting point
Boiling point
Water solubility
Vapor pressure
Henry's Law Constant
Log K_{ow}

PAHs have usually high melting and boiling points. Most of the times the melting points are over 100 ^{0}C and the boiling points can pass over the 200^{o}C. Moreover, PAHs have low vapor pressure and low water solubility. Consequently, the determination of PAHs in aqueous samples is difficult, as their concentration in water is extremely low due to their poor solubility. The vapor pressure tends to decrease as the molar mass increased but increases as the temperature increased. Also, the solubility of PAHs in water decreases as it changes their molecular weight and the number

of the aromatic rings increases [11]. Table 2 below shows the chemical formula, the molecular weight, the melting and the boiling point, the vapor pressure and the log K_{ow} of each one of the nine PAHs that have been characterized as highest pollutants in the surface water.

Table 2: Physical and chemical characteristics of some popular PAHs in the water pollution (US EPA, 1995; ATSDR, 1995).

PAHs	Chemical Formula	Molecular weight	Melting point ^{0}C	Boiling point ^{0}C	Vapor pressure (mm Hg)	Log K_{ow}
Naphthalene	$C_{10}H_8$	128,17	80,26	218	0,087	3,29
Acenapthylene	$C_{12}H_8$	152,20	92-93	265 - 275	0,029	4,07
Anthracene	$C_{14}H_{10}$	178,2	218	340-342	$1,75*10^{-6}$	4,45
Fluoranthene	$C_{16}H_{10}$	202,26	110,8	375	$5*10^{-6}$	4,9
Benzo(b) fluoranthene	$C_{20}H_{12}$	252,3	168,3	No data	$5*10^{-7}$	6,04
Benzo(k) fluoranthene	$C_{20}H_{12}$	252,3	215,7	480	$9,59*10^{-11}$	6,06
Benzo(a) pyrene	$C_{20}H_{12}$	252,3	179-179,3	495	$5,6*10^{-9}$	6,06
Benzo(g,h,i) perylene,	$C_{22}H_{12}$	276,34	273	550	$1,03*10^{-10}$	6,50
Indeno1,2,3-cd] pyrene	$C_{22}H_{12}$	276,3	163,6	530	10^{-10}-10^{-6}	6,58

2.1.2 Polycyclic aromatic hydrocarbons in the Ultraviolet

PAHs have very particular and characteristic Ultraviolet absorbance spectra. UV spectra are uniquely capable of identifying specific PAH molecules. Each ring structure has a unique UV spectrum, as a result each PAH has a different UV absorbance spectrum fact that helps in the identification of each PAH.

Most PAHs are also fluorescent, emitting characteristic wavelengths of light when they are excited [14]. Thus, analyzing PAHs in the ultraviolet can be the best way to identify each one of the PAHs based on their spectrum.

2.1.3 Sources

Generally, PAHs formation can be natural such as forest fires, volcanoes, erosion of sedimentary rocks. Therefore, some PAHs are produced naturally in the environment from chemical or biological transformation of natural organic matter, or from biological processes. PAHs can also be formed by human activities (anthropogenic formation) such as incinerators, smoking, petroleum products, power plants, house heating or its incomplete combustion such as petroleum products and incomplete combustion of fossil fuels, biofuels or other forms of organic matter [15].
PAHs can be separated in three different groups depending on the sources that they are formed:

Pyrogenic PAHs that are formed during the process of pyrolysis, where organic substances are exposed to very high temperatures with low oxygen, or by biomass combustion. These hydrocarbon compounds are associated with the combustion of petroleum, wood, coal. Industrial activities such as coke and steel production have released large amounts of pyrogenic PAHs. Moreover, pyrogenic PAHs are often associated with sediments. Pyrogenic PAHs are usually met in urban areas. Fluoranthene, pyrene and phenanthrene could be characterized as pyrogenic PAHs.

Petrogenic PAHs that are formed during crude oil maturation. These hydrocarbon compounds are associated with petroleum. PAHs from petrogenic sources are not persistent and do not accumulate in sediments. Petrogenic PAHs are formed in the aquatic environment through accidental oil spills, municipal and urban runoff, etc. Naphthalene is present in the highest concentration in crude oil [15]. Phenanthrene, fluorene, and chrysene are also three of the sixteen PAHs that belong to this category. The rate of the petrogenic PAHs degradation decreases as the number of rings increases, for instance naphthalene > fluorene > phenanthrene.

Biogenic / Diagenetic PAHs that are formed during the degradation of vegetative matter. They are produced during the slow transformation of organic materials in lake sediments. Biogenic PAHs are produced by plants, phytoplankton and microorganisms. Perylene is characterized as a biogenic PAH. In addition benzo[b]fluoranthene, phenanthrene and naphthalene may come from land plants [15].

2.1.4 Exposure to Polycyclic aromatic hydrocarbons

Exposure to PAHs is too easy. People are exposed to mixtures of PAHs almost every day by breathing air contaminated with motor vehicle exhaust, cigarette smoke, wood smoke, or fumes from asphalt roads. Moreover, PAHs can be spread into the humans' body from the grilled meat or cereals and vegetables or from foods on which PAHs particles have been inside from the air.

PAHs can cause to people diseases on their skin, irritate the eyes [16]. In addition, the most significant effect of PAHs toxicity to humans is cancer. Increased incidences of lung, skin, and bladder cancers are associated with occupational exposure to PAHs. In the following table is described which of the PAHs are carcinogenic or not. The first column in table 3 is the name of each of the sixteen PAHs and the second column present the carcinogenicity of each one.

Table 3: The effect of each of the sixteen PAHs toxicity (From Neff, 1979; CCREM, 1987; NRCC, 1983; USPHS, 1990).

PAHs	CARCINOGENECITY
Naphthalene	Non Carcinogenic
Acenaphthylene	Non Carcinogenic
Acenaphthene	Non Carcinogenic
Fluorene	Non Carcinogenic
Anthracene	Non Carcinogenic
Phenanthrene	Non Carcinogenic
Fluoranthene	Non Carcinogenic
Pyrene	Non Carcinogenic
Benzo(a)anthracene	Carcinogenic
Chysene	Weakly Carcinogenic
Benzo(b)fluoranthene	Carcinogenic
Benzo(a)pyrene	Strongly Carcinogenic
Indeno(1,2,3-cd)pyrene	Carcinogenic
Dibenzo(a,h)anthracene	Carcinogenic
Benzo(g,h,i)perylene	Non Carcinogenic

2.1.5 Uses of PAHs

Even though PAHs can be harmful to human health, they are used in pharmaceuticals, agriculture and chemical industries. For instance, acenaphthene, phenanthrene and fluorene produce plastics, pesticides and pharmaceuticals products. Some others might be found in the asphalt that is used for the construction of roads.

Also, products that are made of rubber or plastic tool and bicycle handles, shoes, or sports items or tyres may contain PAHs. They are added to the rubber in order to achieve the desired elasticity. Moreover, recycling products from used tyres, road building materials and roof coverings include

PAHs. They are also used as active ingredients in wood preservatives and in protective coats, coatings, and adhesives [17].

2.2 Surfactants

A surfactant is defined as a molecule of dual character (a polar and a nonpolar group). The polar group is hydrophilic and may not be ionized. The nonpolar group is hydrophobic and is made up of a hydrocarbon chain [18].

Surfactants generally are products which are used extensively in different fields in daily life, like cosmetics, paper or in households, industries and institutes [19].

The surfactants are classified according to their dissolution in the water in the following three categories [20]:

Anionic surfactants are dissociated in water in an amphiphilic anion and a cation which is in general an alkaline metal (Na^+, K^+) or a quaternary ammonium. These surfactants are used mostly. If the hydrophile of the surfactant is ionized, then the surfactant is an anionic surfactant and depends on the sign of the charge [18]. On this category of surfactants are the soaps and other carboxylates. Also, in this category are the sulfates which are included in many products for domestic and industrial use such as shampoos, toothpastes, bath creams. As well, the sulfonates which are used in lubricating oils and dry cleaning products, are anionic surfactants. Moreover, in this category are included the lingosulfonates which come from the reaction of wood lignin with bisulfite or sulfate ions during the wood digestion reaction to make the pulp and they are used as clay dispersants in drilling fluids.

Nonionic surfactants are these surfactants that do not ionize in aqueous solution, such as alcohol, phenol, ether, ester, or amide. If the hydrophile is not ionized, then the surfactant is called a nonionic surfactant [18]. They are used in pharmaceuticals, cosmetics and food products. Nonionic surfactants can be found in domestic and industrial products, such as powdered or liquid formulations

Cationic surfactants are dissociated in water into an amphiphilic cation and an anion. As in anionic surfactantas, the hydrophile of the surfactant is ionized in this category too [18]. These surfactants come from natural fatty acids and they are more expensive than anionics. As a result, cationic surfactants are used as bactericides to clean surgery hardware, like desinfectants for domestic and hospital use. These surfactants could sterilize food bottle or containers, usually in industries. Also, they are used as emulsifiers in asphaltic emulsions and coatings, in inks, wood pulp dispersions, magnetic slurry [20].

Amphoteric surfactants have both anionic and cationic centers attached to the same molecule. The anionic part can be variable and include sulfonates. The cationic part is mostly primary, secondary or tertiary amines or quaternary ammonium ions. They are sensitive in pH and behave as anionic or cationic depending on pH.

2.2.1 Anionic surfactants

The linear alkylbenzene sulfonates (LAS) belong to the category of anionic surfactants. They represent the greater portion (50%) at today's world surfactant market. It is rapidly biodegraded under aerobic conditions. LAS toxicity to fish and aquatic organisms is affected by chain length and phenyl ring position. LAS toxicity is decreased as the carbon chain length decreases and as the phenyl group is positioned away from the end of the alkyl chain. The biodegradation of LAS is slower in sea water than in fresh water.

The Directives EC 73/404, 1973a, EC 73/405, 1973b and EC 82/242, 1982a, EC 82/243, 1982b lay down that all anionic and non ionic surfactants present in washing, rinsing and cleaning

products must be tested for primary biodegradation only by the methods that are presented in the Directives. They have to be biodegraded not less than 90% [21].

2.2.1.1 Sodium Dodecyl Benzene Sulfonate (SDBS)

Sodium Dodecyl Benzene Sulfonate (SDBS) is an anionic surfactant soluble in the water, member of the linear alkylbenzenesulfonates (LAS). It is produced as a mixture of sulfonates and it is the major component of laundry detergent cleaning products and in pesticides. Sodium Dodecyl Benzene Sulfonate is white to light yellow flakes, granules or powder.

Moreover, these surfactants are soluble and effective at low concentrations. Sodium Dodecyl Benzene Sulfonate is sensitive to water hardness and not easy to oxidation. It is foam and it can combine with various additives. Sodium Dodecyl Benzene Sulphonate is known for its properties, such as detergency, moistening, foaming [22, 23].

In table 4, some properties about Sodium Dodecyl Benzene Sulfonate are presented, such as the molecular formula and weight, boiling and melting point and solubility. At figure 4, its chemical structure is presented.

Table 4: Physical and chemical properties of Sodium Dodecyl Benzene Sulfonate [24].

Physical and chemical properties	**Values**
Appearance	Solid/Powdered solid
Molecular formula	$C_{18}H_{29}NaO_3S$/ $C_{12}H_{25}C_6H_4SO_3Na$
Molecular weight	348.49 g/mol
Boiling point	Decomposes
Melting point	144.5 ^oC
Solubility	Not available

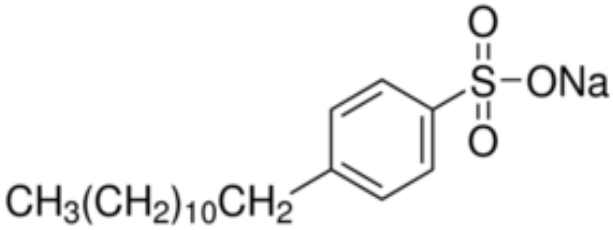


Figure 4: Chemical structure of Sodium Dodecyl Benzene Sulfonate.

Sodium Dodecyl Benzene Sulfonate can affect the human health by breathing in it. This can irritate the nose and the lungs having as a consequence coughing, wheezing or shortness of breath. Also, the contact with this surfactant can irritate the skin and the eyes causing eye damage. In addition, Sodium Dodecyl Benzene Sulfonate decomposes on heating and reacts with acids and acid fumes. Thus, toxic and irritating fumes are produced (sulfur oxides).

There is no limitation on the concentration of SDBS in water, except from the concentration of anionic surfactants measured as MBAS. The maximum permissible concentration of surfactants in drinking water is 0,5 mg/L MBAS.

High Performed Liquid Chromatography HPLC is a good method for analyzing many compounds such as PAHs, surfactants and other categories of the compounds either with low molecular weight either with high, but also to separate ionic and non-ionic surfactant species.

2.3 Chromatography

Chromatography was discovered as an analytical technique in the early twentieth century (1901) and firstly it was used as a method of separating colored compounds. The name Chromatography comes from the greek word 'chroma' which means color, and the word 'graphy' which means writing. The first appearance of the liquid chromatography was by a Russian botanist named Mikhail S. Tswett where columns of calcium carbonate were used to separate mixtures of plant pigments into the pure constituents.

Chromatography is a technique which separates organic and inorganic compounds of a sample in order to figure out what makes this compound. It is a method that is suitable for observing and analyzing mixtures and solvents and it is used to determine unknown substances. With this method the separation of the compounds is achieved by two phases, the stationary phase and the mobile phase. The stationary phase is the phase that doesn't move and the mobile phase is the phase that does move. The mobile phase passes over the stationary phase gathering the components of the compounds that are tested. This technique uses two substances. One substance, a gas or a liquid is the mobile phase, in which transports the solution that is analyzed through the other substance and a liquid or a solid is the stationary phase.

Chromatography is used in qualitative analysis, the identification of individual compounds in the sample, and in quantitative analysis, the measurement of the concentration of a compound in the sample, of biological and chemical substances [25].

There are four major chromatographic separation techniques that are used to separate most of the compounds:

Reversed-phase chromatography. The stationary phase is less polar than the mobile phase. The stationary phase consists of silicon oxide coupled to various groups such as alkyl, phenyl, diols, amino groups, cyano groups and the mobile phase is composed of organic solvent mixtures like methanol, acetonitrile and water. In this phase, there is a strong attraction between the polar solvent and polar molecules in the mixture being passed through the column. The polar molecules travel through the column more quickly than the others [26, 27].

Normal-phase and adsorption chromatography. The stationary phase is more polar than the mobile phase, which consists of non polar solvents. The column is filled with tiny silica particles, and the solvent is non-polar.

Ion exchange chromatography. The separation of the compounds happens due to the electrostatic interactions between the ions and the charged groups of the stationary phase.

Size exclusion chromatography. The separation is based on the shape and the size of the molecules. This separation mode uses in the analysis and the characterization of the polymers. The large molecules first exit the column, and the small molecules delay and exit later [27].

According to the kind of sample that is going to be analyzed, there are four main types of chromatography: Liquid Chromatography, Gas Chromatography, Thin-Layer Chromatography and Paper Chromatography.

Liquid Chromatography examines the water pollution in lakes or rivers by using only liquid samples. It is used to analyze metal ions and organic compounds in solutions. Liquid chromatography uses liquids which may incorporate hydrophilic, insoluble molecules.

Gas Chromatography is used in airports to detect bombs and is used is forensics to compare fibers that have been found on a victim. It is used to analyze fibers on a persons' body and also analyze blood found at a crime scene. In gas chromatography helium is used to move a gaseous mixture through a column of absorbent material.

Thin-layer Chromatography uses an absorbent material on flat glass or plastic plates. This is a simple method to check the purity of an organic compound. It is used to detect pesticide or insecticide residues in food. Thin-layer chromatography is also used in forensics to analyze the dye composition of fibers.

Paper Chromatography is one of the most common types of chromatography. It uses a strip of paper as the stationary phase. Capillary action is used to pull the solvents up through the paper and separate the solutes. It is used to separate histamines and antibiotics [25].

2.3.1 High Performance Liquid Chromatography (HPLC)

By using High Performance Liquid Chromatography, chemical and biological compounds, that are non-volatile, are separated. A small amount of a liquid sample is injected into a tube with porous particles, which are columns who contains the stationary phase. After the loading of the sample, solvents are added, and individual components of the sample are moved through the tube. During the movement of the compounds, interactions between their molecules are generated and finally they are separated until their exit from the tube. There exists a flow-through device, which is called detector that measures their amount and gives the liquid chromatogram [27].

2.3.1.2 How Does High Performance Liquid Chromatography Work

At the beginning, during the mobile phase the solvent is holded in the reservoir. A high-pressure pump is used to generate and drives the sample through the column while a specified flow rate of mobile phase is used in order to reduce the variation in the elution. The sample is injected into the mobile phase from an injector that sends the sample to the HPLC column. The column contains the chromatographic material which is needed for the separation. This material is called the stationary phase. A detector which is located at the end of the HPLC column, is needed to detect the separated compounds as they elute from the HPLC column. The mobile phase exits the detector and can be sent to waste or collected [28].

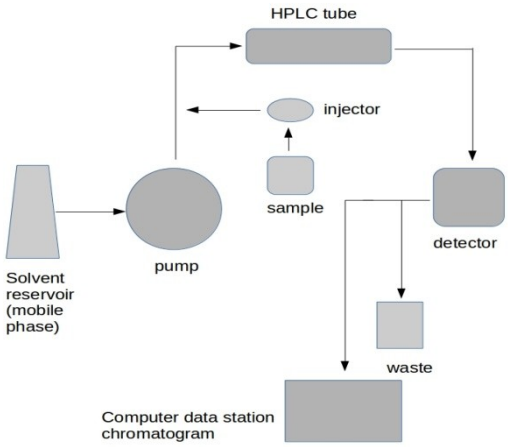


Figure 5: A scheme of how exactly the HPLC works.

The detector is connected with the computer data station, that records the electrical signal in order to generate the chromatogram on its display and to identify and quantitative the concentration of the sample constituents. The output signal that is recorded represents a series of peaks of each compound. Since the sample compound characteristics can be very different, several types of detectors have been developed. For example, if a compound can absorb ultraviolet light, a UV-absorbance detector is used. If the compound fluoresces, a fluorescence detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an evaporative-light-scattering detector [ELSD].

The most powerful approach is the use of multiple detectors in series. For instance, in this thesis a fluorescence detector and a UV-absorbance detector have been used.

2.3.1.3 Chromatogram

A chromatogram is a representation of the separation that has chemically occurred in the HPLC system. It is the signal that the computer station represents.

A series of peaks rising from a baseline is drawn on a time axis. Each peak represents the detector response for a different compound. The chromatogram is plotted by the computer data station. Below, at the figure 4, is a typical chromatogram that shows the signal from the detector. In this chromatogram is the absorption (mAU), as a function of the time (min) that each sample needs to inject to the detector [29]. At the x axis is the time that the substance is eluted in the chromatographer and in y axis is the absorption of each substance.

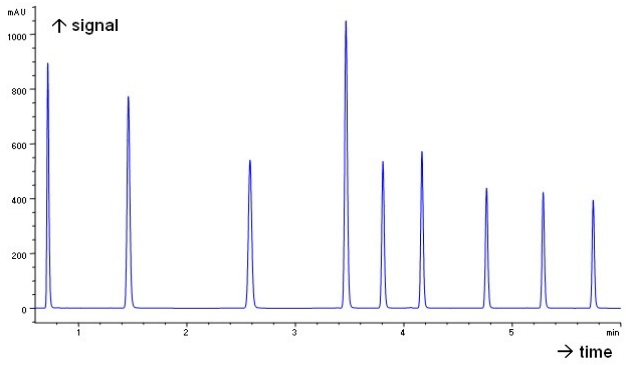


Figure 6: A typical chromatogram of absorption vs. time [29].

2.3.1.4 Retention time – dead time

Retention time and dead time are two main terms in chromatography which are important for the identification of a substance. Both times are recognized in a chromatogram.

The time that a particular compound needs to move from the injector to detector called retention time (t_r). This time is estimated by the time that the sample is injected inside the column until the time that the chromatogram shows the highest peak for that compound. Every compound has its own retention time, in other words the different compounds have different retention times. So, knowing the retention time of a substance helps to identify the peaks. The retention time depends on the pressure, the nature of the stationary phase, the composition of the solvent and the temperature of the column [26].

The time that needs to reach the detector the non retained substance is called dead time (t_m). Figure 7 below, shows how exactly the retention time and the dead time of a compound appear in a chromatogram.

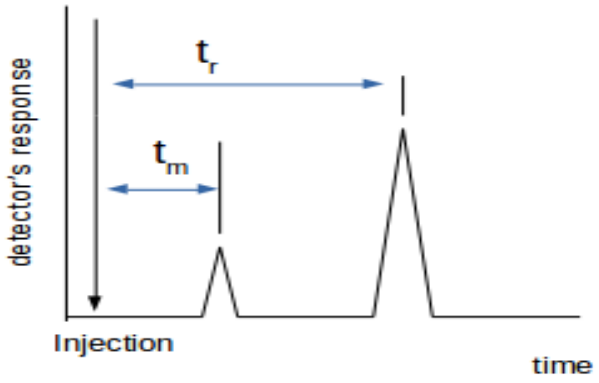


Figure 7: Dead time and retention time.

2.3.1.5 Detection in HPLC

The detection in High Performance Liquid Chromatography uses the chromatography detectors. These devices are able to detect the components of a mixture that have being eluted the chromatography column and finally identify the compounds of a mixture. The detectors sense the presence of the compounds as they elute the column and they give an electric signal to a data station. The detectors in HPLC should be non-distructive, have high sensitivity and stability [30].

(I) Ultraviolet Absorption (UV)

The ultraviolet detector in HPLC measures the absorbance of monochromatic light of wavelengths from 190 nm until 400 nm. An ultraviolet light beam is directed through a flow cell and there is a sensor that measures the light passing through the cell [27]. The amount of the light energy that falls on the sensor will change if the compound elutes from the column that absorbs. This change in the energy drives to a data system and it is recorded. The canal in the HPLC that is recorded the signal of the adsorption is the diode-array detection (DAD) in which a photo diode array detect the light in individual wavelengths. Each photo diode receives a different wavelength and the adsorption of each one and finally a chromatogram with these data is given [31].

(II) Diode Array Detector (DAD)

UV detectors include the diode array detectors. A lamp emits light in the visible range and then it passes from a deuterium lamp, where UV and visible light are added. The polychromatic beam passes the flow shell. The grating splits up the polychromatic beam, which separates into different wavelengths. The intensities of each one are measured by an array or a photodiode. The DAD has high sensitivity and does not need a reference diode [32].

These detectors are suitable for detecting organic molecules that absorb light in the frequency range of the ultraviolet and visible. The absorption of the radiation by a compound depends on the wavelength of the radiation and the groups of the compound.

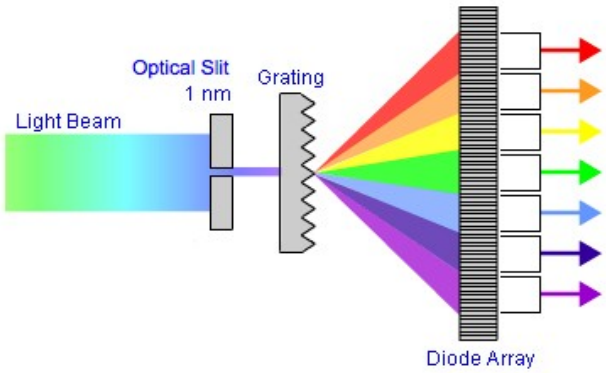


Figure 8: A Diode Array Detector.

(III) Mass Spectroscopy (MS)

A mass spectroscopy detector first ionizes the compound that has been eluted and then it measures the mass of the compound and breaks the molecules of it into smaller pieces. An advantage of this spectroscopy is that sometimes the detector can identify directly the compound because the mass spectrum is unique to every compound.

(IV) Refractive Index Detection (RI)

The Refractive Index detection measures the ability of the molecule to deflect light in a flowing mobile phase. The amount of the deflection depends on the concentration of the compound. Although the RI detector is a worldwide detector but it is not very sensitive.

(V) Fluorescence detection

Fluorescence is the emission of light by a substance that has absorbed electromagnetic radiation. Fluorescence usually appears as visible light and occurs when the exciting radiation is in the ultraviolet or blue light.

When an electron transferred from the excited state to the ground state and light is emitted to it then it is transferred to a higher state. Usually the emitted light has lower energy than the absorbed and the wavelength of fluorescence is longer than the adsorption wavelength [33]. If a molecule emits light 10^{-9} to 10^{-5} seconds after it was illuminated then the process was fluorescence. Fluorescence analysis is suitable for analytes that can be dissolved in solvents like water.

Fluorescence detectors are more sensitive than the others and offer much more selectivity. That's why they can identify compounds at very low concentration levels. Fluorescence detectors find out only those substances that fluorescence.

When the lamp flashes the fluorescing compounds in the sample will luminescence almost simultaneously. The fluorescence detector need only measure over a short period of time after the lamp has been flashed. Fluorescence detector excites the sample with a specified wavelength of light. As a result the compounds fluoresce and emit light at very high wavelengths. A sensor collects only all the emitted light.

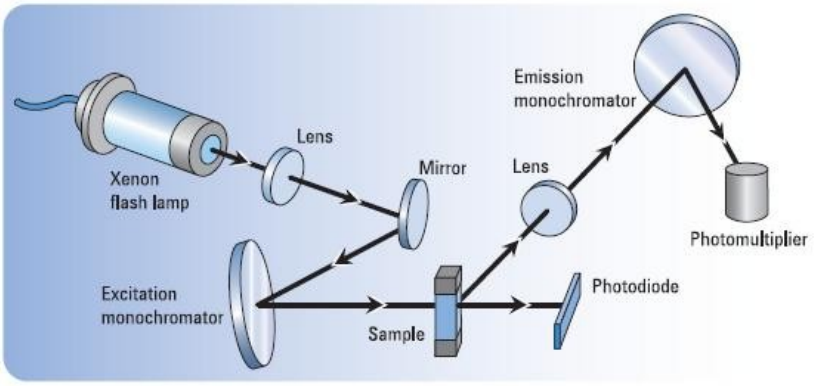


Figure 9: How a fluorescence detector works.

The light from an excitation source passes through a monochromator and strikes the sample. A part of the light is absorbed by the sample and some of the molecules in the sample fluorescence. The fluorescence light emitted to all directions. Some of this fluorescent light passes through a second monochromator and reaches a detector. Fluorescence detector is much more sensitive than visible-ultraviolet detector and when the compound fluoresces, it gives better results.

2.4 Optical spectroscopy

The term spectroscopy was first invented in 1666, when Sir Isaac Newton demonstrated the dispersal of white light through a prism into different colors.

Spectroscopy is the study of interaction between matter and electromagnetic radiation in order to obtain data on the properties of matter. It is a technique that measures the interaction of the light which is emitted, absorbed or scattered by materials. By analyzing the amount of the light which is absorbed or emitted by a sample, we can determine what it's made of and how much of it there is. The techniques that are used in spectroscopy can be classified by the wavelength region of the spectrum, microwave, radiofrequency, infrared, near infrared, visible and ultraviolet, x-ray and γ spectroscopy.

In other words, two things could happen when light hits a sample, either absorption or emission. In absorption, the sample absorbs some of the energy from the light and in emission, the sample is hitted with some light and it emits light of a different wavelength. The percentage of the monochromatic radiation that has been absorbed by the solution of a substance is a function of the concentration of the solution, the light path into the solution, the nature of the solute and the wavelength of the radiation and is given by the Lambert-Beer law:

$$\log I_0 / I = K\, C\, l$$

I_0 = the intensity of the radiation which is incident to the solution.
I = the intensity of the radiation which comes out of solution.
K = the absorption rate.
C = the concentration of the substance in the solution.
l = the length of the distance that makes the radiation into the solution.
$\log I_0 / I$ = absorbance (A)

Spectrophotometer is the instrument that used to measure the intensity of wavelengths in a spectrum of light compared with the intensity of light from a standard source [34]. There are two types of spectrophotometer depending on the range of wavelength of light source [35] :
(a) a UV-visible spectrophotometer, which uses light over the ultraviolet range (190 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum and (b) a IR spectrophotometer, which uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum.

Absorption and fluorescence spectroscopy have been used in this thesis, therefore below are some information of each one respectively.

2.4.1 UV/Vis Absorption spectroscopy

The absorption spectroscopy refers to Ultraviolet and visible (UV-Vis). UV-Vis absorption spectroscopy is the measurement, by using UV spectrophotometer, of a beam of light after it passes through a sample or after reflection from a sample surface. The measurements of the absorption can be at a single wavelength or over an extended spectral range. The diagram in figure 10 shows how a UV spectrophotometer works. The source passes through the wavelength selector and the suitable wavelength is chosen. Subsequently, the sample is injected, passes through a photo detector and the data are collected to a computer.

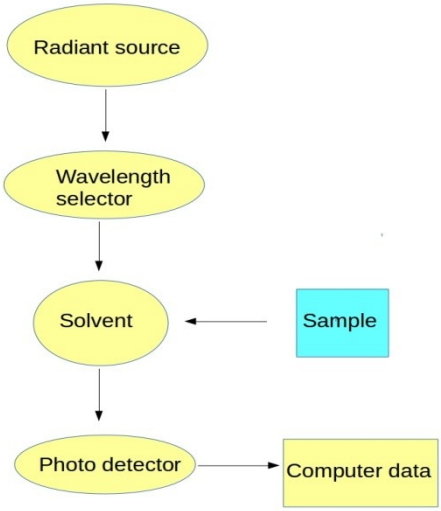


Figure 10: Diagram of how a UV spectrophotometer works.

Absorption spectroscopy complementary to fluorescence spectroscopy; absorption measures the changes from the ground state to the excite state in contrast to fluorescence that measures the changes from the excited state to the ground state. In the following figure, an absorption spectrum and a fluorescence spectrum are presented.

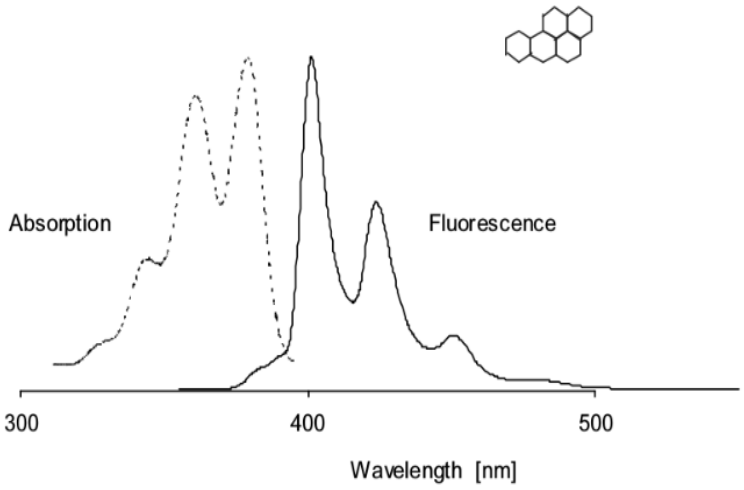


Figure 11: A chromatogram of the absorption and the fluorescence spectra of benzo(a)pyrene.

The absorption spectra and the fluorescence spectra are similar, as the figure 11 shows. First the absorption spectrum appears and then the fluorescence spectrum follows. They have almost the same shape in different wavelengths.

2.4.2 Fluorescence spectroscopy

Fluorescence spectroscopy is a technique which is used for environmental analyses and especially in High Performance Liquid Chromatography due to its ability in measurement of even very low concentrations of particular compounds. The detection with a fluorescence detector has higher sensitivity (10-1000 times higher that the UV detector) and higher selectivity than others and has also the adaptability to field measurements [36].

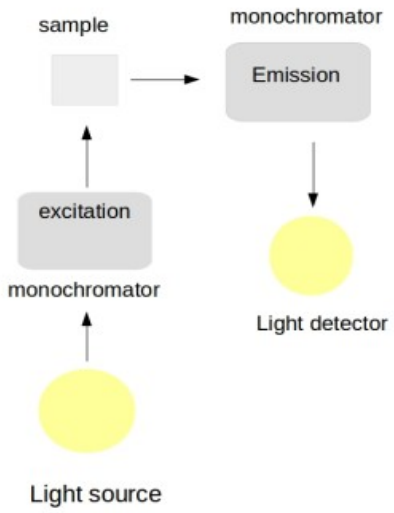


Figure 12: Diagram of detection by using a fluorescence detector.

It is necessary to select an excitation and an emission wavelength when a fluorescence detector is used. The detection of any compound depends on the wavelength that has been chosen in the fluorescence detector because each compound can be detected at different wavelength, 280 or 340 nm, etc. Otherwise, the compound could be missed if the wavelength is not the right in order to detect the substance [37]. Fluorescence occurs when an atom or a molecule passes through vibrational relaxation to its ground state. The particular frequencies of excitation and emission are dependent on the molecule or atom.

$$S_o + hv_{ex} = S_1$$

where, hv is a photon energy (h is the Plank's constant and v is the frequency of the light), S_o is the ground state and S_1 is the first excites state. The fluorescence quantum yield (Φ) gives the efficiency of the fluorescence process. It calculated by the following equation:

$$\Phi = emitted\ photons\ /\ absorbed\ photons$$

The maximum fluorescence quantum yield is 1. The fluorescence quantum yield (Φ) could also be estimated by the excited state decay rates:

$$\Phi = k_f / \Sigma_i k_i$$

where k_f is the rate of spontaneous emission of radiation and the denominator is the sum of all rates of excited state decay for each deactivation process [38].

In the following figure, an excitation and an emission chromatogram appear. The excitation happens at the firstly wavelengths and the emission follows to the next wavelengths. The excitation reflects most 1st order light in the ultra violet range around 250 nm since the emission reflects better in the visible range around 400nm. Fluorescence always occurs after a little time of the excitation to high wavelengths has occurred [39].

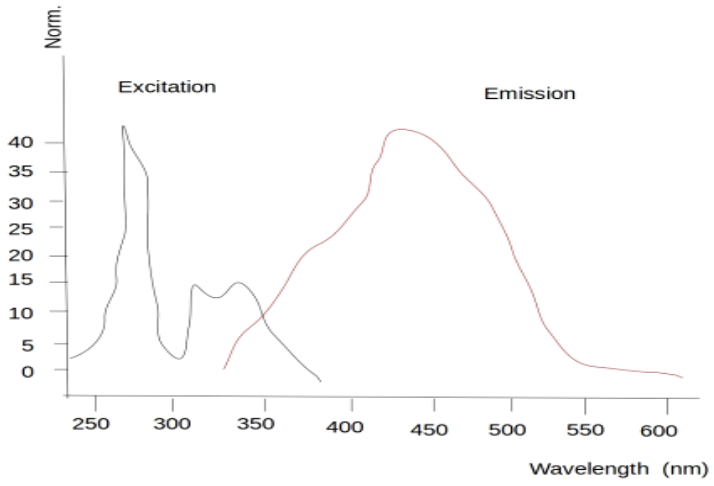


Figure 13: Excitation and Emission spectra.

The advantages of the fluorescence spectroscopy are that the sample needs little time for preparation and the results are fast due to the sensitive of the fluorescent detector which can detect contamination in very low ppb. The use of fluorescence detectors allows measurements in both

excitation and emission spectra, fact that could help to the identification of a substance. For a suitable fluorescence spectrum, the excitation wavelength is set to the desired analytical wavelength and the emission is measured over the UV-VIS measuring range.

Fluorescence of a chromophore is influenced by factors such as temperature, pH, hydrogen-bonding, metal ions and by the presence of other solutes. These factors influence fluorescence intensity and quantum efficiency.

2.5 Solid-Phase Extraction

Solid phase extraction (SPE) is basically used for sample preparation. This technique is popular for the extraction and isolation of a compound from a complex matrix. In this thesis, solid phase extraction has been used as a preparation of the samples before using Liquid Chromatography.

Solid phase extraction is an extraction technique based on the selective separation of one or more components between two phases, one of which is a solid sorbent (known as stationary phase) and the other phase usually is a liquid (known as a mobile phase), where the components that are analyzed adsorb to the solid. The two phases are separated by filtration or decanting or a similar process. When these components are adsorbed on the solid phase, they are desorbed by washing with one or more solvents [40]. Figure 14 below shows the procedure that is followed to the solid phase extraction.

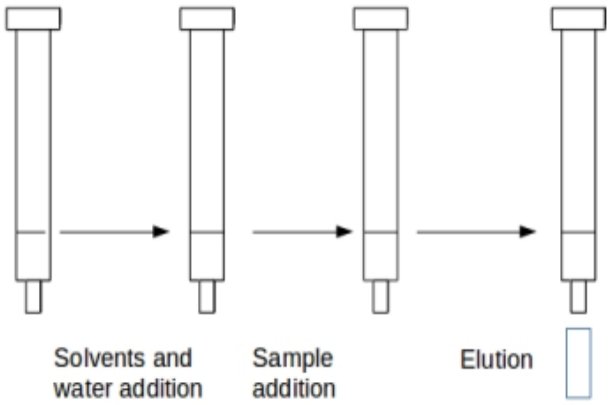


Figure 14: Solid phase extraction process.

The solid sorbent, which is used during the solid phase, is packed into a tube/syringe, as it appears in figure 14, in order to form a bed with good flow distribution characteristics. From this bed, the analyzed liquid sample passes. Subsequently, after the whole liquid sample has been passed, the solvents pass through the bed and the compounds are eluted.

There are four steps of solid phase extraction method, as figure 15 indicates. The first step is the conditioning (a), in which the sorbent is more compatible with the sample solution. The second one is the adsorption (b), in which the sample is injected into the syringe. The third step is the washing (c), in which the wash solution elutes the interferences (impurities). Finally, the fourth step is the elution (d), in which the compounds of interest are eluted. In this step belongs the removal of the solvents from the sample, usually by gas (N_2) evaporation.

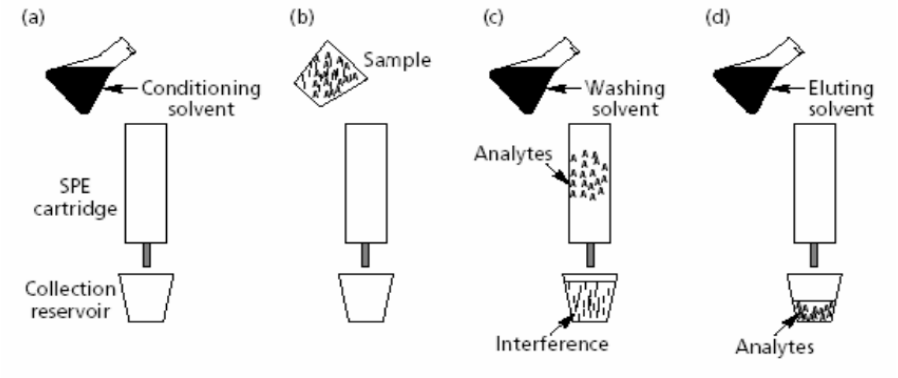


Figure 15: The four steps of solid phase extraction.

There are four primary types of solid phase extraction method [41]:

a) reversed phase,
b) normal phase,
c) ion exchange and
d) adsorption.

The separation in reversed phase involves a polar sample matrix, which is the mobile phase and a non polar stationary phase. In contrast to reversed phase, the normal phase extraction involves a non polar analyte, like acetone or hexane, during mobile phase and a polar stationary phase. In reversed phase the hydrophobic interactions between the analyte and the sorbent becomes through the van der Waals forces. In normal phase the hydrophilic interactions are between polar functional groups of the analyte and polar groups on the sorbent surface. Ion exchange can be used in compounds that are negative (anionic compounds) or positively (cationic compounds) charged. This extraction type contains the electrostatic attraction of the charged functional group on the compound to the charged group. Finally, in the last type which is the adsorption, hydrophobic and hydrophilic interactions could be applied, depending on which solid phase is used each time.

The efficiency of the method depends on the sample volume, the degree of contamination, complexity of sample matrix, quantity of compounds of interest and the type and solvent strength of sample matrix [41].

There are many advantages of using the solid phase extraction technique. First of all, a major benefit is the sample simplification. The large number of interfering substances in a sample matrix usually makes the analysis very difficult. The compounds of interest could be analyzed better due to the fact that the interferences are simplified with this technique. Another benefit is the matrix effects reduction. With this technique ion suppression is reduced and is enhanced in mass spectrometer applications. The interferences from the sample matrix are removed with the solid phase extraction, so the compound ion can form properly. This method also cleans away the interferences from the compound, minimizes with this way the ion enhancement. Moreover, the solid phase extraction method can separate the different classes of the compounds, such as their polarity. Also, an important advantage of this technique is that compounds with extremely low concentration could be analyzed [42]. Therefore, it is a technique with low cost, non toxic and free from impurities.

CHAPTER 3

METHODOLOGY AND EXPERIMENTAL SETUP

In this chapter, the study area, and the sampling points are presented firstly. Subsequently, the analytical methods for the determination of physicochemical parameters are described. Also, the overall preconcentration process of samples prior to liquid chromatography. The parameters of the liquid chromatography method are analyzed.

3.1 Study area

The study area, as in chapter 1 referred, is Almiros river (Fig. 16). The water of the river penetrates into deep and is mixed with the seawater. Its catchment area is not defined due to the complex geology and hydrogeology. There are different approaches about the size that according to Arbif [43] is 300 km^2 and according to Lambrakis [44] is 500 km^2. The wetland of Almiros includes the spring, the river flow and the artificial channel of the power plant. The spring is in the bottom of a lake with diameter 70-90 m and depth more than 20 m. The flow rate of the spring ranges from 4 m^3/s during hot seasons to 20 m^3/s [45] and according to Maramathas [46] it reaches 70-80 m^3/s during cold seasons. Reed-beds, tamarisk trees, sandy coastal zone and built-up areas are also included.

Figure 16: Picture of Almiros river (The red arrows present the route that Almiros flows).

3.2 Sampling and sampling points

Nine samplings have been accomplished on January, March, April, May, June, July, August, October and November at four different points across the river each month (Fig. 17).

Figure 17: The four sampling points on map of Almiros river.

In more detail, the first point was near the karstic spring and after the dam that has been constructed to control the flow rate as it seems to figure 18 (point 1). The second point was situated near touristic units and leisure activities, a parking space and as figure 19 indicates (point 2) below, lots of reeds and ducks make their appearance there. There was another one sampling point between them but it was not possible to approach it after the first two months, so it is not included in the results.

Figure 18: Sampling point 1

Figure 19: Sampling point 2

Figure 20: Sampling point 3

Figure 21: Sampling point 4

The third and the fourth points were after the artificial channel, which passes through the power plant and ends to the sea. The third sampling was in the exit of the plant, as figure 20 shows, representing the estuary of the river. The electrical power plant uses water for cooling its machines. The last point of the sampling, the fourth, was in the coastal area, where the river water has been mixed with the sea water, near the sand, as figure 21 indicates.

All the samples were collected in 2,5 L amber bottles. The bottles were prewashed with tap water, deionized water, 5% HCl solution, tap water and finally deionized water. They weren't used any detergents. The samples were analyzed immediately after the arrival in the laboratory. Otherwise they were kept at 4^{o}C. All the analyses were completed in 3 days period.

3.3 Experimental part

3.3.1 Measurement of physicochemical parameters

i. pH /EC/ TDS/ Salinity

The physicochemical parameters that were measured firstly are: conductivity, pH, salinity and Total Dissolved Solids (TDS). They were measured by using of a pH meter (Hach) connected with a pH electrode and a conductivity electrode.
The other parameters like total hardness, alkalinity and chlorides were measured with the process of titration.

ii. Hardness

The procedure in all the samples was the same for the measurement of the hardness of water. For the determination of the water hardness, 5 ml of each one of the samples were diluted with 20 ml of deionized water (5/25). Moreover, 1 ml ammonia (NH_3) (Fluka) and one indicator buffer tablet (Merck, with titriplex solutions, contains Ammonium chloride, Hexamethylenetetramine) were added in each one. The sample was titrated with ethylenediaminoacetetic acid (EDTA) 0,01 M (Fluka), until the final point in which the color was changed, from pink was become green. The volume of the EDTA solution, which has been consumed, was used to estimate the hardness by the equation:

$$mg/L\ CaCO_3 = (\ ml\ EDTA * 1000 / ml\ of\ the\ sample\ )$$

ml EDTA = the final volume minus the initial volume of the EDTA
ml of the sample = 25 ml

The result of the above equation was multiplied with 5, because the water sample was diluted five times.

iii. Alkalinity

For the determination of alkalinity the same procedure was followed for all the water samples. 50 ml of each one of the samples were mixed with three drops of bromocresol green solution

(Fluka), giving a light blue color to the solution. The sample was titrated with hydrochloric acid (HCl) (Sigma-Aldrich) until the color was changed to light yellow. The equation that has been used to estimate the alkalinity in each sample is:

$$mg/L\ CaCO_3 = ml\ HCl * N * 50000 / ml\ of\ the\ sample$$

ml HCl = the final volume minus the initial volume of the HCl
N = 0.1 , the normality of HCl
ml of the sample = 50 ml

iv. Chlorides

Chlorides in the water sample were also determined with titration. The indicator that had been used was the potassium chromate (K_2CrO_4) (Fluka) which gives to water a yellow color. For the determination of the chlorides, 5 ml of the sample and 1 ml of potassium chromate (Merck) were mixed and titrated with silver nitrate ($AgNO_3$) (Fisher). The titration was ended when the yellow color was turned to brown. The equation for the calculation of the chlorides is:

$$mg/L\ Cl^- = \alpha * 35.45 * N * 1000 / ml\ of\ the\ sample$$

N = 0.2184, the concentration of the $AgNO_3$
α = ml $AgNO_3$
ml of the sample = 5 ml

3.3.2 Data analysis

The data that were obtained from physicochemical parameters were plotted with Microsoft Excel using 3D diagrams. Also, each physicochemical parameter was plotted with Microsoft Excel using line diagrams for all the samples for each sampling period.

Moreover, they were treated by statistical data processing in Matlab in order to study the correlation between the parameters. Pearson's Correlation Coefficient (rxy) was used for analyzing the correlation between the physicochemical parameters for each month.

$$rxy = Cov(x,y) / \sqrt{Var(x)} * \sqrt{Var(y)}$$

where Cov is the covariance and Var is the variance.

$$Var(x) = \Sigma (xi - \bar{x})^2 / N - 1 = SS_{xx} / N - 1$$

$$Cov(x,y) = \Sigma (xi - \bar{x}) * \Sigma (yi - \bar{y}) / N - 1 = SS_{yy} / N - 1$$

When values of r ranging from -1 to -0,75 and 0,75 to 1, then the relation is perfect-strong relation of the two variables. variables. If the r value is in the regions -0,75 to -0,5 and 0,5 to 0,75, the two variables have a moderate relation. Finally, values of r in the ranges -0,5 to -0,25 and 0,25 to 0,5 or -0,25 to 0,25, indicate a weak or no linear relation between the two variables. This data analysis has been already used successfully for the classification of storm water and sea water samples [47].

3.3.3 Chromatographic instrumentation and conditions

The chromatograph (Agilent Technologies 1200 series) was equipped with micro-vacuum degasser, quaternary pump, diode array detector (DAD), fluorescence detector (FLD) connected in series. The column was a Zorbax Eclipse XDB-C18 with dimensions 4,6×150 mm and 5 μm particle size.

The solvents that were used, as the mobile gradient were water (H_2O) and acetonitrile (CH_3CN) (J.T.Baker, HPLC Far UV/Gradient Grade). The injection volume was 50 μL. With a small injection volume, all the sample molecules arrive at the head of the column at almost the same time and as they pass through the column the peaks remind the Gaussian shape. The flow rate was 1 ml/min and the max Pressure was 400 bar and the min Pressure was 0 bar. The method was lasted for 40 minutes and a variety of emission signals and absorption signals were used. In the following table, all the parameters that were chosen on the chromatographer are presented.

Table 5: HPLC Parameters.

Solvents	**Time**	**Acetonitrile %**
	0.00	10.0
	5.00	10.0
	15.00	70.0
	35.00	90.0
	40.00	90.0
Standard injection volume	50 μL	
Flow	1 ml / min	
Duration	40 min	

Signals

Diode array detector (DAD): For the determination of SDBS the signal at 225 nm was recorded.

Fluorescence detector (FLD): Several excitation-emission scans were performed in order to decide for the optimum excitation and emission wavelengths. Finally, the detection of the 16 PAHs was performed at excitation wavelength of 260 nm and emission wavelength of 390 nm.

3.3.4 Sample pretreatment

Prior to chromatography, the pretreatment of samples included three steps: filtration, solid phase extraction and evaporation**.** Subsequently, all the samples were filtered by a 0.45 μm filter, so that to ensure that any microorganism which might be present inside the water sample was removed. Afterwards, the filtered samples should be prepared for the analyzing by the liquid chromatography. The procedure which was followed was the condensation of each sample by using

solid phase extraction. Firstly, for conditioning 10 ml of acetonitrile (CH_3CN) (Merck, gradient grade), 10 ml of methanol (CH_3OH) (Merck, gradient grade) and 10 ml of deionized water were added in each cartridge (Oasis Hydrophilic Lipophilic Balance HLB, 200mg/ 12cc). Then 500 ml from each sample were extracted slowly (5 mL/min) in its own syringe each one of the samples. When all the volume of the sample was passed through the syringes, 5 ml of acetonitrile (CH_3CN) and deionized water (10/90) (v/v) were added for washing. For 15 min the cartridges were dried under vacuum (-15psia). Finally, the solvents were added, 4 ml of dichloromethane, 3 ml of acetonitrile and 3 ml of acetate, in each cartridge. The mixture of them was eluted in the same container.

The eluted compounds were completely evaporated with nitrogen. Finally, 0,5 ml of the evaporated sample with acetonitrile and water (50/50) were added in order to get in the liquid chromatography machine.

A blank sample with deionized water has also been run with the same procedure with the river samples. The peaks of the blank sample were subtracted from the peaks of the samples.

3.3.5 Standard samples

Standard samples of PAHs and SDBS were prepared in different concentrations from dilution of the following stock solutions:
a) 200 ppm solution of SDBS (company, technical grade) was prepared by dissolving of 0,02 gr SDBS in 100 ml deionized water (50 ml) and methanol (50 ml).
b) 10 ppm solution of 16 Polynuclear Aromatic Hydrocarbons dissolved in acetonitrile (Sigma-Aldrich).

Eight different concentrations of sixteen PAHs were made within the range 0,025-7 ppb and six concentration of samples of SDBS solution within the range 5-50 ppb for the method calibration. The standard samples were subject to the same preconcentration procedure by SPE as described for the samples. The identification of SDBS and PAH was based on the combination of spectral data and retention time.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter presents the results of the measurements of the physiochemical parameters of the river water samples and the results of the analyzing samples by liquid chromatography. First, the basic physiochemical parameters are presented as they were mentioned in Chapter 3.3.2, such as pH and alkalinity. Then the results are shown the analysis for the detection of SDBS and PAHs through HPLC.

For each water sample, the physiochemical parameters are presented that were measured directly after the sampling as well as the results of the chromatography analysis for each of our four sampling locations and for all nine samplings throughout the sampling period.

4.1 Physicochemical parameters

The values of the physicochemical parameters that were measured are shown to the following tables (table 6 - 12). In addition, the seasonal change of these parameters are pictured in the following charts (fig. 30 – 36).

Table 6: pH measurements of Almiros river.

Month	Point 1	Point 2	Point 3	Point 4
January	7,56	7,77	7,73	7,80
March	7,35	7,47	7,89	7,99
April	7,20	7,30	8,09	8,05
May	7,47	7,65	8,00	8,00
June	7,64	7,61	8,02	8,00
July	7,20	7,58	7,95	8,05
August	7,42	7,73	8,11	8,09
October	6,98	7,33	7,89	7,79
November	6,69	7,00	7,91	8,00

Table 7: Alkalinity (ppm $CaCO_3$) for Almiros river.

Month	Point 1	Point 2	Point 3	Point 4
January	150	140	155	145
March	155	155	175	155
April	170	150	145	140
May	160	140	140	140
June	140	140	140	150
July	150	170	150	150
August	160	140	140	160
October	150	150	160	140
November	140	140	140	150

Table 8: Conductivity (mS/cm) measurements of Almiros river.

Month	Point 1	Point 2	Point 3	Point 4
January	9,69	9,76	38,4	39
March	10,19	10,22	42,9	42,7
April	13,36	13,38	58,7	58,5
May	15,03	15,04	44,6	44,3
June	16,01	15,99	51,6	51,4
July	16,98	16,93	58,6	58,4
August	17,59	17,58	59,8	59,7
October	18,03	18,02	57,7	57,8
November	18,39	18,47	53,3	57,1

Table 9: Total dissolved solids (g/L) measurements of Almiros river.

Month	Point 1	Point 2	Point 3	Point 4
January	5,31	5,32	23,4	23,8
March	5,56	5,58	26,5	26,4
April	7,54	7,55	38,8	38,7
May	8,5	8,48	28,6	28,6
June	9,03	9,01	32,6	32,5
July	9,62	9,59	37,7	37,5
August	10,00	9,99	38,6	38,5
October	10,26	10,26	37,00	37,1
November	10,48	10,54	33,8	36,6

Table 10: Salinity ($‰_O$) measurements of Almiros river.

Month	Point 1	Point 2	Point 3	Point 4
January	5,50	5,50	24,40	24,80
March	5,70	5,80	27,60	27,50
April	7,80	7,80	40,30	40,20
May	8,80	8,80	29,70	29,70
June	9,40	9,30	33,90	33,70
July	10,00	9,90	39,30	39,20
August	10,40	10,40	40,10	40,00
October	10,60	10,60	38,50	38,50
November	10,90	10,90	38,10	38,00

Table 11: Chlorides (ppm) for Almiros river.

Month	Point 1	Point 2	Point 3	Point 4
January	4180	3329	14168	15794
March	4025	4800	16800	18039
April	4645	4955	21446	22994
May	5110	5032	15872	15639
June	5729	5574	18194	18504
July	6193	6038	21988	21678
August	5729	5884	22297	21678
October	6658	6348	21059	22142
November	6813	6658	21059	21368

Table 12: Hardness (ppm $CaCO_3$) of Almiros river.

Month	Point 1	Point 2	Point 3	Point 4
January	1380	1200	4930	5060
March	1620	1400	5580	5410
April	2220	2200	9800	9900
May	1720	1300	5200	5200
June	1880	1890	5860	5920
July	2000	2020	6920	6880
August	2040	2060	7180	7140
October	2180	2040	6860	7060
November	2620	2400	7280	7240

From the measurements of pH in table 6 and the measurements of hardness in table 12, it is observed that in points 1 and 2 the pH values are decreased on April compared with the other months while the measurements of hardness are increased at these points at the same month.

The measurements of conductivity, salinity and chlorides may indicate sea intrusion due to tidal flow as it seems from tables 8, 10 and 11 respectively. The salinity values in points 3 and 4, on April and August, present a sudden increase. The same thing happens with the conductivity values at these two points. The values of conductivity are also increased in points 3 and 4 during the same months.

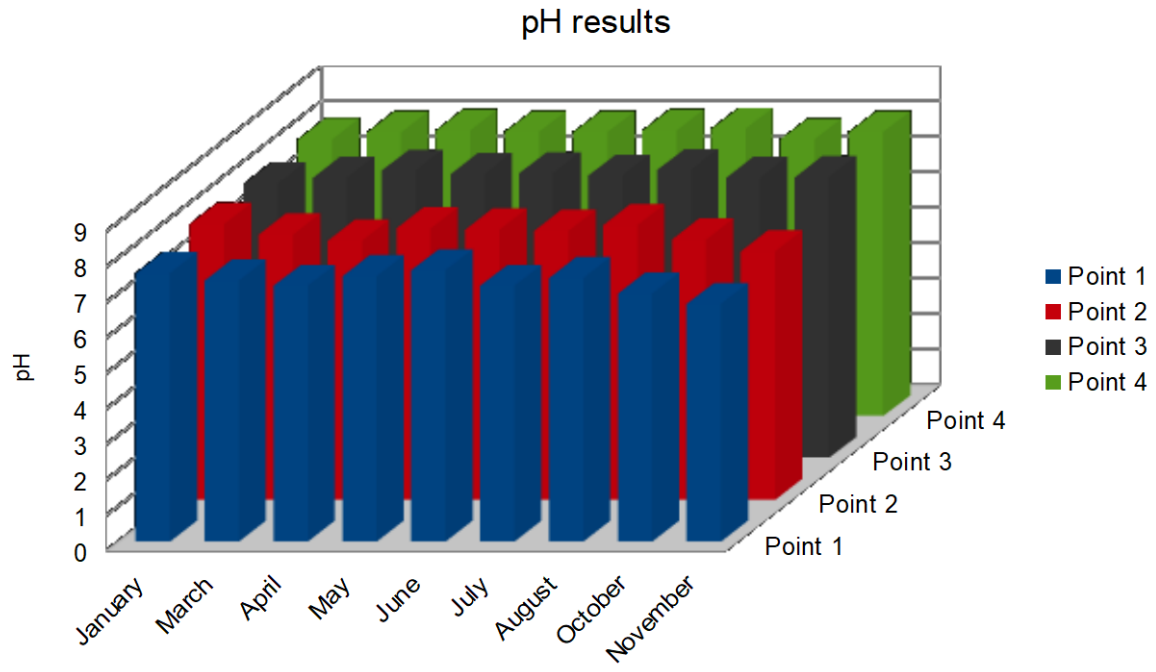


Figure 22: pH measurements of all the samples over the sampling period.

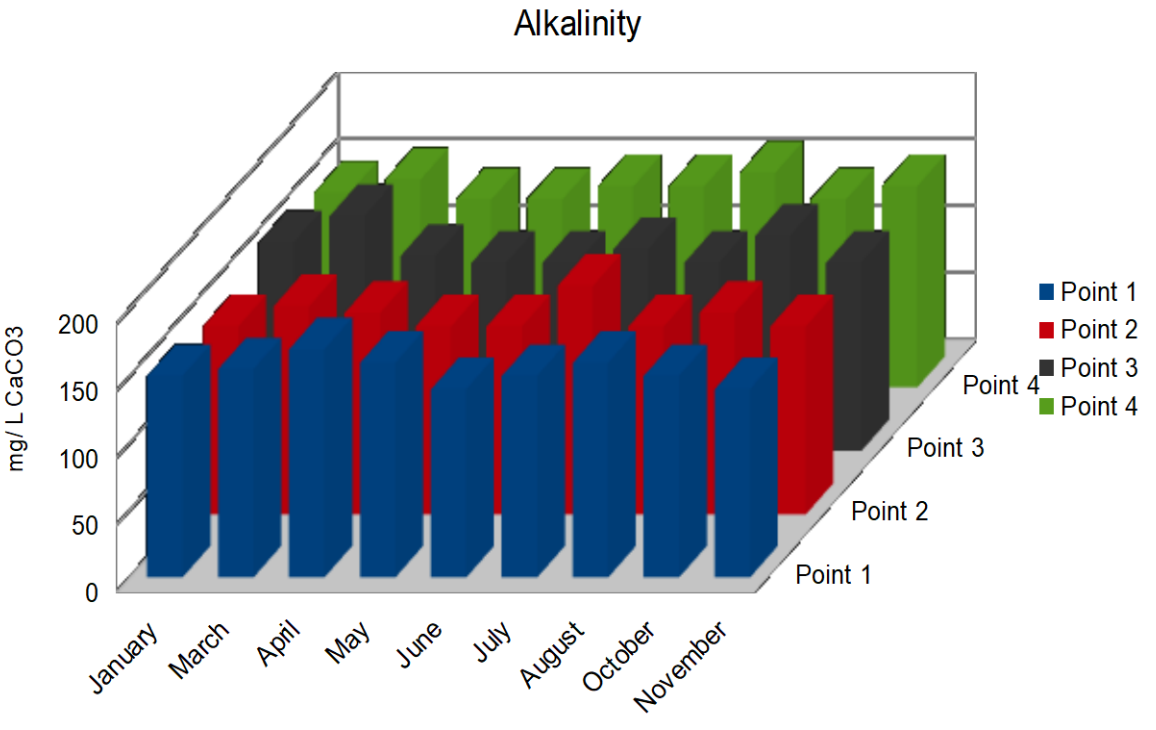


Figure 23: Alkalinity measurements of all the samples over the sampling period.

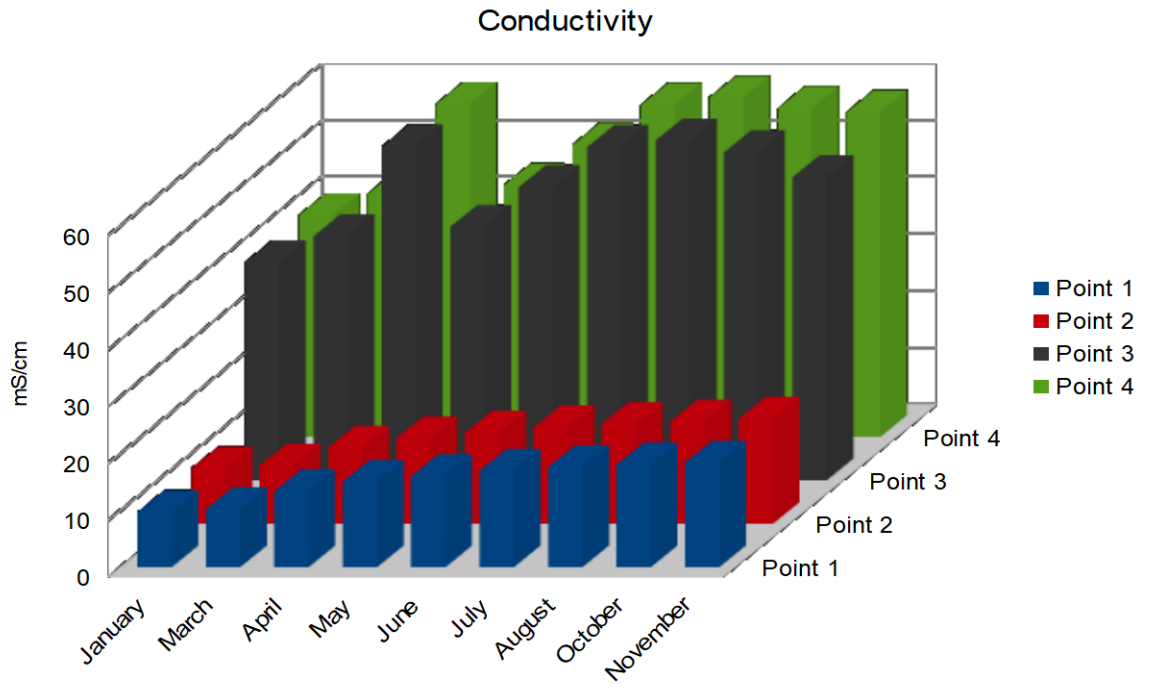


Figure 24: Conductivity measurements of all the samples over the sampling period.

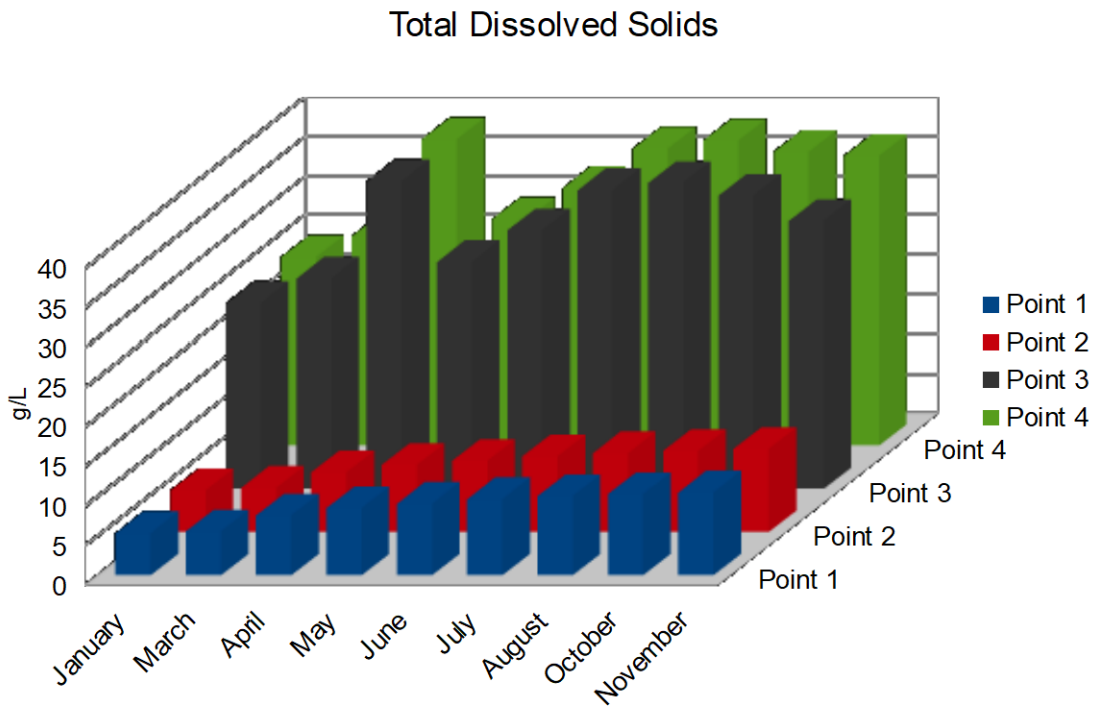


Figure 25: Total dissolved solids measurements of all the samples over the sampling period.

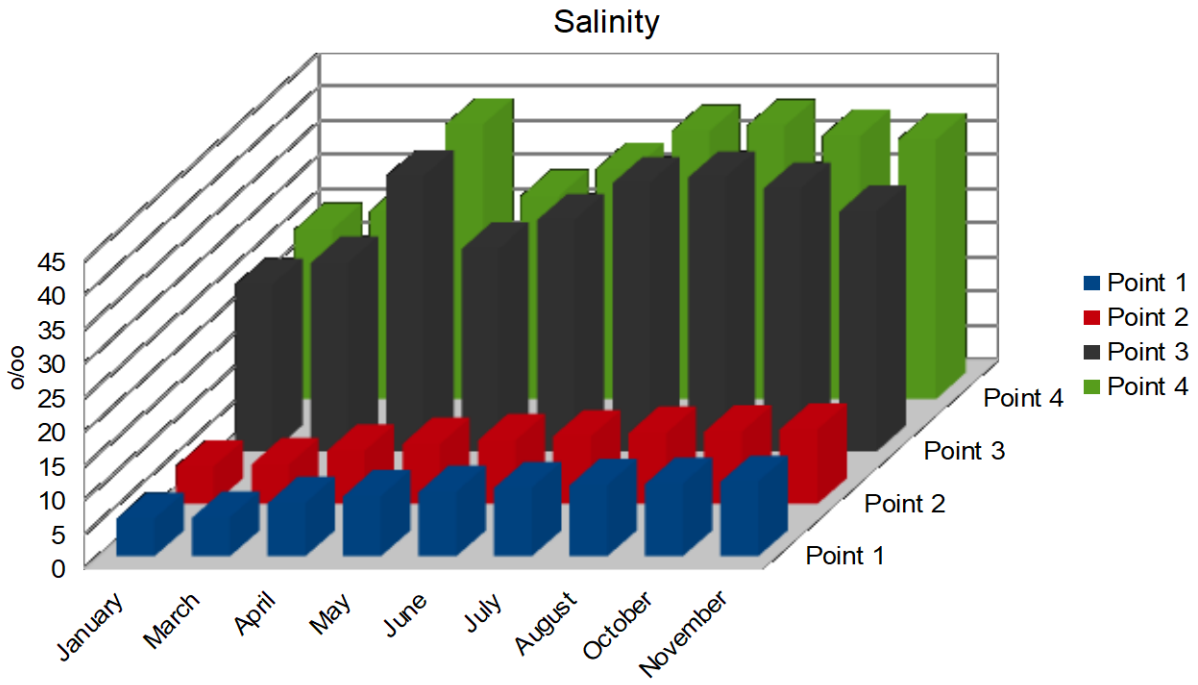


Figure 26: Salinity measurements of all the samples over the sampling period.

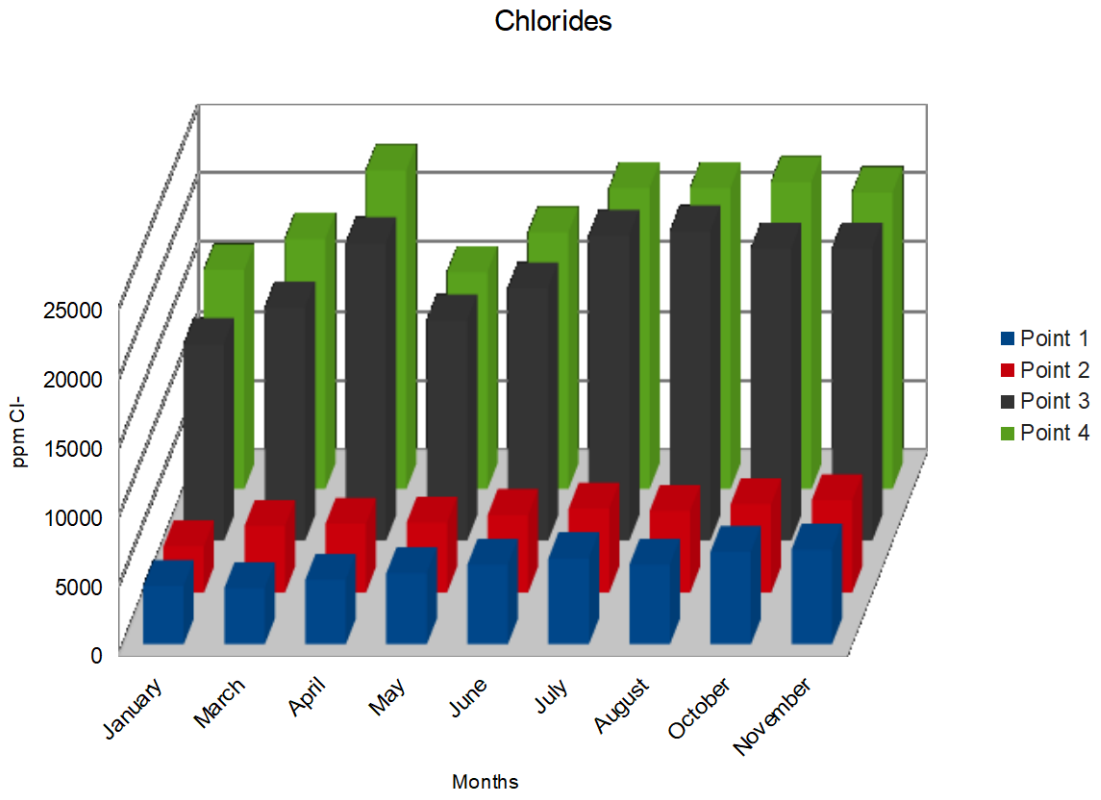


Figure 27: Chlorides measurements of all the samples over the sampling period.

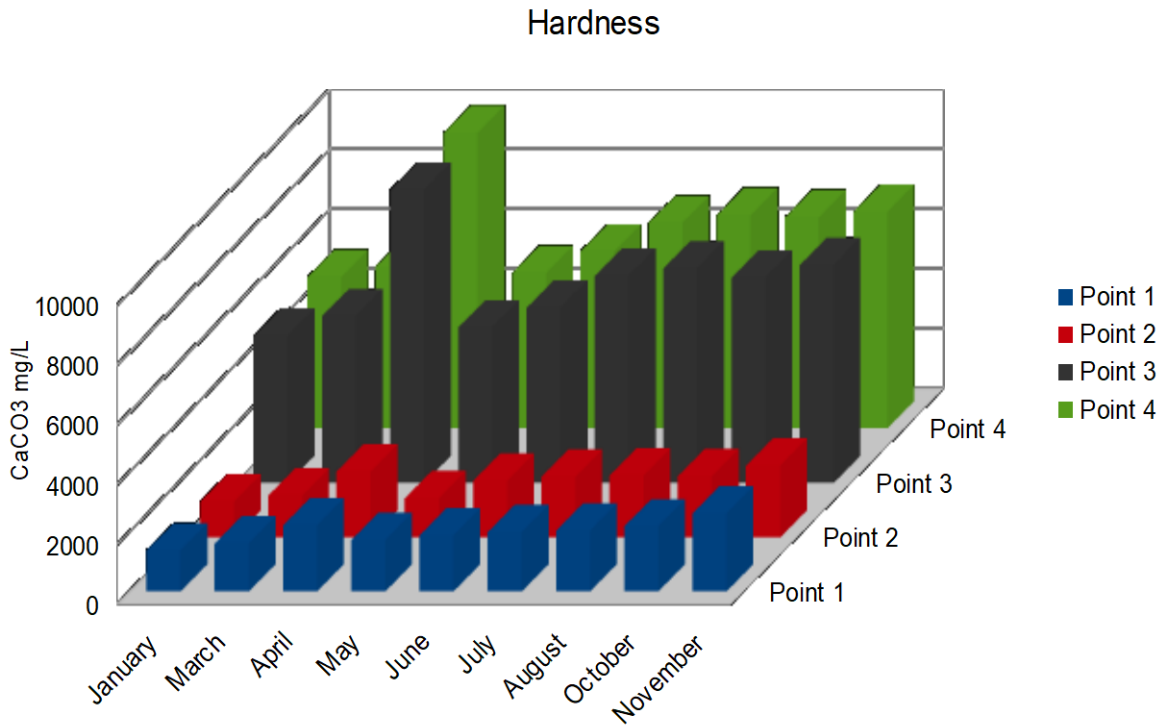


Figure 28: Hardness measurements of all the samples over the sampling period.

The pH values of the samples that were analyzed showed small changes during the year. The lowest pH values, as figure 22 indicates, were measured in points 1 and 2 during October and November. The same pH values were observed in points 3 and 4 for all the sampling period. In addition, during the same month, pH is increased from sample 1 to sample 4. The spatial variation happens because of salinity. The same was observed in the occasion of Loudias river in the North Greece that there was a pH increase at the mouth of the river [48].

The chloride concentrations have been increased continuously from January to November in points 1 and 2, as figure 27 shows. But, in points 3 and 4 maxima concentrations in chlorides were observed in April and August. The sampling of May however, shows similar concentration with March and from May to August the concentrations tend to increase. In the last two samplings, the chloride concentrations are decreased.

The concentrations of conductivity show the same trend with the chloride concentrations. That is evident from figure 24, where the highest concentration is observed on April in points 3 and 4. Also, during the summer period the conductivity values were increased in the same points, which is due to the concentration of chloride ions in Almiros river.

Almost, the same behavior follows the values of TDS concentrations. As observed in figure 25, the highest concentrations have been measured in points 3 and 4. Moreover, the concentrations of TDS are the same in points 1 and 2 with an increasing trend.

The salinity concentrations indicate the same tendency with conductivity, chlorides and total dissolved solids. The salinity values in points 3 and 4 were the highest on April, as it shows figure 26. Between points 1 and 2, there are no differences in salinity, only a slight increase. Higher salinity values occur mostly during dry seasons (summer months) as it has been stated by many researchers [49]. This is mostly the result of the evaporation. Also, the salinity at the estuary of the river is 4-5 times higher than the salinity at the spring. Although, more measurements are necessary,

an evaluation can be done that the type of estuary is more a vertically well-mixed estuary and less a partially mixed estuary.

Figure 23 shows that all the points have the same alkalinity during a month. On April, an increase is observed in all samples. As well, the alkalinity values in point 2 are increased on July in contrast to the other points.

As far as the concentrations of hardness concerned, figure 28 shows that they are increased in all points during the sampling period. But the highest values are observed on April in all points. At the same month, in point 1 appears an increase in hardness concentration, as with alkalinity concentration. This could indicate that the hardness increase in this point is because of carbonate hardness.

Unfortunately, there is limited literature about Almiros River. There are data about chloride series from 1990 - 2001. Comparing our measurements of the physicochemical parameters with the measurements conducted by Anastasiadis [50] and Arfib [51], we notice that most of the measurements in this thesis are higher. The salinity, conductivity, hardness values are higher than those found in Anastasiadis research while the values of pH, alkalinity and chlorides are close to this research. Salinity has been increased the last decades all over the world, leading to the conclusion that it is a global problem of major concern. It is strongly connected with the sea-level rise and the climate change [52].

According to Giannarou [45] the higher the flow rate, the lower the salinity and chlorides in the spring (point 1). The flow rate was high the cold season (rainy period) and the salinity was low. These results agree with our measurements for seasonal variation of salinity and chloride although there are not presented flow rate data. There are no previous results in bibliography about the estuary of the river in order to be compared with the results of the present study.

In addition, the comparison with the occasion of Karamana river in India shows that the pH values are lower (ranging from 6,5 to 6,9) than these in Almiros river. Also, the values of alkalinity are lower in this river in India. In contrast to the conductivity values that they are higher in Karamana river than Almiros river. In Karamana river the conductivity varies from 92 to 252 mhos [53].

4.2 Chromatographic analysis

4.2.1 Method validation

4.2.1.1 Linearity, Reproducibility and Detection limits

At least four standard samples were used in order to calculate the calibration curves except from napthalene and acenapthylene. The calibration curves were prepared using linear regression analysis. The regression coefficients (R^2) are presented in table 13.

The method detection limits (MDL) were calculated using the standard deviations obtained from three measurements of a blank sample multiplied by 3 and divided by the slope. They are also listed in table 13. The reproducibility of the method expressed as RSD (%) was calculated by measuring three replicates of a standard sample. The retention time for each compound as shown in table 13, is the mean value from at least three measurements for each compound. Also, the standard deviation for RT is presented in parenthesis in the same table.

Table 13 Calibration data of sixteen PAHs and SDBS.

PAHs	EQUATION	R^2	MDL	RSD (%)	RT (±SD)
Naphthalene	y=0,6087x+0,4348	1	-	1,59	20,07
Acenapthylene	y=14x-54	1	5,30	1,91	20,25
Acenaphthene	y=300,22x+190,03	0,9986	0,0282	3,76	20,43 (0,68)
Fluorene	y=584,91x+270,65	0,9941	0,141	3,23	20,67 (0,89)
Phenanthrene	y=7,0299x-1,6045	0,9937	-	2,27	21,86 (1,00)
Anthracene	y=16,075x+8,4888	0,9942	0,470	3,47	22,30 (1,04)
Fluoranthene	y=410,09x+272,57	0,9956	0,0490	4,56	23,27 (1,54)
Pyrene	y=56,729x-14,346	0,9989	0,461	4,16	23,95(0,69)
Chrysene	y=402,43x+470,77	0,9998	0,0330	4,70	24,73 (1,12)
Benzo (a) anthracene	y=314,66x+385,33	0,999	0,0689	4,80	24,64 (1,10)
Benzo(b)fluoranthene	y=94,085x-28,879	0,9968	0,488	4,67	27,17 (1,27)
Benzo(k)fluoranthene	y=115,88x-14,618	0,9982	0,234	4,66	27,79 (1,11)
Benzo (a) pyrene	y=256x-61,238	0,9956	0,523	4,59	28,61 (1,02)
Dibenzo (a,h) anthracene	y=100,55x-19,082	0,9899	0,367	4,53	30,25 (1,39)
Benzo (g,h,i)perylene	y=8,4242x+3,5849	0,9975	0,896	3,25	31,21 (0,58)
Indeno [1,2,3-cd] pyrene	y=63,878x+25,508	1	0,840	3,76	32,27 (1,25)
SDBS	y=4,698x+98,224	0,9941	2,71	12,3	-

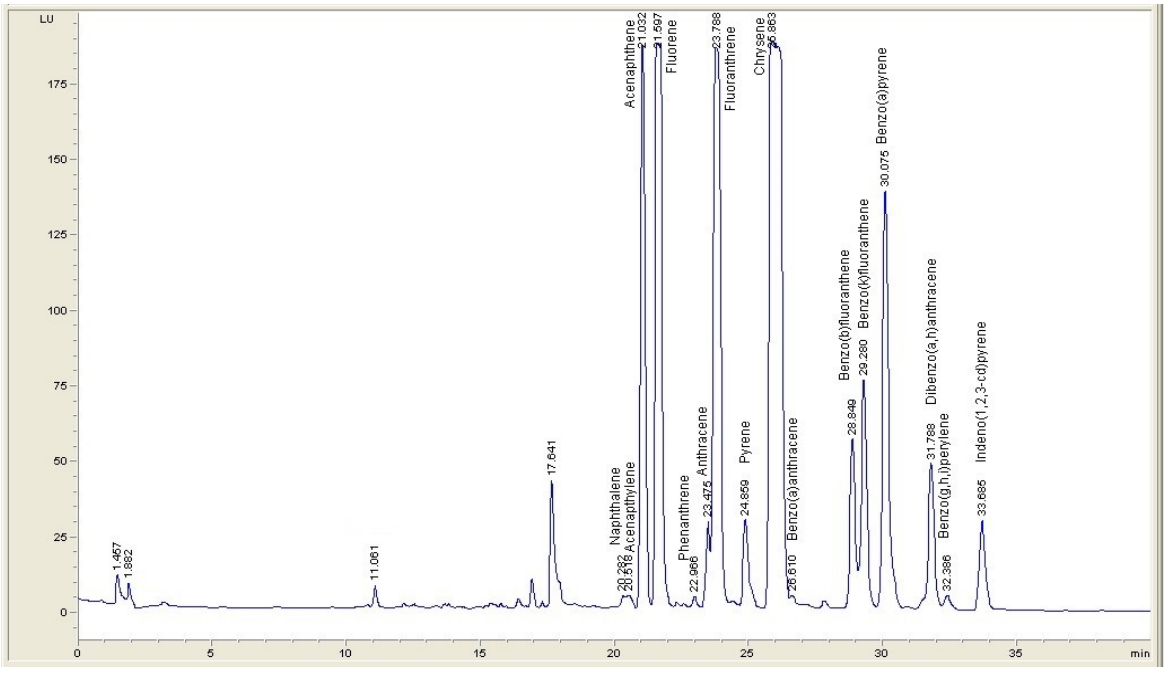


Figure 29: Chromatogram for the standard samples for 5 ppb PAHs concentration.

4.2.1.2 Recovery

The mean recoveries of the method were studied by spiking water samples at different fortification levels as shown in table 14. The recoveries for SDBS are presented in table and the recoveries of the PAHs in table .

Table 14 : Recovery of PAHs and SDS.

COMPOUND	RECOVERY (%)
Naphthalene	27,9
Acenapthylene	10,7
Acenaphthene	63,3
Fluorene	8,53
Phenanthrene	155,3
Anthracene	68,3
Fluoranthene	66,4
Pyrene	12,0
Chrysene	127,3
Benzo (a) anthracene	5,85
Benzo(b)fluoranthene	95,7
Benzo(k)fluoranthene	77,7
Benzo (a) pyrene	88,7
Dibenzo (a,h) anthracene	89,5
Benzo (g,h,i)perylene	35,6
Indeno [1,2,3-c d] pyrene	53,2
SDBS	119,2

Naphthalene is a very volatile compound and Acenapthylene has the weakest fluorescence which makes their detection extremely difficult.

4.3 SDBS residues in samples

The concentrations of SDBS in the samples are listed in table 15. As it is shown SDBS were detected in almost all samples except from points 2, 3 and 4 on January. During April the same

concentrations were detected in the sampling points. June and July had high concentrations of SDBS. Also, during August and October SDBS were detected in all samples, except the sample in point 3.

Table 15: Results of SDBS.

SAMPLING MONTH	POINT	DETECTED (ppb)
January	1	67,7
January	2	-
January	3	-
January	4	-
March	1	49,5
March	2	28,3
March	3	28,4
March	4	50,3
April	1	10,8
April	2	10,8
April	3	14,0
April	4	16,1
May	1	29,9
May	2	21,7
May	3	24,0
May	4	39,5
June	1	98,5
June	2	63,6
June	3	6,55
June	4	35,7
July	1	3,99
July	2	54,9
July	3	124,3
July	4	15,3
August	1	53,2
August	2	46,6
August	3	6,34
August	4	29,9
October	1	20,2
October	2	21,0
October	3	5,06
October	4	3,57
November	1	25,5
November	2	32,3
November	3	29,5
November	4	4,21

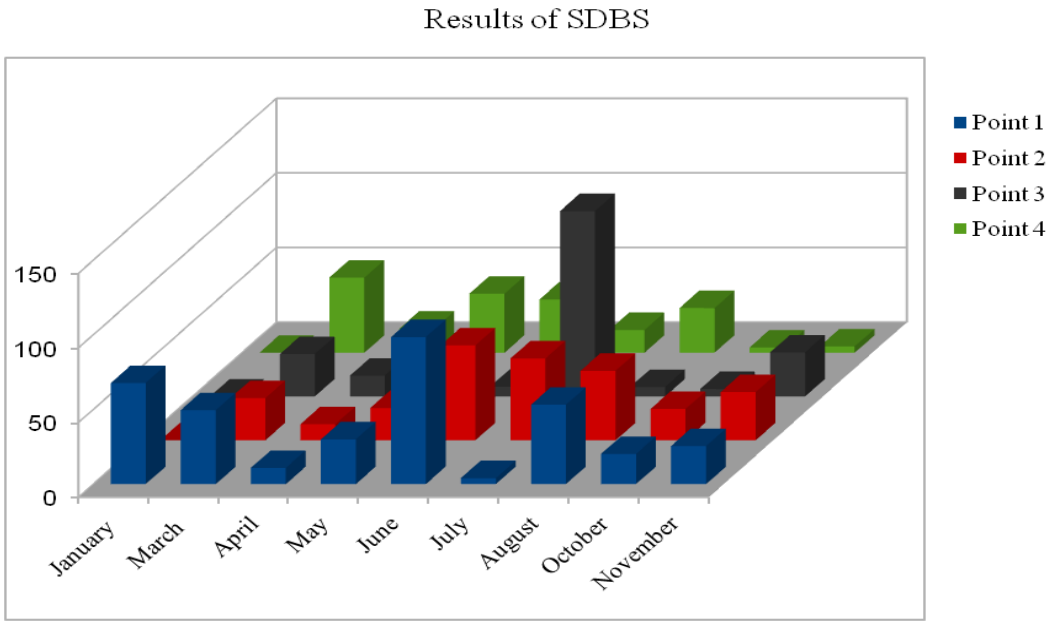


Figure 30: Graph of the results of the detected SDBS for each point for each month.

There are many studies about the concentration of anionic surfactants in surface and coastal water. But the majority of them determine the anionic surfactants as MBAS. There are less studies referring to the concentration of LAS detected by HPLC method.

Kikuchi and coworkers [54] measured LAS concentrations between 0,8 and 29,9 ppb in Tokyo Bay water, with the highest concentration detected in coastal areas. The detection method was high performance liquid chromatography with fluorophotometric detection.

In two Japanese bays, Katsuura and Moriura, the concentration ranged from 0,003 to 0,016 mg LAS/L [55] and 0,008 mg LAS/L was measured in coastal waters near Hiroshima [56].

Hon-nami and Hanya [57] determined the LAS/MBAS ratios in Tama River water and water from Tokyo Bay to be 0.4 to 0,85 and less than 0,2, respectively. A combination of gas-liquid chromatography and mass spectrometry were used.

The concentration and biodegradation of anionic surfactants in the Hudson estuary of the New York City area have been studied [58], to estimate MBAS, and specifically LAS, concentrations in saline waters as well as biodegradation rates at various salinities. In surface samples from the Hudson River estuary with salinities ranging from 1,3 to 23 ‰, MBAS levels ranged from 0,02 to 0,19 ppm and were directly correlated at the sampling points (sewage outfalls) with the efficiency of sewage treatment of the effluents discharged into the estuary.

4.4 PAH residues in samples

The following table shows the concentrations of the PAHs that were detected.

Table 16: Results of PAHs.

Sampling month	Point	PAHs	Concentration (ppb)
January	1	Pyrene	1,89
January	2	Pyrene	2,18
June	2	Fluoranthene	<MDL
June	2	Pyrene	0,97
July	4	Fluoranthene	<MDL
August	1	Fluoranthene	<MDL
October	1	Fluoranthene	<MDL
October	1	Pyrene	4,89
October	2	Pyrene	0,75
October	2	Benzo (b) fluoranthene	0,74
October	2	Dibenzo (a,h) anthracene	0,71
October	2	Benzo (g,h,i) perylene	-

The determination of benzo (g,h,i) perylene could not be done because it was coeluted with another compound.

It is clear that the sampling points 1 and 2 are the most polluted compared with points 3 and 4. Moreover, point 2 compared with point 1 is more polluted. Finally, from the sampling months October seems to be the most polluted.

Fluoranthene and pyrene were the abundant PAHs. Both of them are pyrogenic PAHs that are associated with the combustion of petroleum, wood, coal. Industrial activities such as coke and steel production have released large amounts of pyrogenic PAHs. Moreover, pyrogenic PAHs are often associated with sediments. Pyrogenic PAHs are usually met in urban areas.

Okoro [59] found that the concentrations of PAHs in Ekpan Creek in the Niger Delta were from pyrogenic origin and included five PAHs (phenanthrene, anthracene, fluoranthene, pyrene, chrysene and benzo(a)anthracene). The concentrations of these PAHs was between 0.02439-0.2836 mg/L.

In a research at Eleme in Nigiria, the concentration of seven PAHs, phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(k)fluoranthene, and indeno (I,2,3-cd pyrene), was ranged between 0-2.5 x 10^{-3} ng/L. Benzo(a)pyrene, which is a toxic PAH was only observed at concentration levels of 2.01 x 10^{4} ng/L and 1.98 x 10^{4} ng/L in surface waters in the area. Also, at Ahoada East, in Nigiria a research was done as a comparison area to Eleme. The only PAHs that was found were benzo(k) fluoranthene, benzo(a)pyrene and indeno(1,2,3-cd) pyrene. The highest total PAH concentration was 8.39 x 10^{3} ± 1.46 x 10^{4} ng/L and was less than 2.21 x 10^{4} ± 2.76 x 10^{4} ng/L that was found in Eleme surface waters [60].

According to a research in Iran, PAHs were detected in the Caspian seawater. The results of the concentration of seven PAHs were ranged from 0,63 ppb to 14,11 ppb. Acenaphthene concentration was 1,24 ng/mL, anthracene concentration was 2,06 ng/mL and fluoranthene concentration was 10.71 ng/mL. If we compare these concentrations of PAHs with the detected concentrations of PAHs in Almiros, we observe that in the Caspian the PAHs concentrations are higher than Almiros River [61].

In a study by Dionex [62] naphthalene only was detected in a tap water sample in concentration of 0,46 ppb below their method detection limit (1,17 ppb).

During October the concentration of benzo (b) fluoranthene was 0,74 ppb. In a research in surface water of Al-Dalmaj marsh in Iraq , the same compound had concentration 0,32 - 1,63 mg/L. The highest concentration of pyrene in Al-Dalmaj marsh was 13,82 mg/L in contrast to Almiros river in which pyrene had 4,89 ppb [63].

A research for the determination of PAHs in surface water, at the Doce and Piracicaba Rivers in Brazil, has found Acenapthylene (from 25,9 to 391,3 ng/L), Phenanthrene (from 27,8 to 873,9 ng/L), Fluoranthene (from 10,0 to 180,9 ng/L), and Pyrene (from 9,5 to 209,6 ng/L) at higher concentrations. The emissions from diesel combustion, coal stoves, vehicle exhaust, and urban dusts were the sources of the PAHs that have been detected [64]. In the present research, the concentration of pyrene was ranging from 0,75 to 4,89 ppb, almost the half concentration from the two rivers in Brazil.

Moreover, in Todos Os Santos Bay, Brazil, the total PAH concentrations were ranged from 0,0029 to 0,1079 ng/L in surface waters, with mean value of 0,0344 ng/L. The two-ring and three-ring PAHs, naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene, with maximum concentrations were ranged from 0,0015 to 0,0271 ng/L, were generally the most abundant PAHs. These PAHs in surface water in Todos Os Santos Bay were mainly due to petrogenic inputs [65].

4.5 Fluorescence data in samples

The peaks that were detected by fluorescence are discussed furthermore in this chapter. Fluorescence has been used for the characterization of river and sea water. Natural organic matter (NOM) consists mainly of humic substances and proteins. These compounds have fluorescence properties. According to the excitation and emission, the analysis separate to regions, which each one correspond to different organic matter [66]. In table 17, the regions are described.

Table 17: Fluorescence regions (from Chen et al.,2003).

Excitation	Emission	Region
220 - 250	280 - 332	Aromatic proteins I
220 - 250	332 - 380	Aromatic proteins II
220 - 250	380 - 580	Fulvic acid - like
250 - 470	280 - 380	Microbial by products
250 - 470	380 - 580	Humic acid - like

The peaks that are present in the river Almiros have been categorized according to their retention time and to their emission spectra.

Retention time:

In the following table 18 the retention time of the fluorescence peaks of each point for all the sampling months is presented. A blank sample was first subtracted from all the river samples. The chromatograms of the four points for the nine months of the sampling are presented in the Appendix (figures 37-45). As it is clear from the table, on July and October the samples show heterogeneity in contrast to the other months, where the four points appear to consist of more similar peaks between them.

Table 18: Retention time (min) of the peaks in the fluorescence detector for each sample. Each column corresponds to the points of the sampling.

JANUARY 20 peaks				MARCH 9 peaks				APRIL 2 peaks			
Point 1	**Point 2**	**Point 3**	**Point 4**	**Point 1**	**Point 2**	**Point 3**	**Point 4**	**Point 1**	**Point 2**	**Point 3**	**Point 4**
				2,73		2,431					
3,432		3,735			3,149		3,046				
				12,974							
15,194			15,083								
		16,26	16,25								
18,446	18,34		18,63								
19,59	19,306	19,194	19,756								
22,936	22,462										
		26,271									
27,851	27,218		27,987								
		28,73				28,473					
				31,149		31,143	31,113	31,381	31,278		

MAY 12 peaks				JUNE 16 peaks				JULY 10 peaks			
Point 1	**Point 2**	**Point 3**	**Point 4**	**Point 1**	**Point 2**	**Point 3**	**Point 4**	**Point 1**	**Point 2**	**Point 3**	**Point 4**
				2,528				2,62	2,06		
	3,578				3,569	3,63	3,52				
								6,47			
15,798		15,35	15,37	15,735				15,03	15,4		
		16,267	16,257	16,245	16,031	16,75	16,78	16,50			
								18,8	18,02		
		19,057		19,202		19,51	19,40		19,8		
22,052					22,314						
					24,492					--------	24,103
				28,675							
31,114	31,02	30,983	31,033								

AUGUST 11 peaks				OCTOBER 15 peaks				NOVEMBER 18 peaks			
Point 1	**Point 2**	**Point 3**	**Point 4**	**Point 1**	**Point 2**	**Point 3**	**Point 4**	**Point 1**	**Point 2**	**Point 3**	**Point 4**
		2,78	2,979	1,176	1,176				2,085	1,944	1,946
			3,62				3,49				3,06
						4,53				4,573	4,03
										5,777	5,536
											6,781
				9,56				10,154	9,805	9,42	9,39
				12,046				12,002	11,743	11,97	11,3
12,68										1	12,80
18,16	18,88		18,20			18,6					

	19,68					19,2	19,4						
22,53				22,92									
				24,80	24,35								
28,49					28,249								
					29,419								
					31,08								

As presented in table 18, there are common peaks that have been detected as PAHs, as is already mentioned in the previous section. Also, there is a common peak at 31 minutes for three months (March, April, May and October). The peak of October is a mixture of a PAH and another compound. More peaks were appeared during the cold season (January, November and October) than during the hot season.

Moreover, the November peaks did not show any similarity with the peaks of the other months suggesting different consistency.

During July, August and to a less extent on May there are spatial differences between the sampling points.

Emission spectra:

In addition, the emission spectra of each peak for each minute of the samples for all the sampling period have given significant information. These spectra are presented in the Appendix (figure 55-87).

Taking into account the emission spectra, it is concluded that during March, April, June, July, August there are similar peaks. The majority of the peaks (60,2 %) appear to have a maximum emission at 370 nm. This is mostly due to humic like and microbial by products (Chen,2003). These compounds have been found to represent 53% of the total fluorescence (Penru) while aromatic proteins account for a smaller percent.

Although November samples have different consistency as it is clear from retention times, most of the spectra of the peaks have an emission maximum at 370 nm. In table 19, these peaks are grouped according to their emission maximum and to the sampling month and presented.

Table 19: Number of peaks and their emission spectra.

Maximum emission (nm)	**Number of peaks J+Mr+Ap+Ma+Jn+Jl+Au+O+N**	**Proportion (%)**
410	2+0+0+0+0+0+0+0+0 =2	1.8
390	2+0+0+0+0+0+0+2+5 =9	8.0
370	4+7+2+11+14+7+9+7+7 =68	60.2
310	5+0+0+0+0+2+1+0+3 = 11	9.7
PAH	2+0+0+0+2+1+1+6+0 =12	10.6

Unseparated/ several	5+2+0+1+0+0+0+0+3 =11	9.7
Sum	20+9+2+12+16+10+11+15+18 =113	100.0

Nine peaks have a maximum excitation at 390 nm, all of them the colder months. Two peaks from January show spectra with maximum emission at 410 nm, two with emission at 390 nm and five with maximum emission at 310 nm. During November there were no peaks at 410 nm, five at 390 nm and three at 310 nm.

As it is prementioned, the smaller number of peaks has been detected in April and March samples, while the higher number of peaks has been detected in January and November samples. These months belong to cold (wet) periods. Monthly variation is mostly obvious in winter and late autumn. This happens because less reactions (photochemical oxidation, microbial degradation) and less evaporation happen these months.

Salinity does not show to affect to a great extent the spatial variation. Usually when the salinity is increased between 25 and 30‰, it causes a blue shift towards shorter wavelengths [67]. This could result from structural changes in compounds that include: a) reduction in the extent of the electron system, such as decrease in the number of aromatic rings, reduction of conjugated bonds in a chain structure or conversion of a linear ring system to a non-linear system and b) elimination of functional groups such as carbonyl, hydroxyl and amine [68].

4.6 Correlation between the physicochemical parameters

Another point of view for Almiros river is offered by the following graphs. These present the physicochemical measurements of the samplings for all the points through the entire period of the samplings. In each graph, there is one point for each one of all the months. For instance, point 1 for all months is shown in the graph 1 Jan, 1 Mar, 1 Apr, etc.

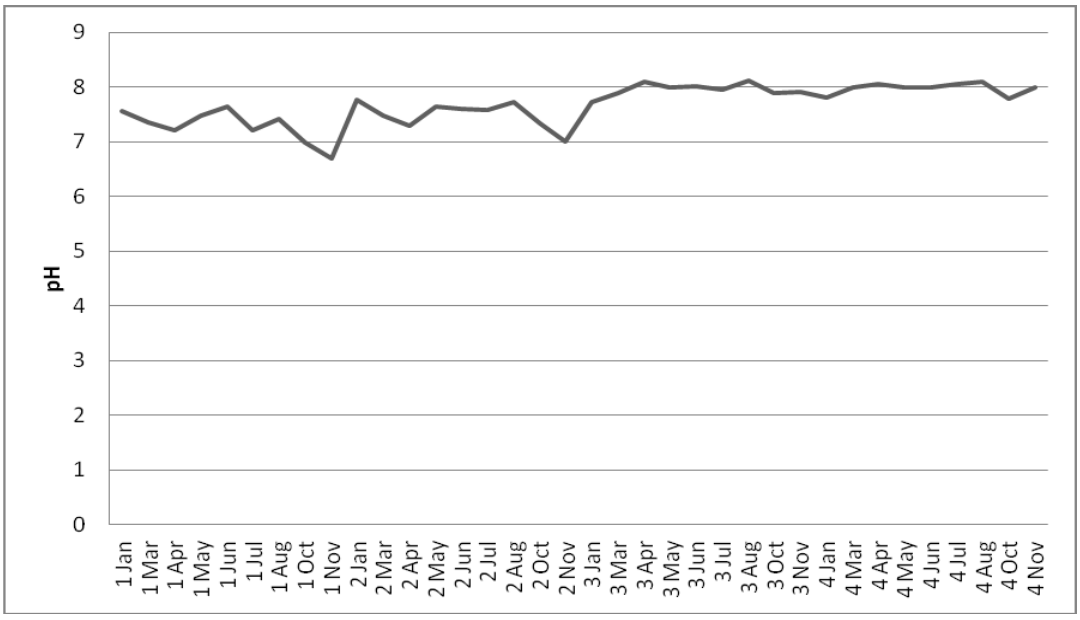


Figure 31: Graph of pH for all the points through the months of samplings.

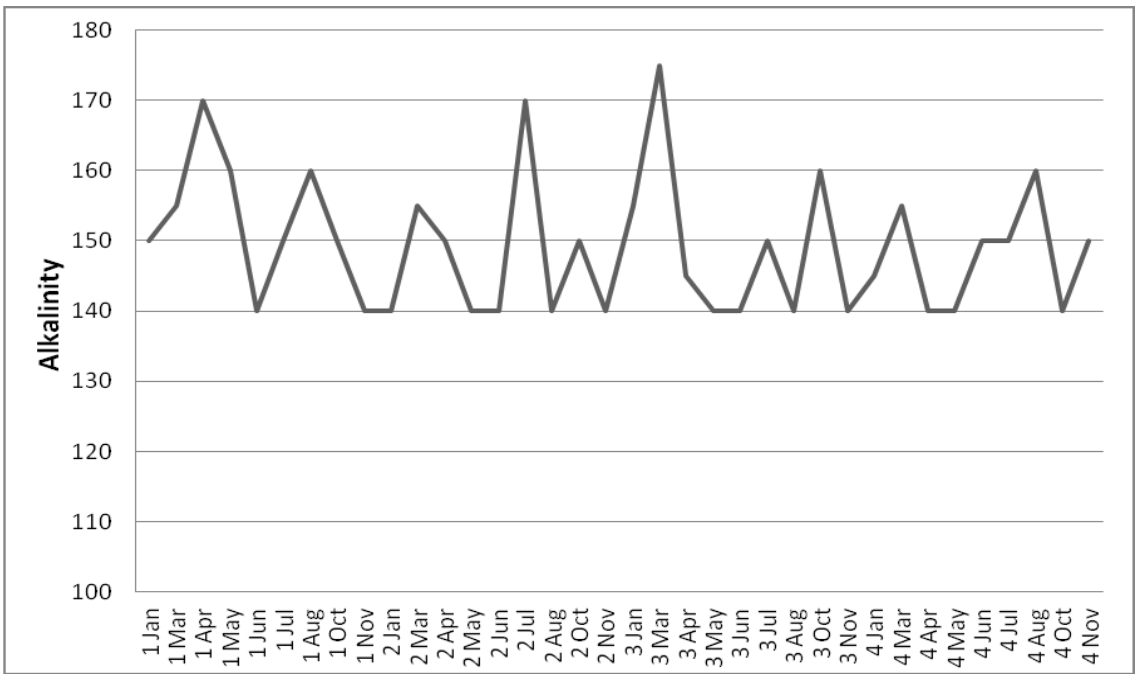


Figure 32: Graph of alkalinity for all the points through the months of samplings.

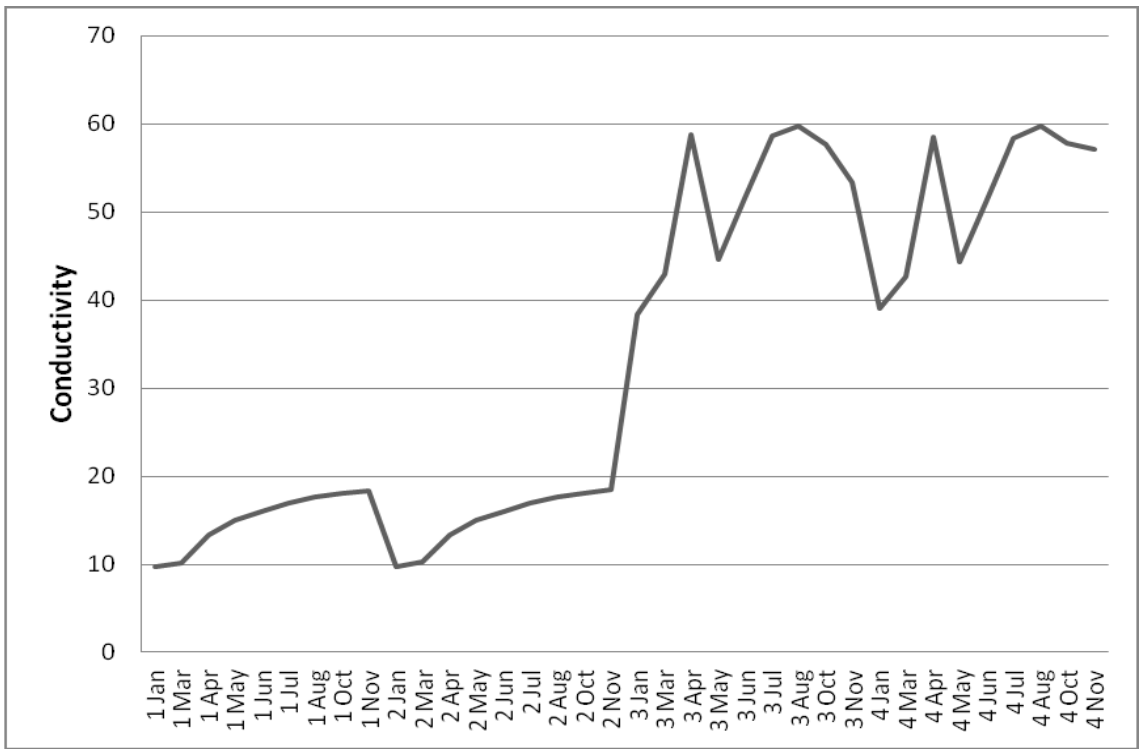


Figure 33: Graph of conductivity for all the points through the months of samplings.

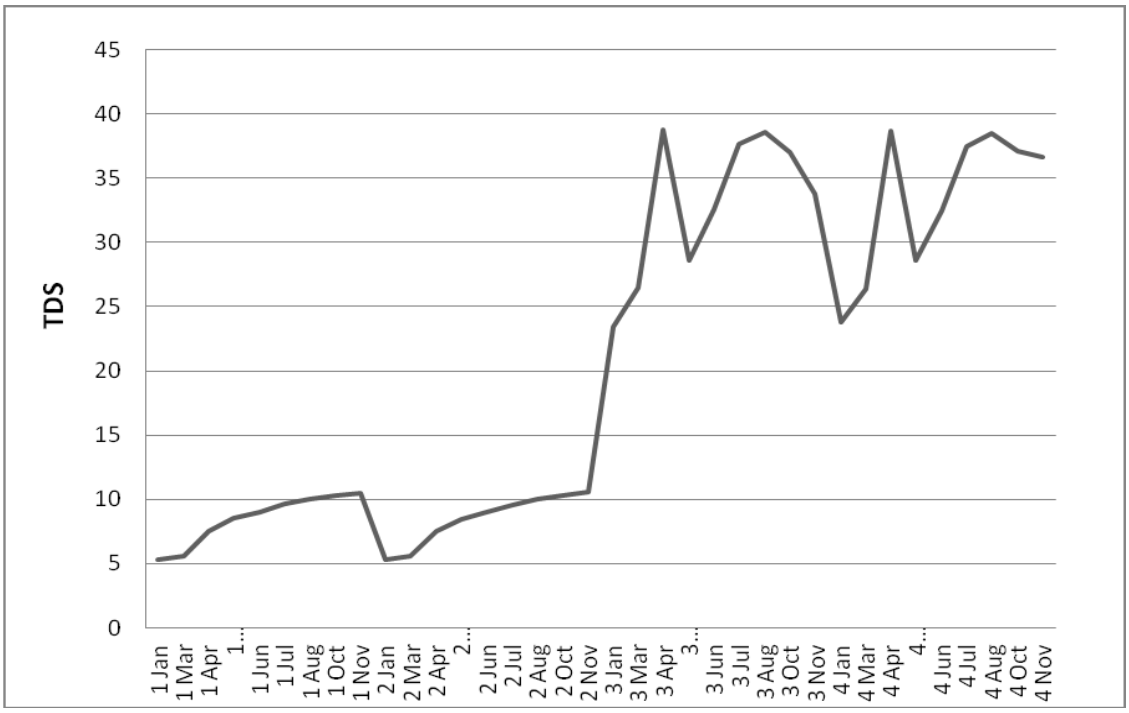


Figure 34: Graph of total dissolved solids for all the points through the months of samplings.

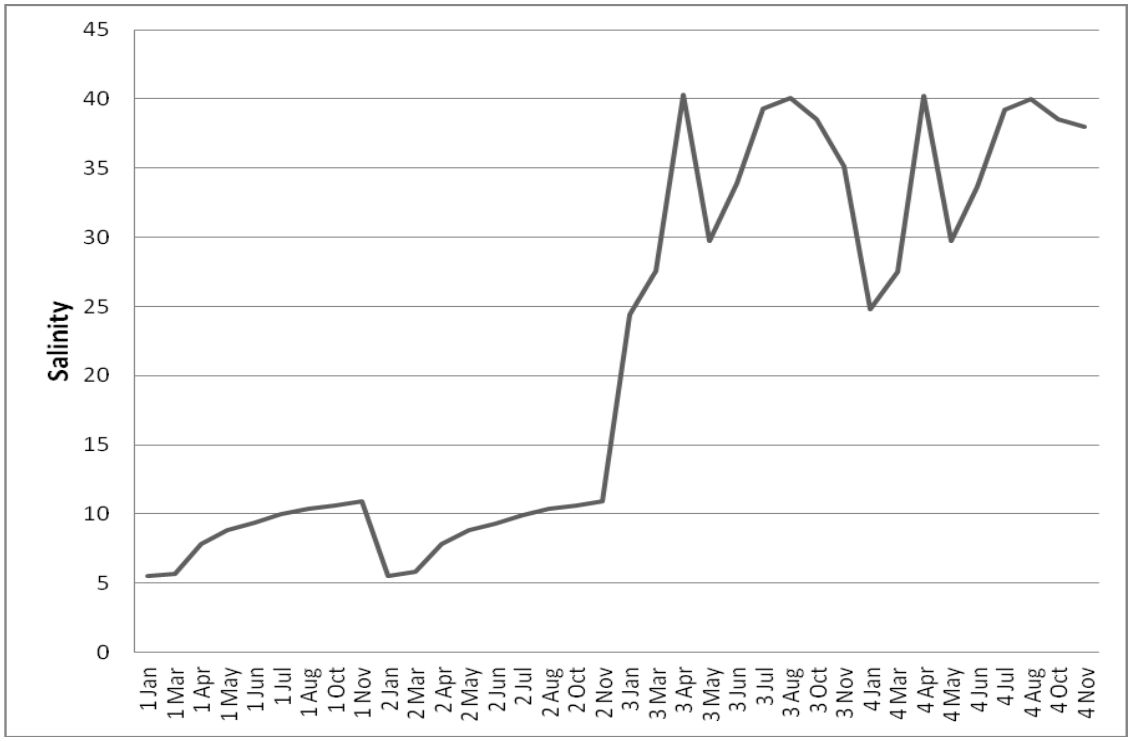


Figure 35: Graph of salinity for all the points through the months of samplings.

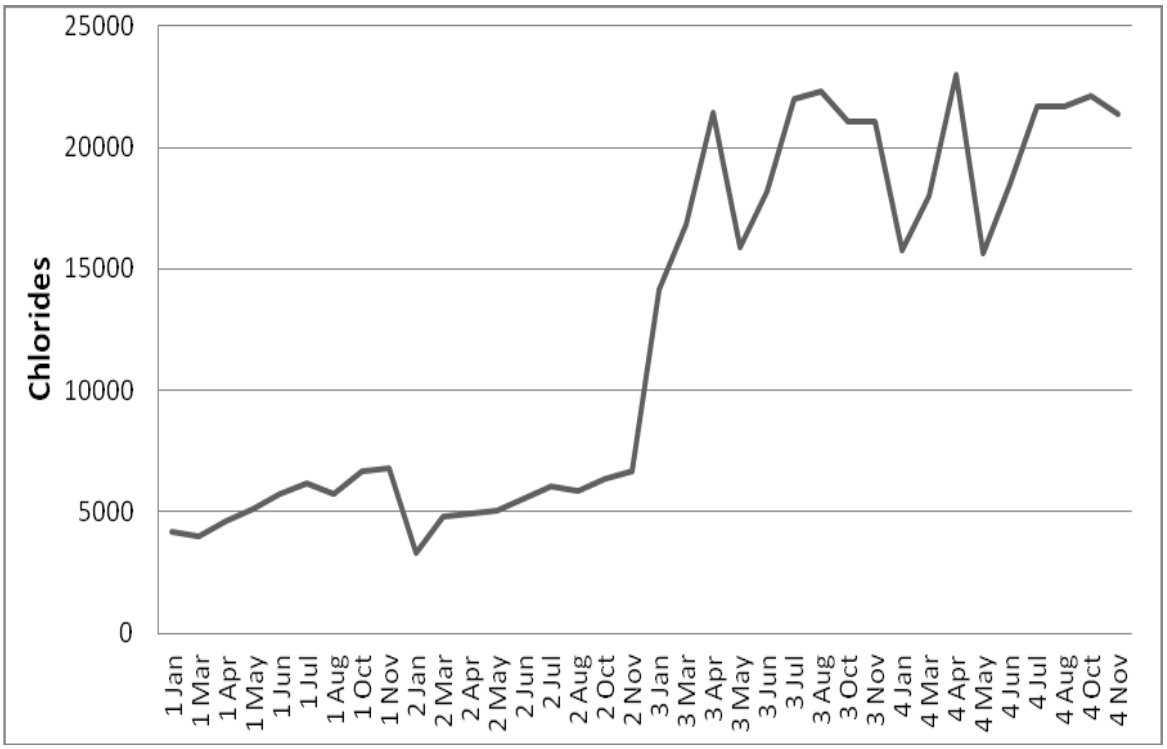


Figure 36: Graph of chlorides for all the points through the months of samplings.

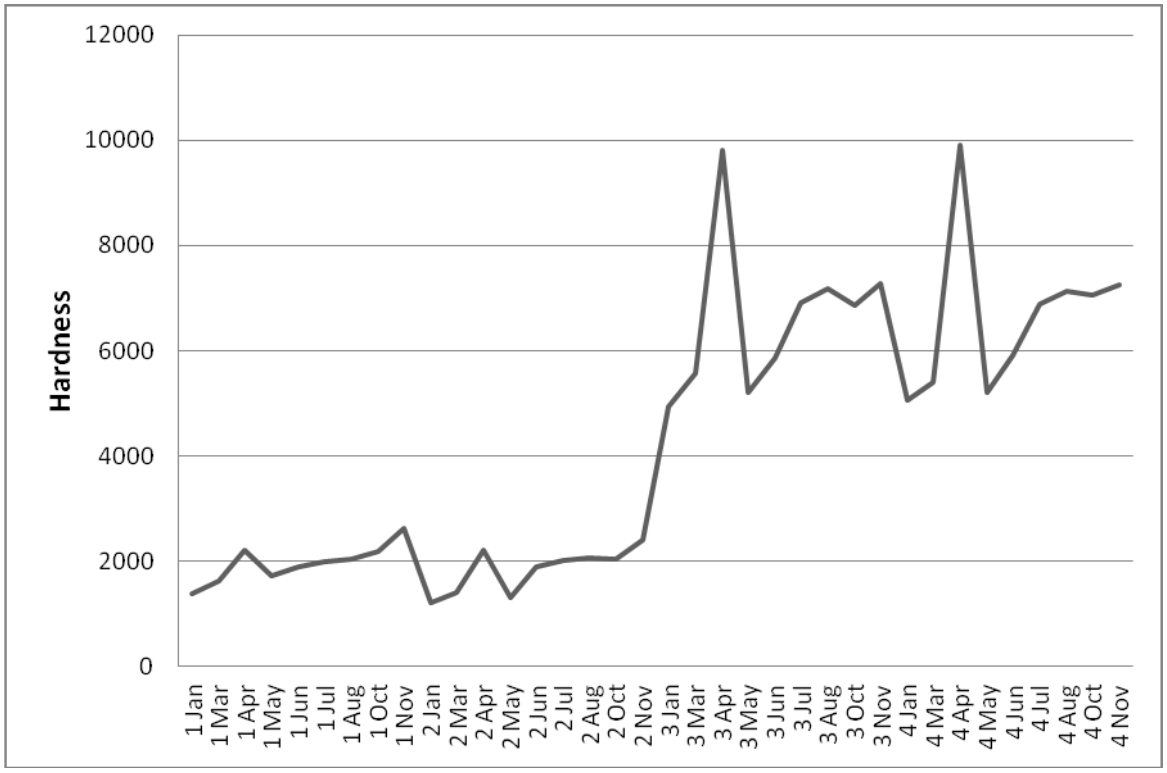


Figure 37: Graph of hardness for all the points through the months of sampling

As the above graphs indicate, points 1 and 2 are similar. The same occurs in points 3 and 4. In all the physicochemical measurements these points of the sampling have almost the same appearance in the graphs. Except alkalinity which appears similarity in all points almost for all the months.

In addition, the tables 20 - 28 present the correlation between the physicochemical parameters through the months. The correlation is a measure of how well two parameters fit. For each month the physicochemical parameters are compared between.

Table 20: Correlation coefficients between physicochemical parameters for January.

January	pH	Conductivity	TDS	Salinity	Hardness	Alkalinity	Chlorides
pH	1,000	0,6388	0,6383	0,6375	0,6201	-0,0312	0,6279
Conductivity	0,6388	1,000	1,000	1,000	0,9994	0,5523	0,9953
TDS	0,6383	1,000	1,000	1,000	0,9995	0,5527	0,9954
Salinity	0,6375	1,000	1,000	1,000	0,9995	0,5527	0,9954
Hardness	0,6201	0,9994	0,9995	0,9995	1,000	0,5663	0,9972
Alkalinity	-0,0312	0,5523	0,5527	0,5527	0,5663	1,000	0,5418
Chlorides	0,6279	0,9953	0,9954	0,9954	0,9972	0,5418	1,000

Table 21: Correlation coefficients between physicochemical parameters for March.

March	pH	Conductivity	TDS	Salinity	Hardness	Alkalinity	Chlorides
pH	1,000	0,9815	0,9816	0,9819	0,9715	0,4095	0,9928
Conductivity	0,9815	1,000	1,000	1,000	0,9989	0,5299	0,9968
TDS	0,9816	1,000	1,000	1,000	0,9989	0,5292	0,9969
Salinity	0,9819	1.000	1,000	1,000	0,9988	0,5291	0,9970
Hardness	0,9715	0,9989	0,9989	0,9988	1,000	0,5530	0,9923
Alkalinity	0,4095	0,5299	0,5292	0,5291	0,5530	1,000	0,4689
Chlorides	0,9928	0,9968	0,9969	0,9970	0,9923	0,4689	1,000

Table 22: Correlation coefficients between physicochemical parameters for April.

April	pH	Conductivity	TDS	Salinity	Hardness	Alkalinity	Chlorides
pH	1,000	0,9958	0,9958	0,9958	0,9952	-0,8130	0,9926
Conductivity	0,9958	1,000	1,000	1,000	0,9999	-0,7681	0,9978
TDS	0,9958	1,000	1,000	1,000	0,9999	-0,7681	0,9978
Salinity	0,9958	1,000	1,000	1,000	0,9999	-0,7680	0,9978
Hardness	0,9952	0,9999	0,9999	0,9999	1,000	-0,7686	0,9985
Alkalinity	-0,8130	-0,7681	-0,7681	-0,7680	-0,7686	1,000	-0,7843
Chlorides	0,9926	0,9978	0,9978	0,9978	0,9985	-0,7843	1,000

Table 23: Correlation coefficients between physicochemical parameters for May.

May	pH	Conductivity	TDS	Salinity	Hardness	Alkalinity	Chlorides
pH	1,000	0,9607	0,9604	0,9606	0,9352	-0,7815	0,9591
Conductivity	0,9607	1,000	1,000	1,000	0,9967	-0,5775	1,000
TDS	0,9604	1,000	1,000	1,000	0,9968	-0,5768	0,9999
Salinity	0,9606	1,000	1,000	1,000	0,9968	-0,5774	0,9999
Hardness	0,9352	0,9967	0,9968	0,9968	1,000	-0,510	0,9971
Alkalinity	-0,7815	-0,5775	-0,5768	-0,5774	-0,510	1,000	-0,5731
Chlorides	0,9591	1,000	0,9999	0,9999	0,9971	-0,5731	1,000

Table 24: Correlation coefficients between physicochemical parameters for June.

June	pH	Conductivity	TDS	Salinity	Hardness	Alkalinity	Chlorides
pH	1,000	0,9980	0,9980	0,9982	0,9973	0,5462	0,9975
Conductivity	0,9980	1,000	1,000	1,000	0,9999	0,5741	0,9997
TDS	0,9980	1,000	1,000	1,000	0,9999	0,5749	0,9998
Salinity	0,9982	1,000	1,000	1,000	0,9999	0,5726	0,9997
Hardness	0,9973	0,9999	0,9999	0,9999	1,000	0,5860	0,9999
Alkalinity	0,5462	0,5741	0,5749	0,5726	0,5860	1,000	0,5913
Chlorides	0,9975	0,9997	0,9998	0,9997	0,9999	0,5913	1,000

Table 25: Correlation coefficients between physicochemical parameters for July.

July	pH	Conductivity	TDS	Salinity	Hardness	Alkalinity	Chlorides
pH	1,000	0,9093	0,9092	0,9088	0,916	-0,1981	0,9057
Conductivity	0,9093	1,000	1,000	1,000	1,000	-0,5780	0,9999
TDS	0,9092	1,000	1,000	1,000	1,000	-0,5780	0,9999
Salinity	0,9088	1,000	1,000	1,000	1,000	-0,5793	0,9999
Hardness	0,916	1,000	1,000	1,000	1,000	-0,5750	0,9999
Alkalinity	-0,1981	-0,5780	-0,5780	-0,5793	-0,5750	1,000	-0,5830
Chlorides	0,9057	0,9999	0,9999	0,9999	0,9999	-0,5830	1,000

Table 26: Correlation coefficients between physicochemical parameters for August.

August	pH	Conductivity	TDS	Salinity	Hardness	Alkalinity	Chlorides
pH	1,000	0,9225	0,9225	0,9226	0,9237	-0,2899	0,9254
Conductivity	0,9225	1,000	1,000	1,000	1,000	-0,0011	0,9997
TDS	0,9225	1,000	1,000	1,000	1,000	-0,0016	0,9997
Salinity	0,9226	1,000	1,000	1,000	1,000	-0,0017	0,9997
Hardness	0,9237	1,000	1,000	1,000	1,000	-0,0059	0,9998
Alkalinity	-0,2899	-0,0011	-0,0016	-0,0017	-0,0059	1,000	-0,0239
Chlorides	0,9254	0,9997	0,9997	0,9997	0,9998	-0,0239	1,000

Table 27: Correlation coefficients between physicochemical parameters for October.

October	pH	Conductivity	TDS	Salinity	Hardness	Alkalinity	Chlorides
pH	1,000	0,9359	0,9358	0,9361	0,9258	0,0966	0,9250
Conductivity	0,9359	1,000	1,000	1,000	0,9994	-0,0018	0,9987
TDS	0,9358	1,000	1,000	1,000	0,9994	-0,0026	0,9987
Salinity	0,9361	1,000	1,000	1,000	0,9994	-0,0000	0,9986
Hardness	0,9258	0,9994	0,9994	0,9994	1,000	-0,0291	0,9998
Alkalinity	0,0966	-0,0018	-0,0026	-0,0000	-0,0291	1,000	-0,0507
Chlorides	0,9250	0,9987	0,9987	0,9986	0,9998	-0,0507	1,000

Table 28: Correlation coefficients between physicochemical parameters for November.

November	pH	Conductivity	TDS	Salinity	Hardness	Alkalinity	Chlorides
pH	1,000	0,9813	0,9812	0,9809	0,9723	0,6114	0,9787
Conductivity	0,9813	1,000	1,000	1,000	0,9963	0,6353	0,9983
TDS	0,9812	1,000	1,000	1,000	0,9957	0,6408	0,9978
Salinity	0,9809	1,000	1,000	1,000	0,9958	0,6406	0,9979
Hardness	0,9723	0,9963	0,9957	0,9958	1,000	0,5722	0,9995
Alkalinity	0,6114	0,6353	0,6408	0,6406	0,5722	1,000	0,5896
Chlorides	0,9787	0,9983	0,9978	0,9979	0,9995	0,5896	1,000

During the months March till November, the pH values have a perfect strong relation (0,90 - 0,97) with the values of conductivity, TDS, salinity, hardness and chlorides. But on January, pH values have a moderate relation (0,62 - 0,64) with the values of conductivity, TDS, salinity, hardness and chlorides.

pH values have a moderate relation (-0,81 - 0,61) with alkalinity values on April, May, June and November in contrast to January, March, July, August and October. These months pH values indicate a weak relation (-0,03 - 0,41) with alkalinity values.

As concern as the relation between alkalinity values and the values of conductivity, TDS, salinity, hardness and chlorides, they have a moderate relation during January, March, April, May, June and November. The rest months (July, August and October) the relation between them is no linear.

From the observation of the values of conductivity, TDS, salinity, hardness and chlorides, it is concluded that there is a perfect strong relation between these four parameters during all the months of the sampling.

Thus, the lowest correlation between pH values and the rest of the parameters was observed on January. While the highest correlation between pH values and the other parameters, was observed on March, April, June and November. Generally, alkalinity has showed very low correlation with the other parameters. From the comparison of correlation between alkalinity and pH, it is clear that the highest positive correlation is on June and on November.

During November not only the values of pH were lower in contrast to the other months, as they were presented in chapter 4.1 (table 6), but also the values of alkalinity were low, as chapter 4.1 (table 7) shows. Thus, on November is the best positive correlation between alkalinity values and pH values, which means that low pH values and low alkalinity values give better correlation. The strongest negative correlation between alkalinity and pH has been observed during April and May.

Alkalinity can be increased when pH is decreased. One way that alkalinity increases is by the dissolution of calcium carbonate [$CaCO_3$] to carbonate [Ca^{2+} and CO_3^{2-}]. The dissolution of calcium carbonate decreases pH and increases alkalinity due to absorption of CO_2. Also, alkalinity can be increased by anaerobic degradation processes, like denitrification and sulfate reduction. These processes consume hydrogen ions, which means that pH decreases and alkalinity increases. On the other hand, alkalinity decreases when H^+ ions increase, such as in anaerobic degradation processes. The following chemical reactions explain the relationship between alkalinity and pH.

(a) $CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$ (at neutral pH values)

(b) $CaCO_3 + H^+ \leftrightarrow Ca^{2+} + HCO_3^-$

(c) $CaCO_3 + 2H^+ \leftrightarrow Ca^{2+} + CO_2 + H_2O$

When CO_2 is dissolved in the water and the water comes into contact with the atmosphere, the equilibrium is moving to the right, thus HCO_3^- are increased, as the reaction a shows. Addition of CO_2 to a solution can affect the alkalinity in carbonate minerals, which have contact with water. By the dissolution of carbonate rocks and the increasing of H^+, the equilibrium goes towards the right, thus HCO_3^- are increased. So, alkalinity concentration is increased according to the reaction b. When pH values are low, then the concentration of bicarbonate will be high. When carbonate minerals, water and atmosphere are in equilibrium, the reaction (c) indicates that pH and calcium ions are connected. When pH is low, then the calcium ions concentration is high. So, when pH value is high, bicarbonate and carbonate ions are increased [69].

As concern as the other parameters, conductivity, total dissolved solids, salinity, hardness and chlorides show extremely high correlations as it was expected.

CONCLUSIONS

The conclusions that are obtained from the results of this study are the following:

- The parameters TDS, electrical conductivity, salinity and chlorides have a very strong correlation between them for all months.

- The salinity is increased from January to November in sampling points 1 and 2. There were extremely maximum concentrations in 3 and 4 on April. The salinity is increased from the springs (point 1) to the mouth of the river (point 4).

- Generally, greater spatial variations were observed during July, August and October while during April there were smaller. This is ascertained by the smaller correlation between the alkalinity and the other physicochemical parameters and by the fluorescent compounds that are present in the samples.

- During the dry periods there are less fluorescent compounds than the rainy (wet) periods. This happens because of the higher temperatures that there are more photochemical reactions, microbial degradation or evaporation. The most fluorescent compounds (60%) belong to the humic-like type and microbial byproducts type.

- Polycyclic Aromatic Hydrocarbons (PAHs) were detected mostly in points 2 and 1. October was the most polluted month. Their concentrations were lower than the EC limits for inland water.

- Anionic surfactants as LAS were detected in almost all samples and months except from January in points 2, 3, 4.

- The river is polluted by activities from the surrounding area, like burning reeds or tree branches. Moreover, the pollution is caused by illegal disposal of wastes, such as tires and lot of domestic equipment, as the river is closed to the national central road in Heraklion.

- The water from its springs is mixed with higher salinity water in small quantities.

- The estuary of the river is a well-mixed type estuary.

APPENDIX

Emission spectra of sixteen PAHs

The spectra of each one of the sixteen PAHs in the fluorescence detector are presented in Appendix.

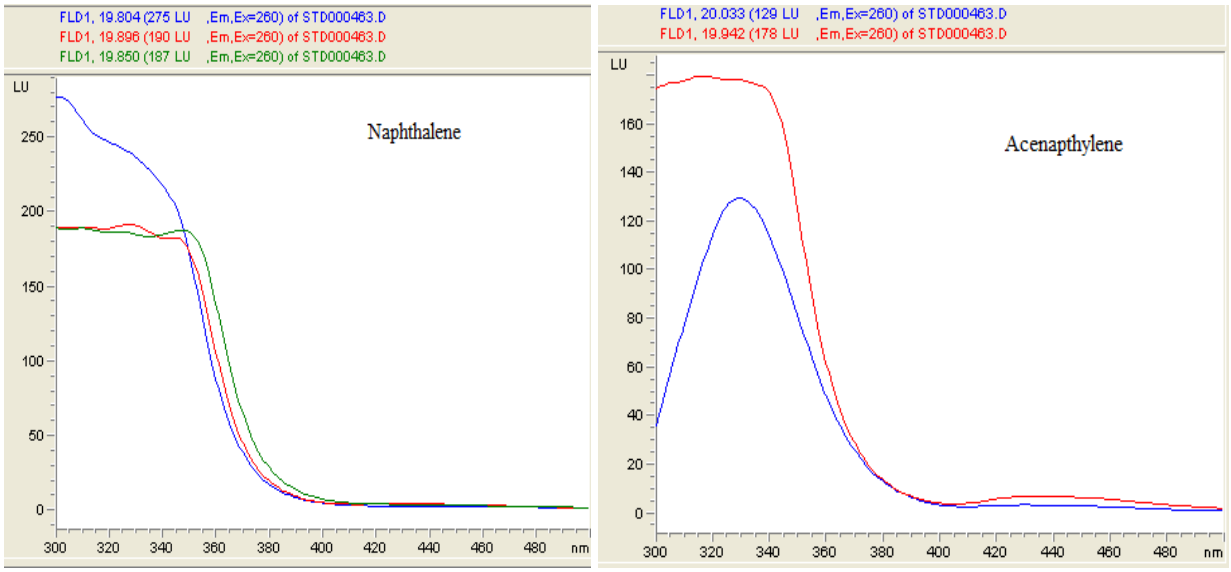


Figure 38: The emission spectra of Naphthalene and Acenapthylene.

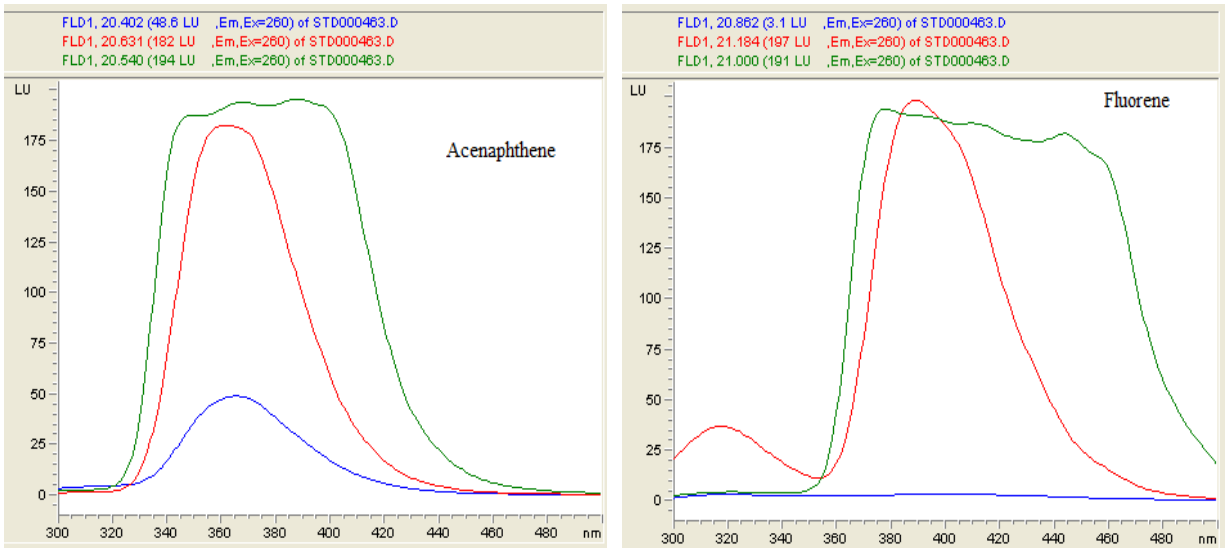


Figure 39: The emission spectra of Acenaphthene and Fluorene.

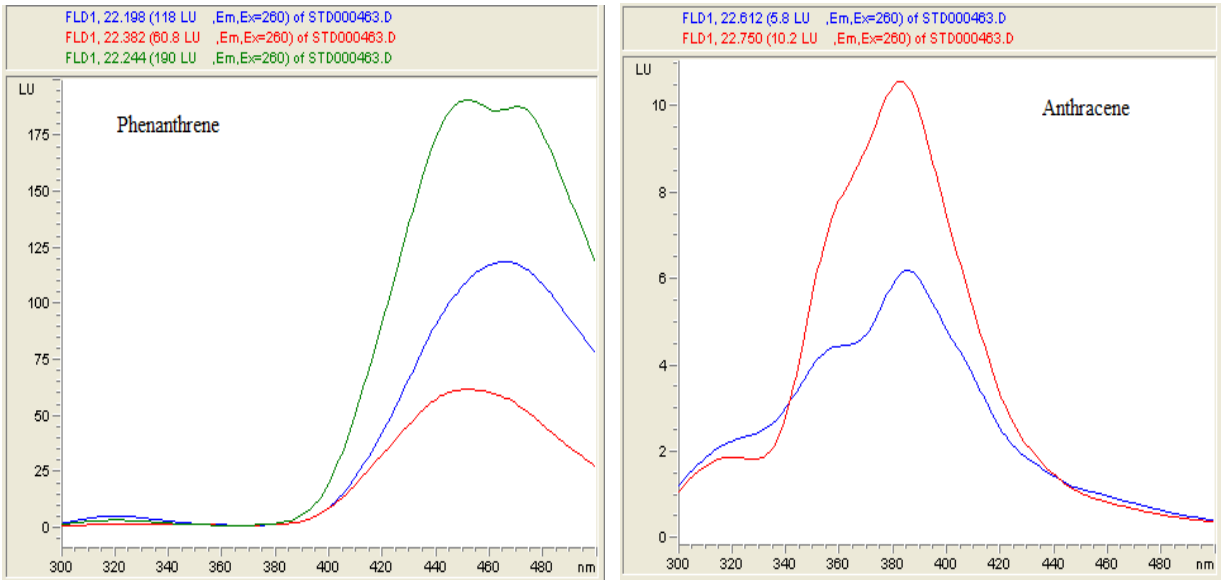


Figure 40: The emission spectra of Phenanthrene and Anthracene.

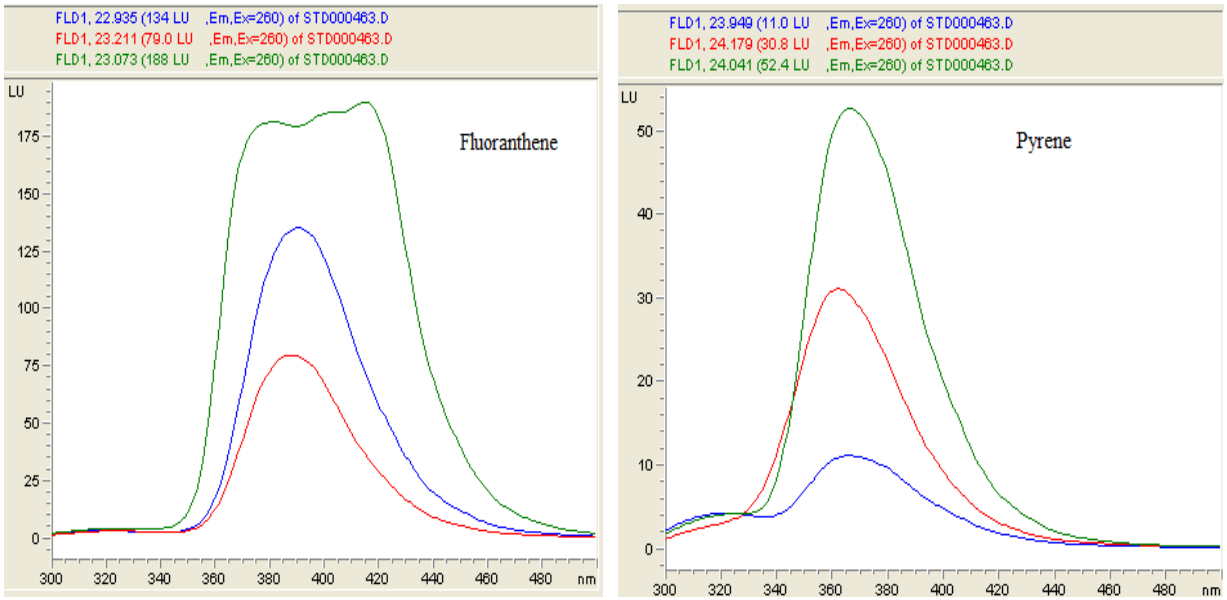


Figure 41: The emission spectra of Fluoranthene and Pyrene.

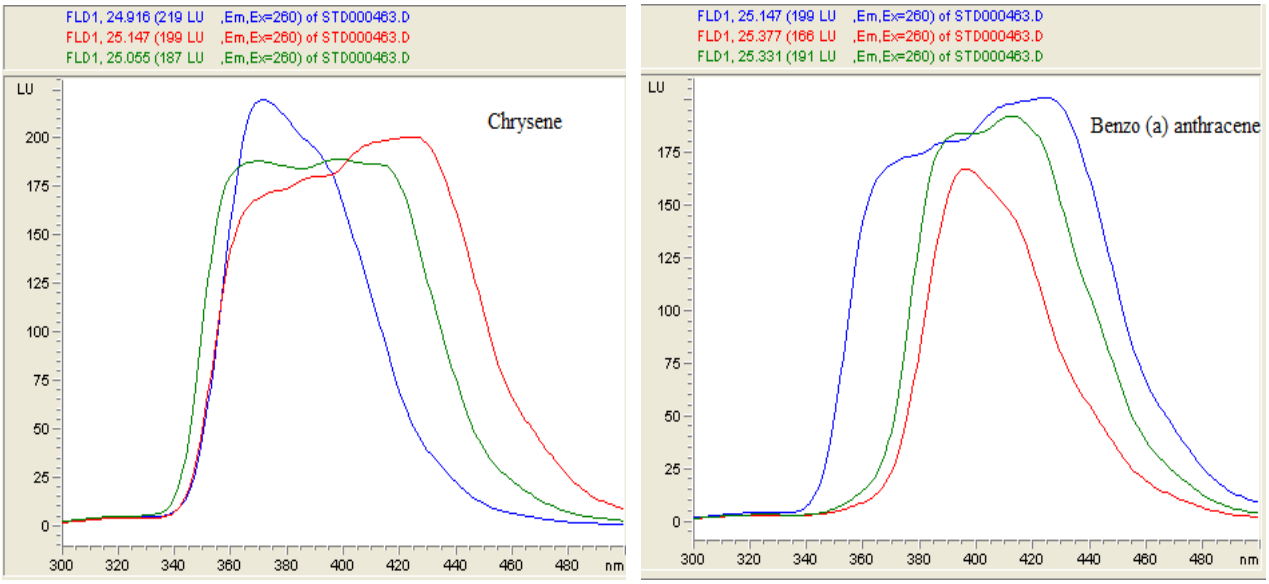


Figure 42: The emission spectra of Chrysene and Benzo (a) Anthracene.

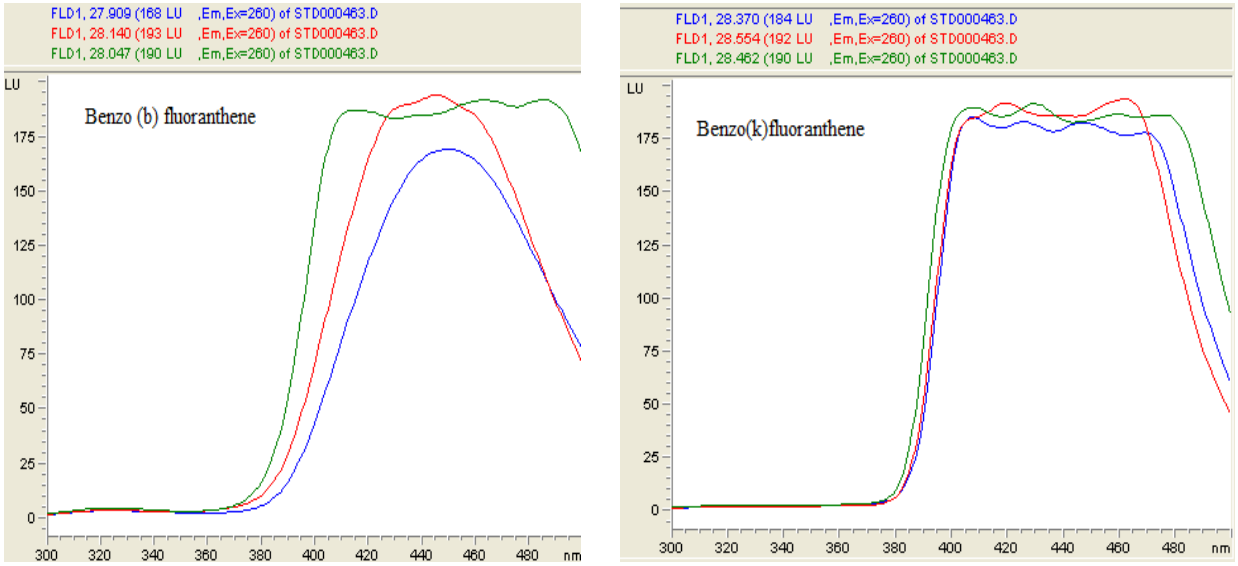


Figure 43: The emission spectra of Benzo (b) fluoranthene and Benzo (k) fluoranthene.

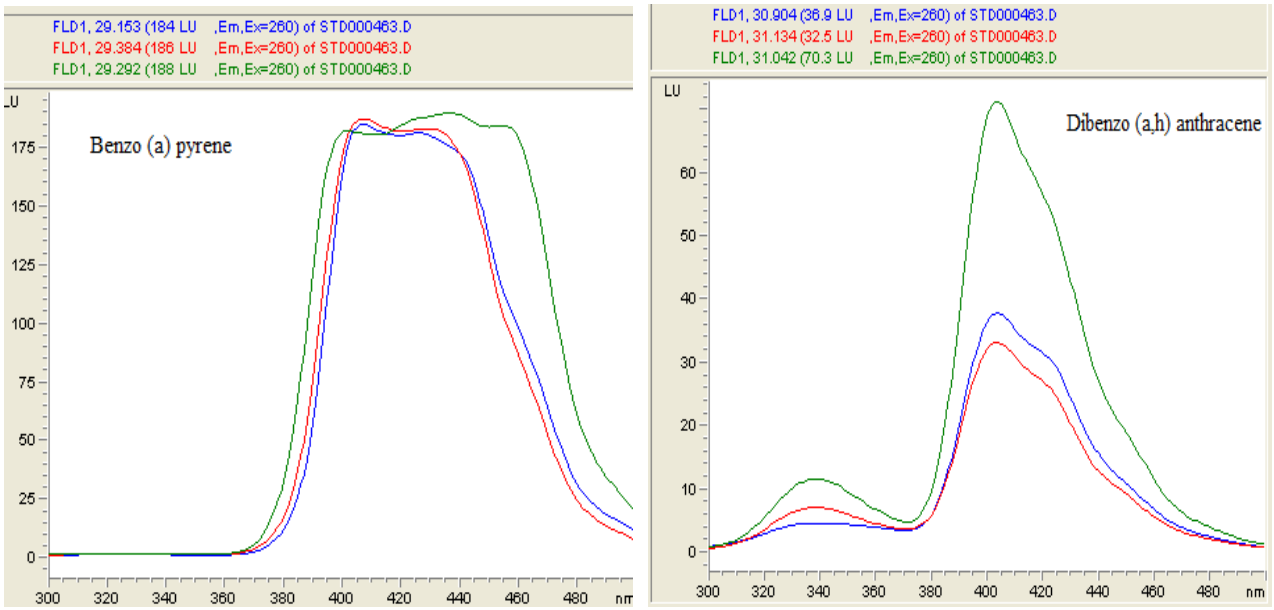


Figure 44: The emission spectra of Benzo (a) pyrene and Dibenzo (a,h) anthracene .

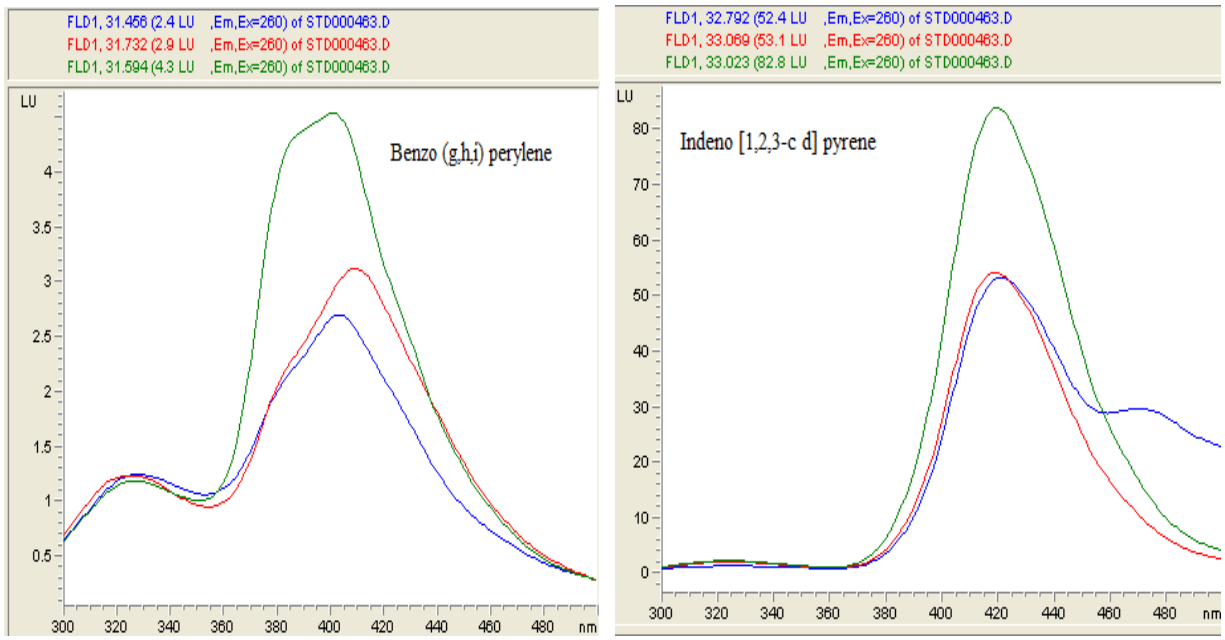


Figure 45: The emission spectra of Benzo (g,h,i) perylene and Indeno [1,2,3-cd] pyrene.

Chromatographic signals from FLD

Subsequently, the chromatograms, in figures 46 till 54, for the four points for each one month separate are presented in the Appendix. Each chromatogram refers to each one of the months of samplings and shows the chromatogram for the four different points at the fluorescence detector.

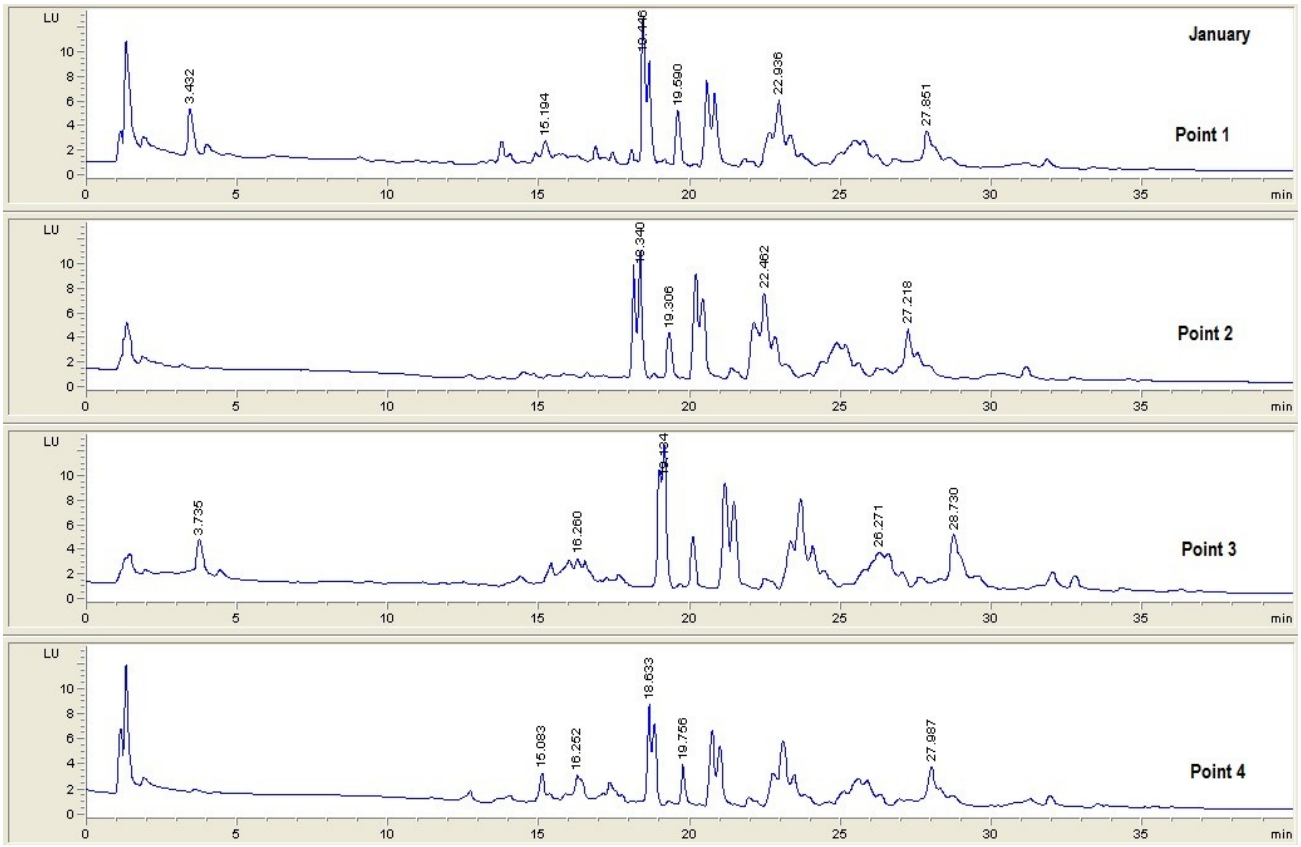


Figure 46: Chromatogram of all the points on January.

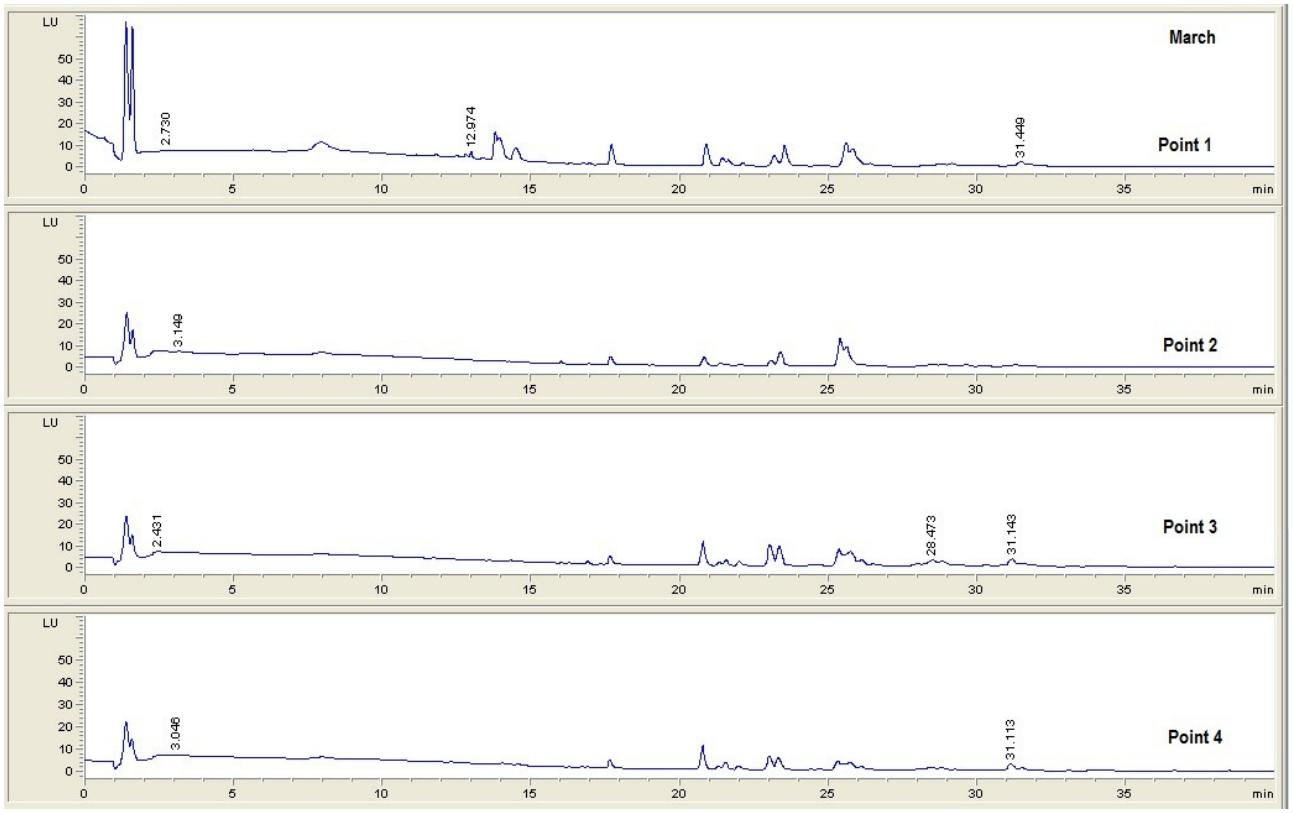


Figure 47 : Chromatogram of all the points on March.

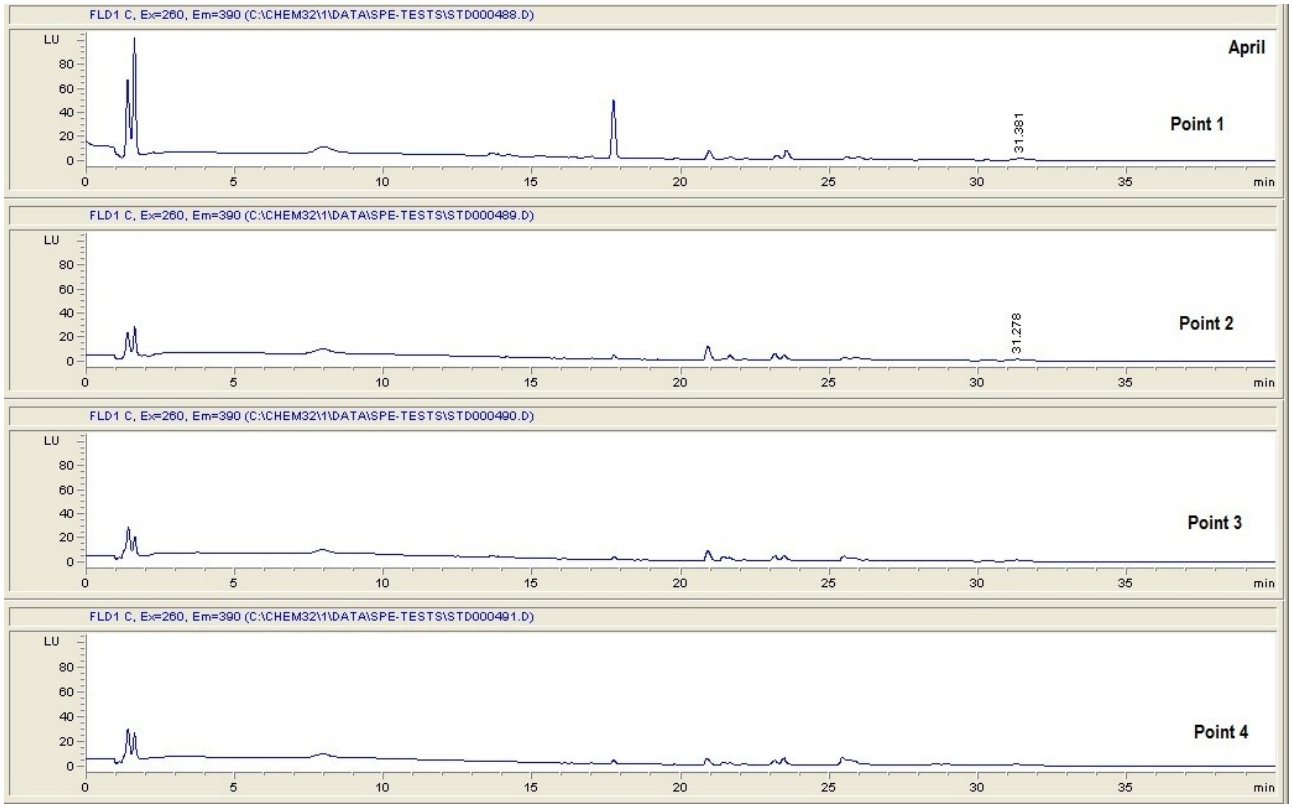


Figure 48 : Chromatogram of all the points on April.

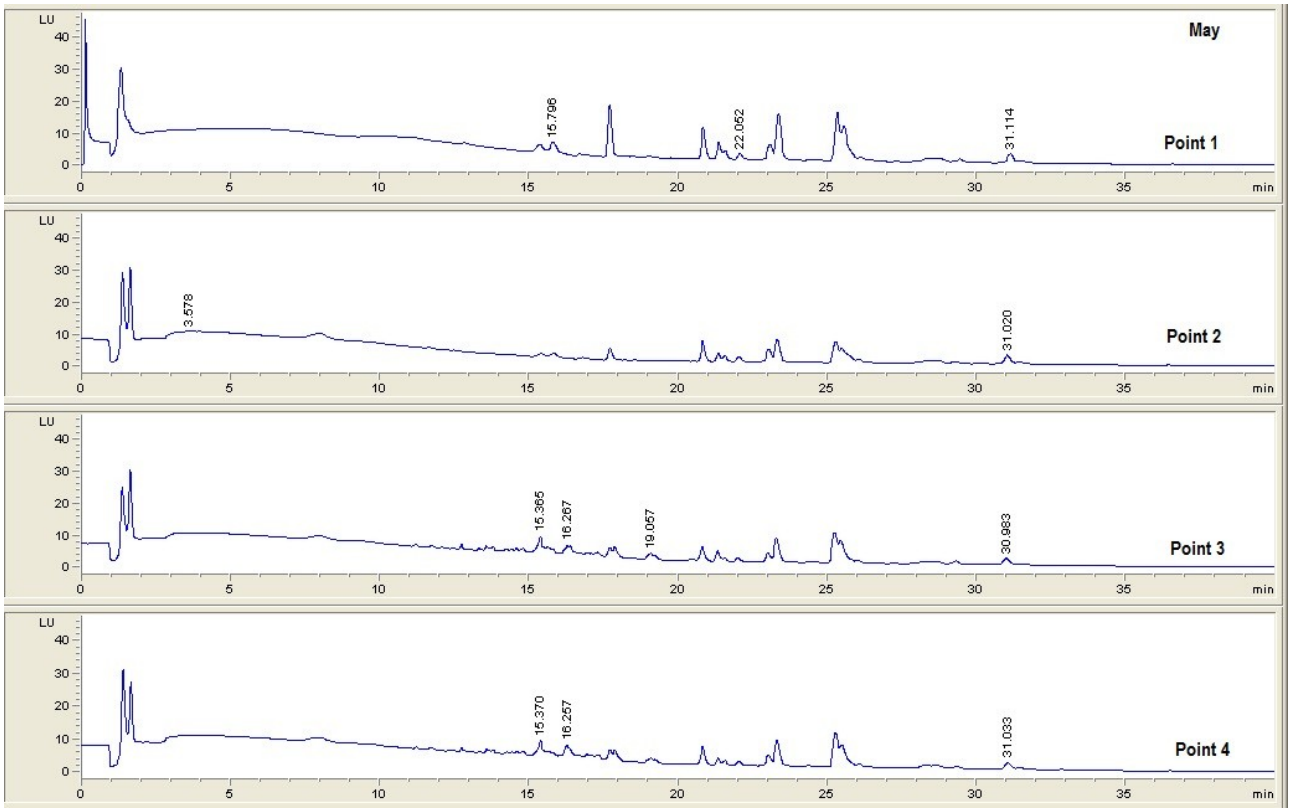


Figure 49 : Chromatogram of all the points on May.

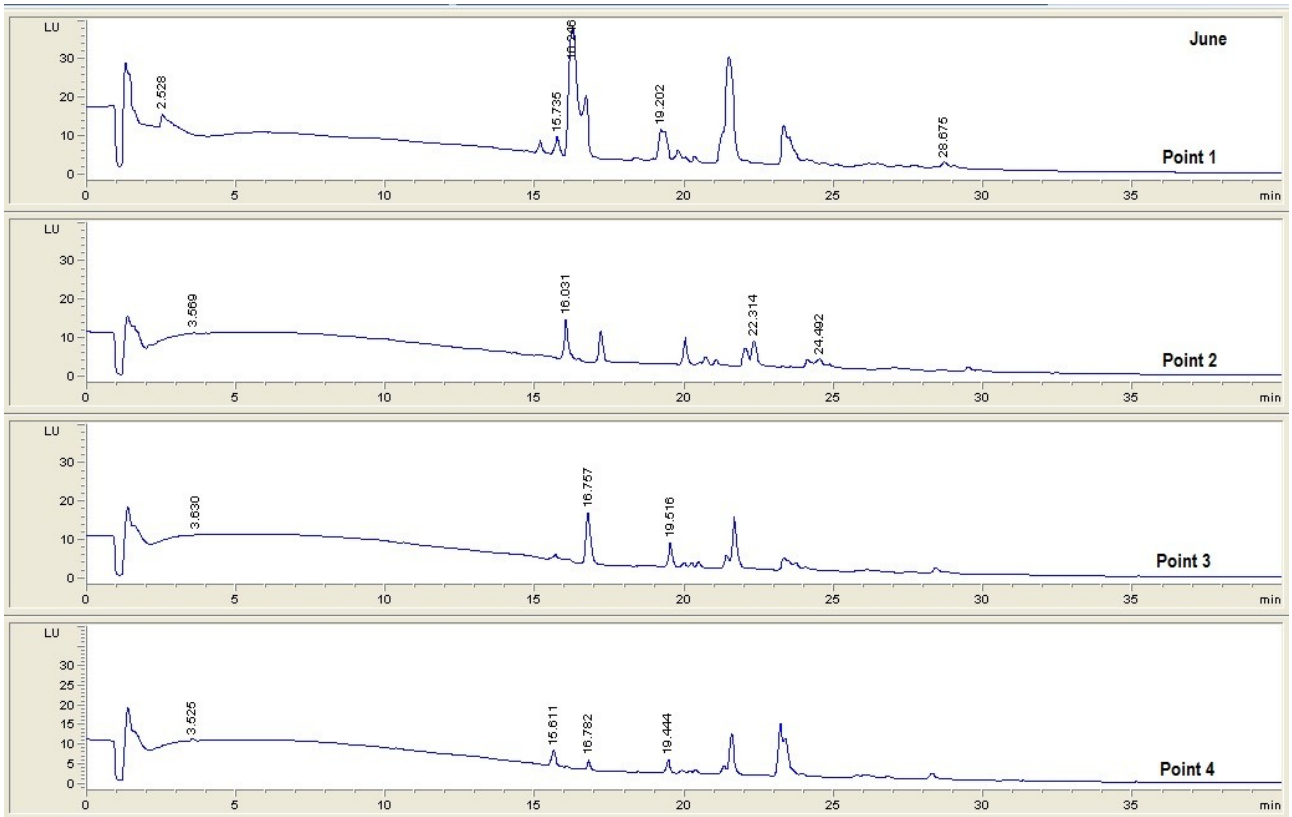


Figure 50 : Chromatogram of all the points on June.

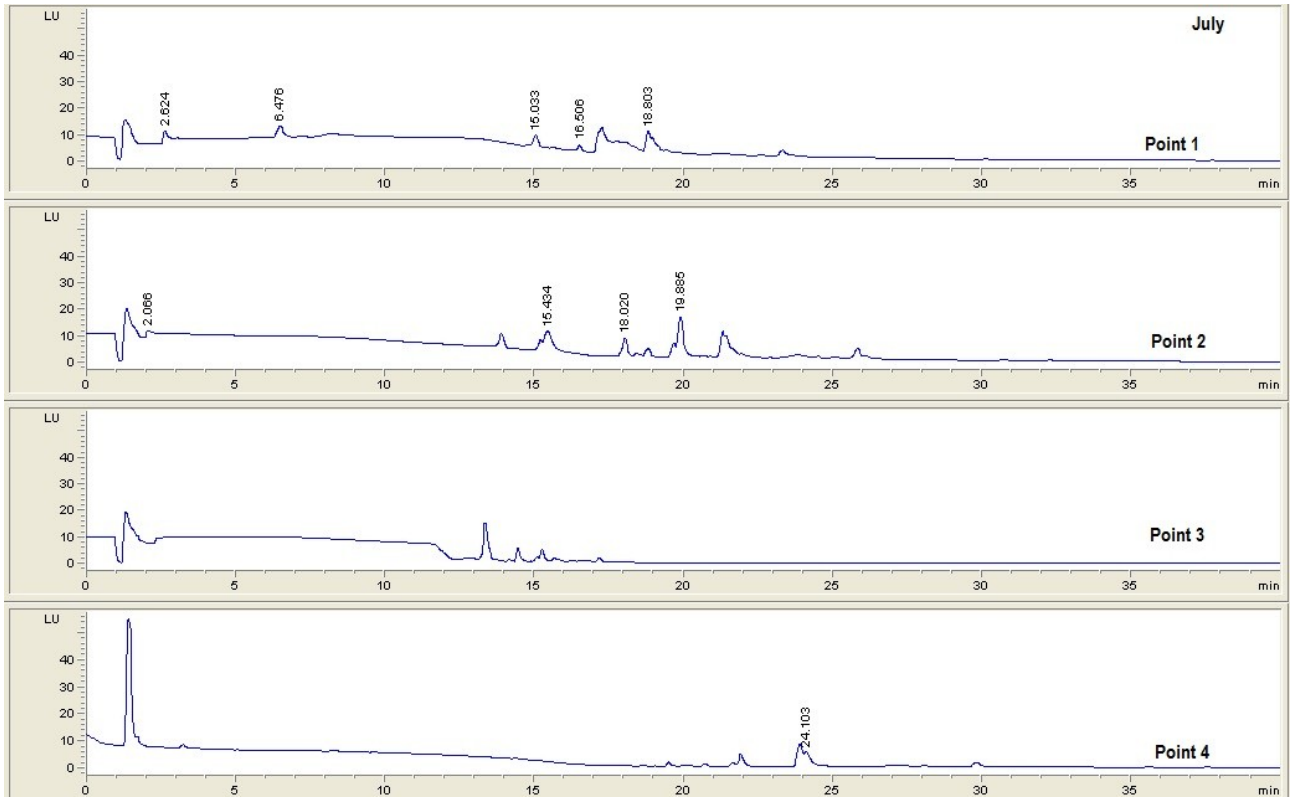


Figure 51 : Chromatogram of all the points on July.

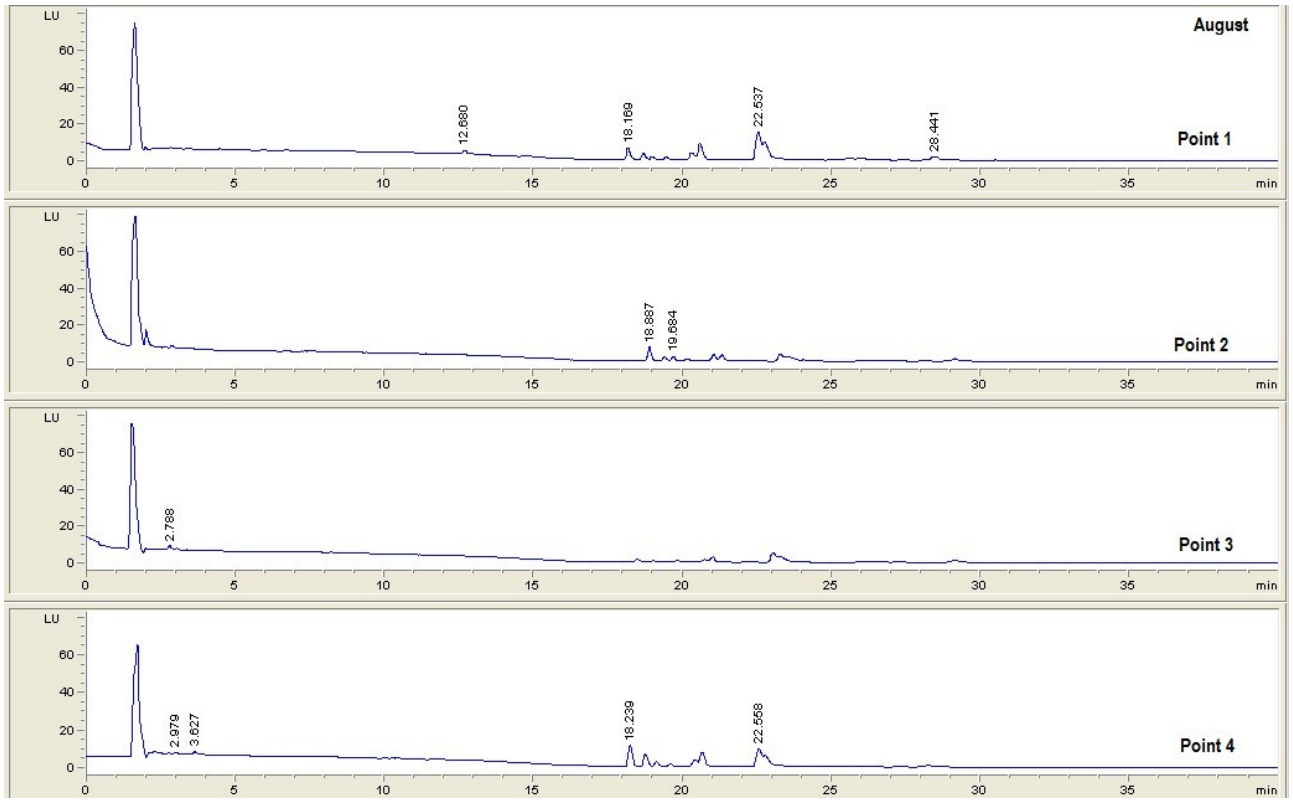


Figure 52 : Chromatogram of all the points on August.

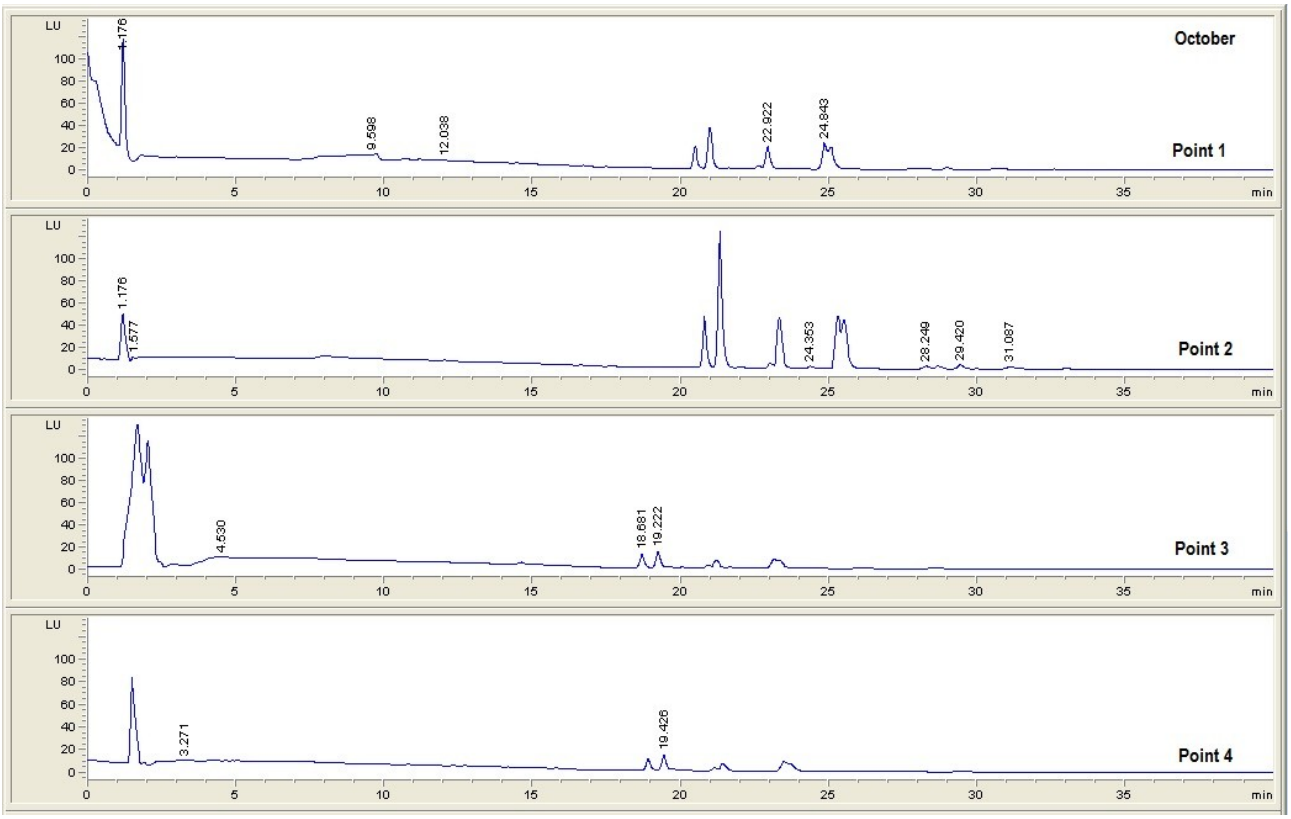


Figure 53 : Chromatogram of all the points on October.

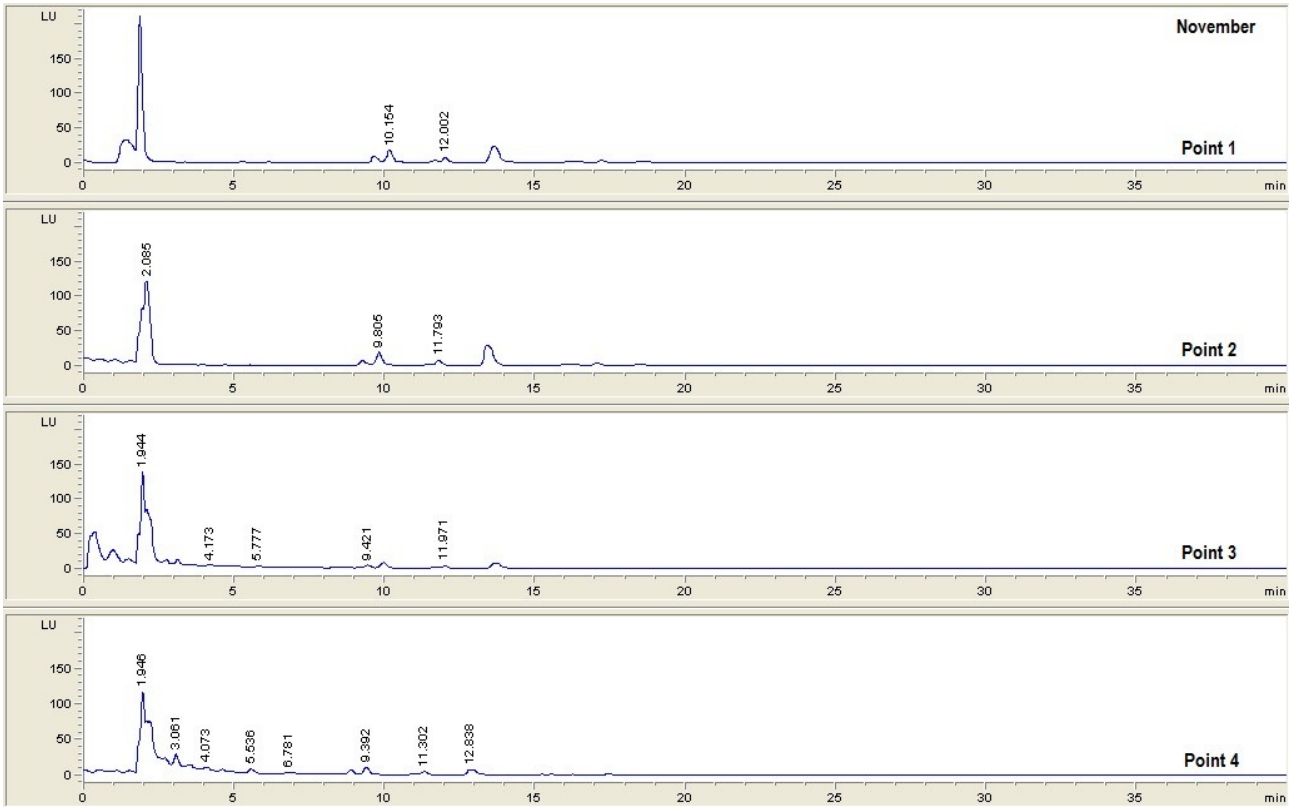


Figure 54 : Chromatogram of all the points on November.

Emission spectra for all the points from FLD

The emission spectra for each peak of each point of each month are presented below. These spectra are displayed in fluorescence detector.

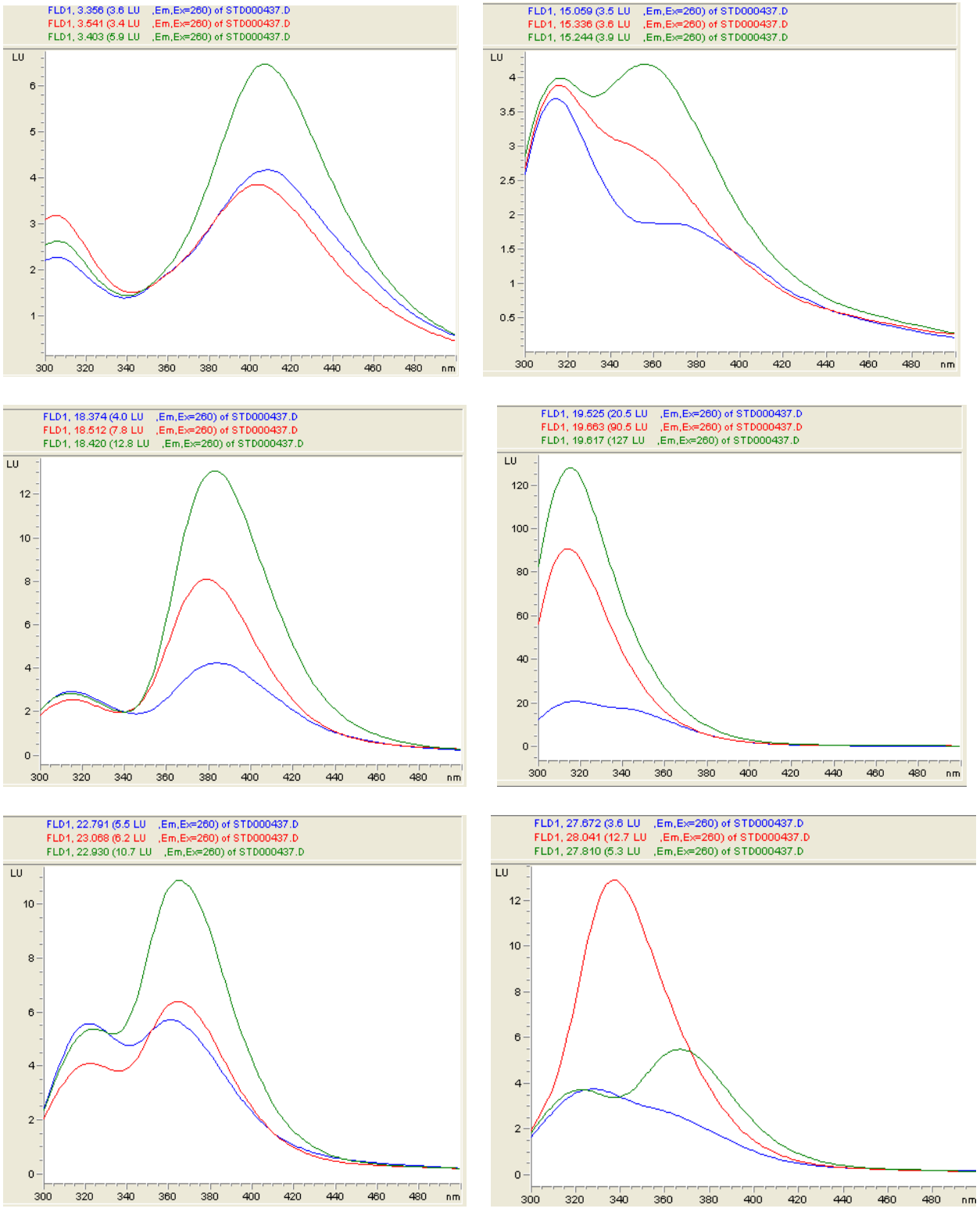


Figure 55: Emission spectra of January's peaks for point 1 in fluorescence detector.

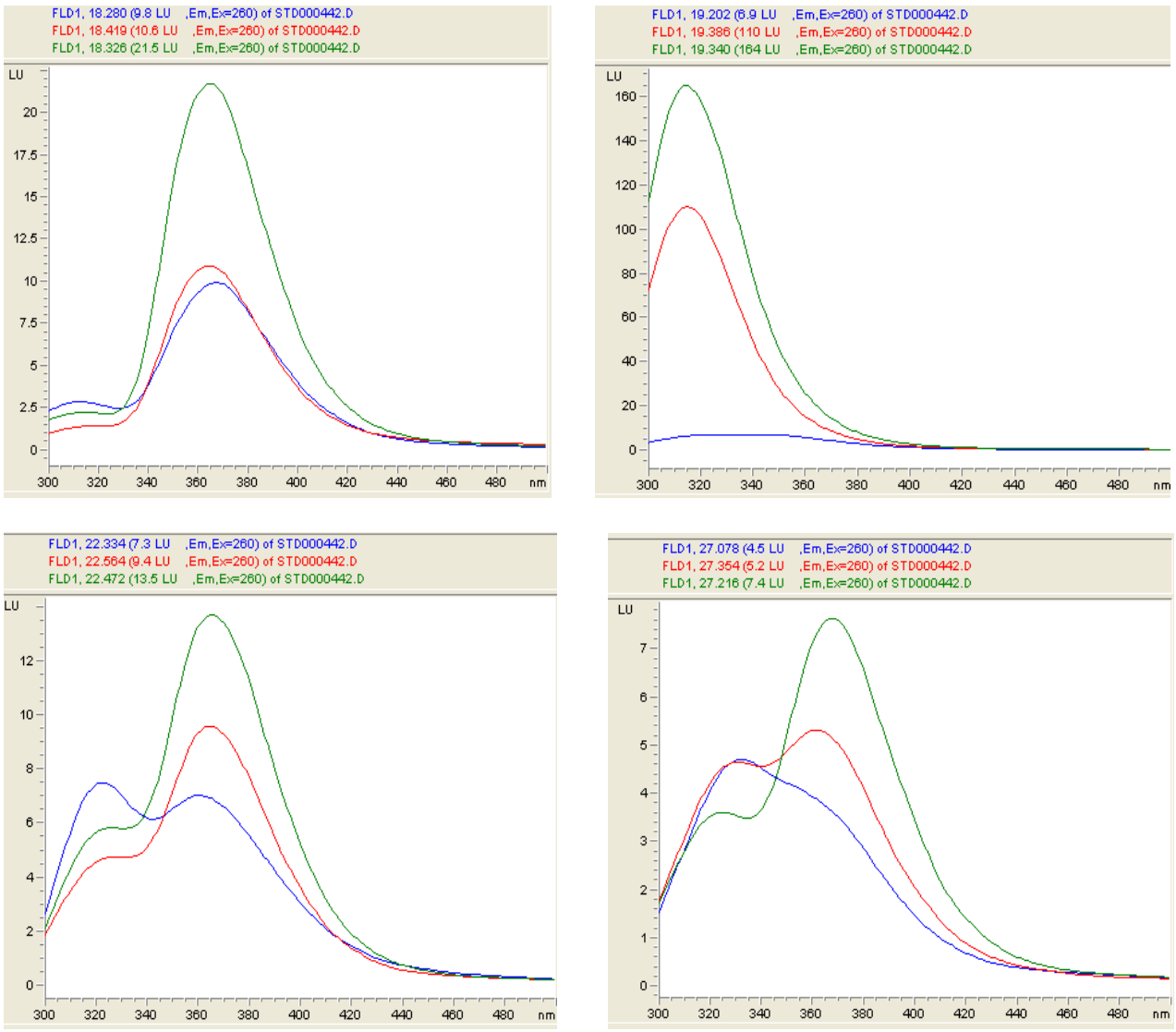
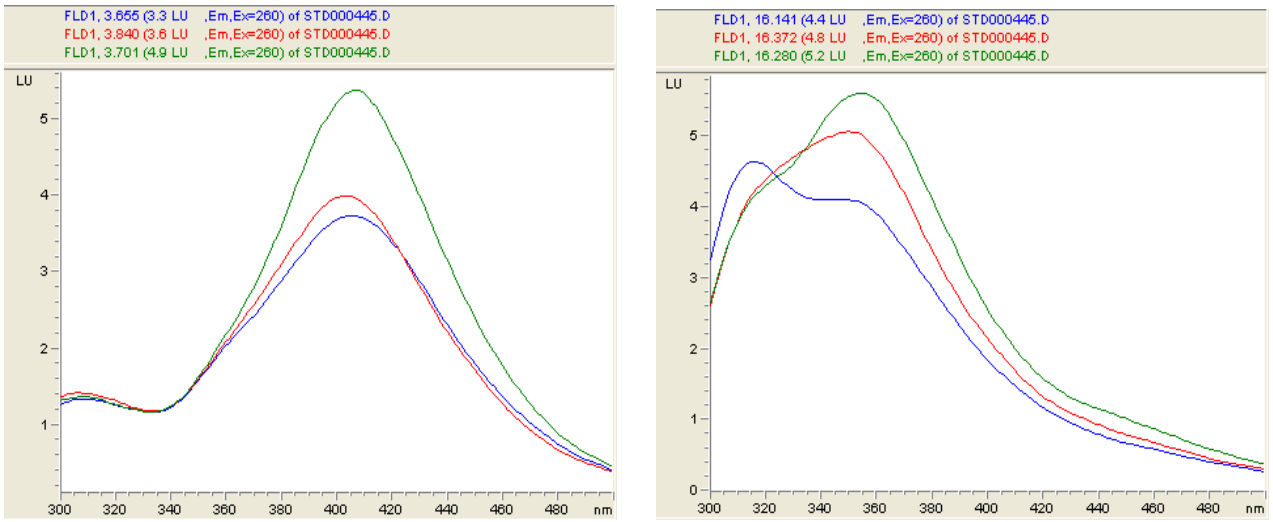


Figure 56: Emission spectra of January's peaks for point 2 in fluorescence detector.



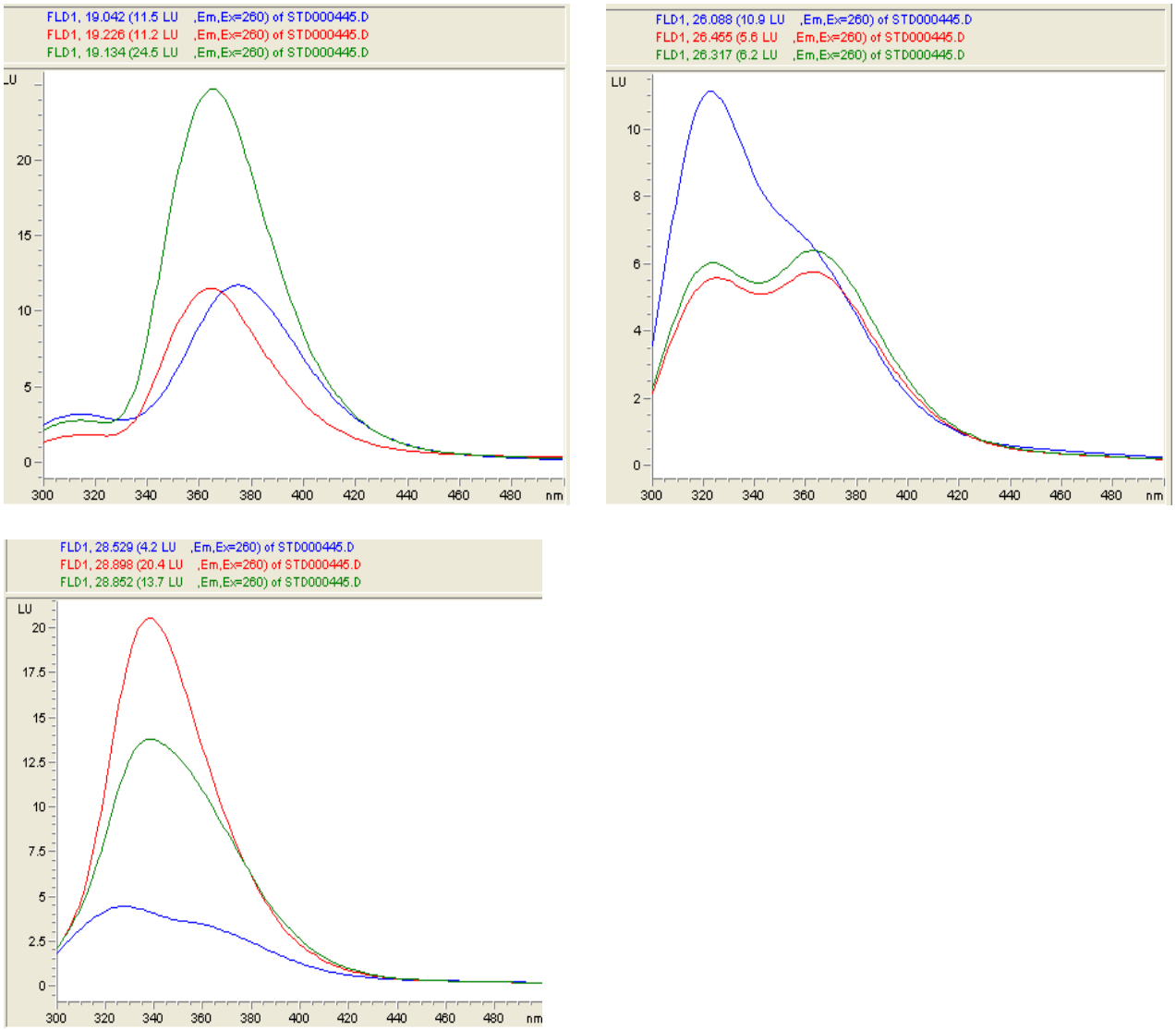
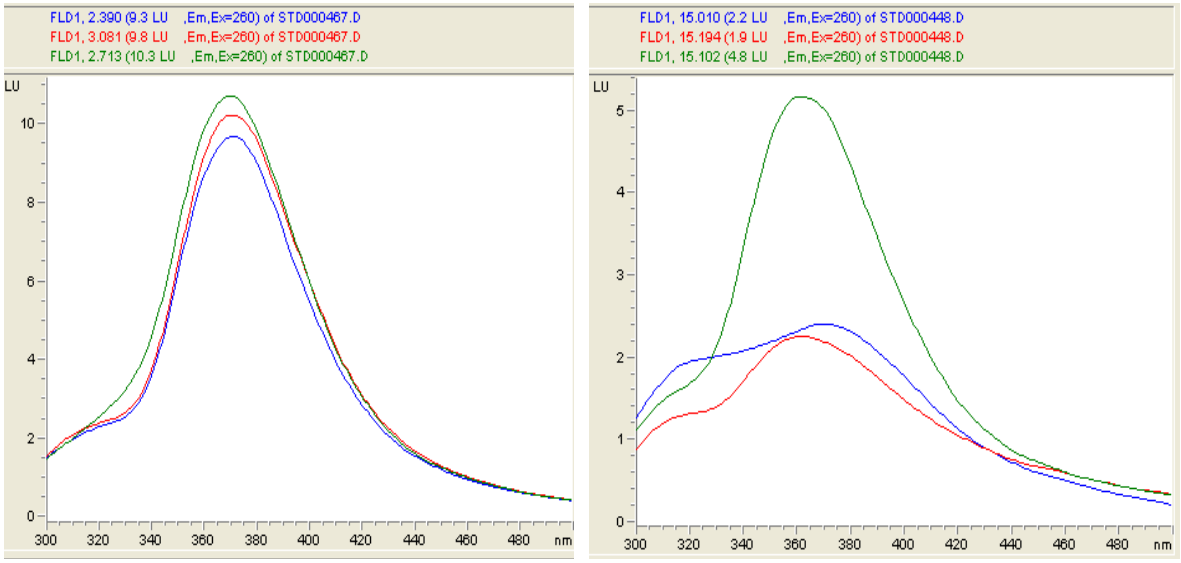


Figure 57: Emission spectra of January's peaks for point 3 in fluorescence detector.



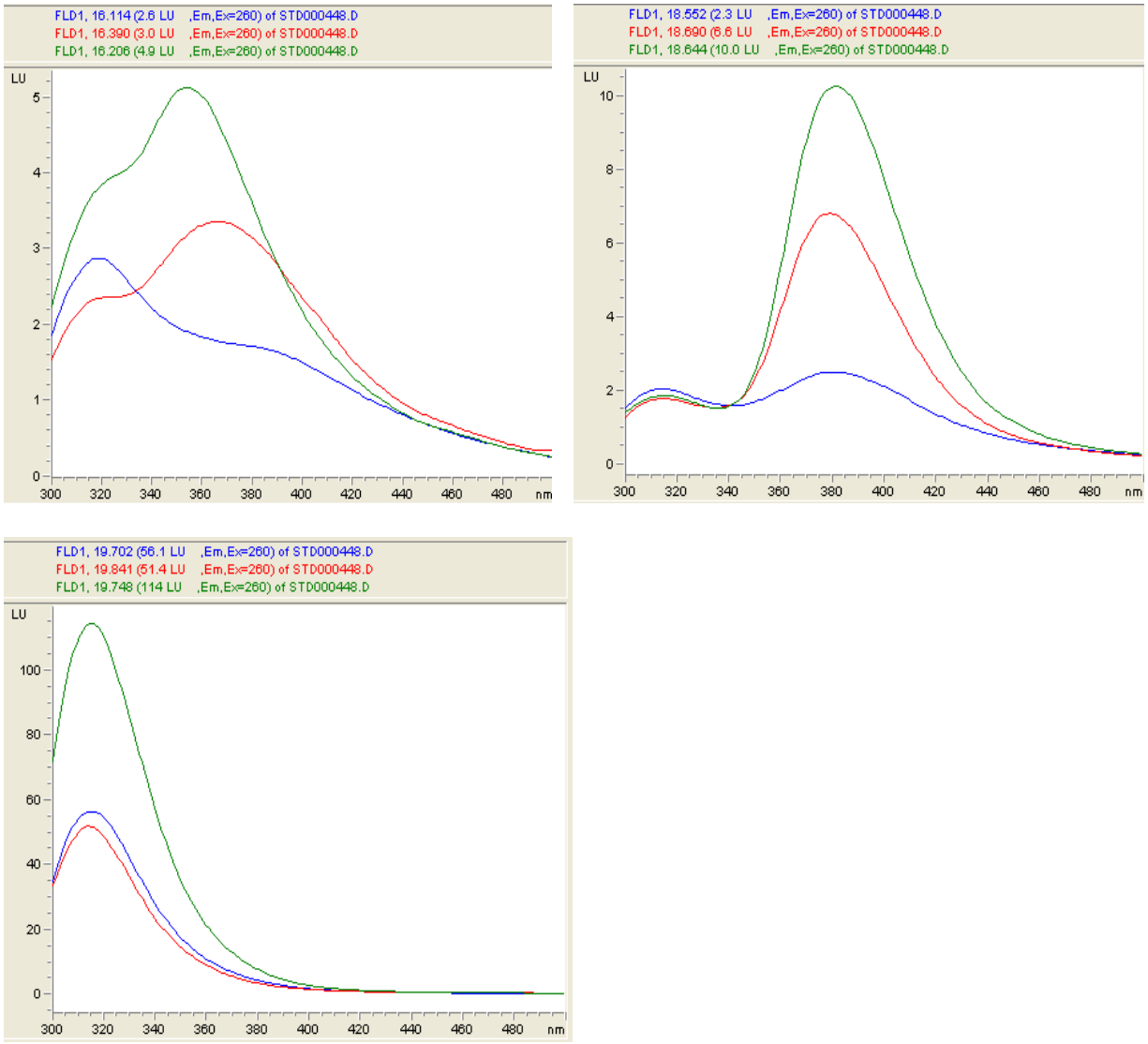
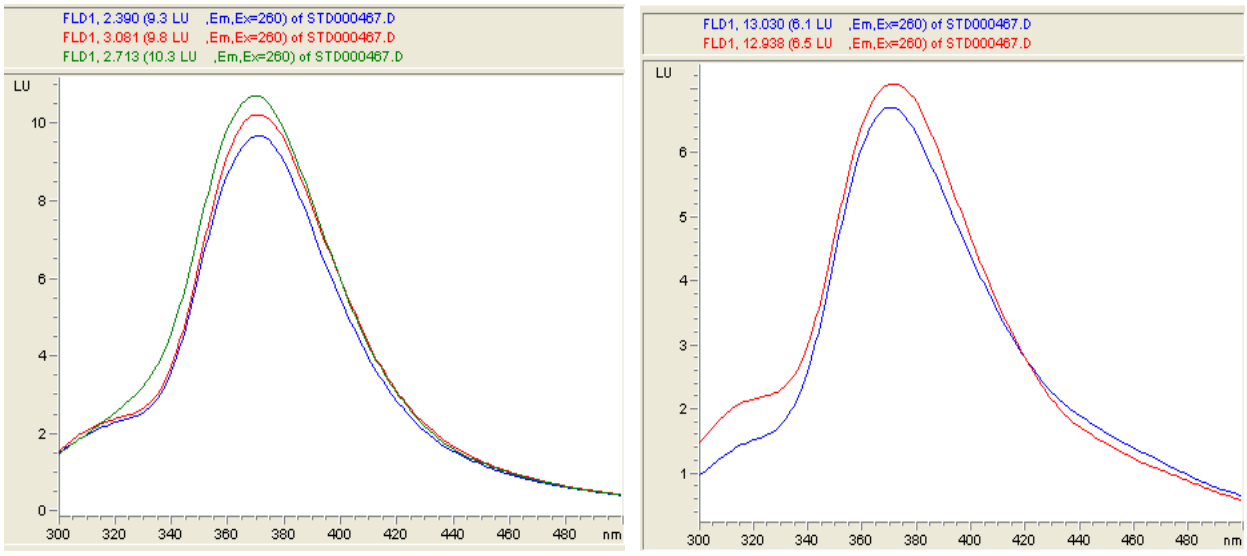


Figure 58: Emission spectra of January's peaks for point 4 in fluorescence detector.



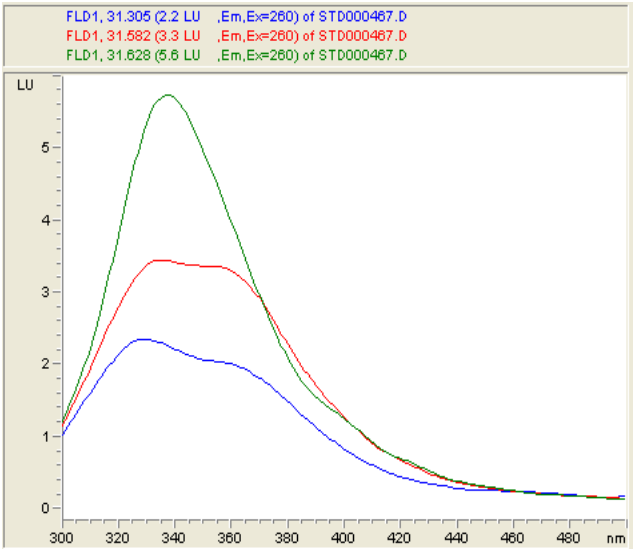


Figure 59: Emission spectra of March's peaks for point 1 in fluorescence detector.

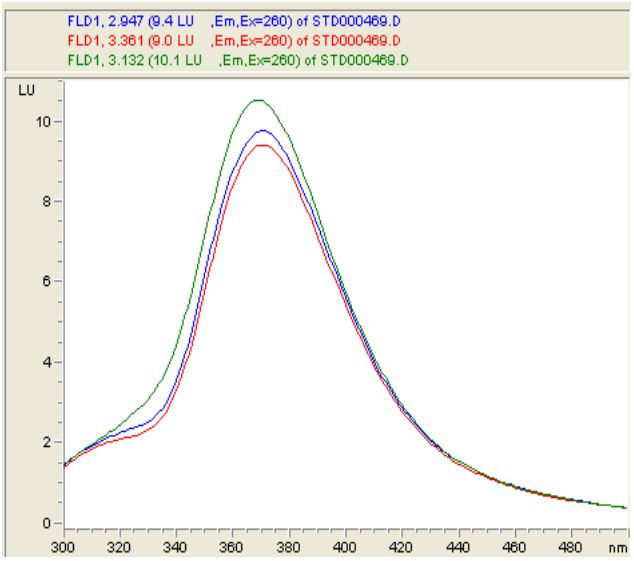


Figure 60: Emission spectra of March's peaks for point 2 in fluorescence detector.

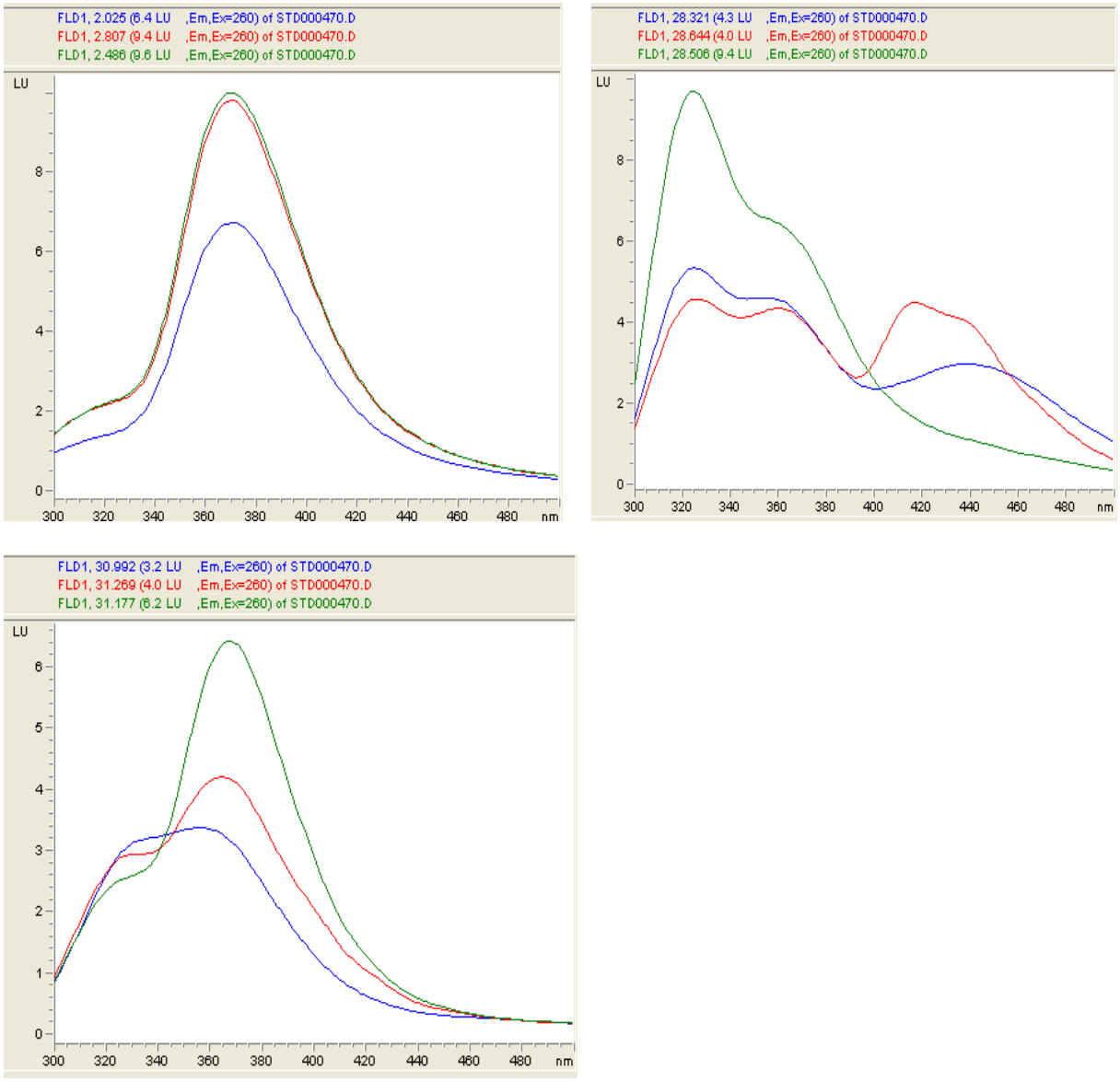


Figure 61: Emission spectra of March's peaks for point 3 in fluorescence detector.

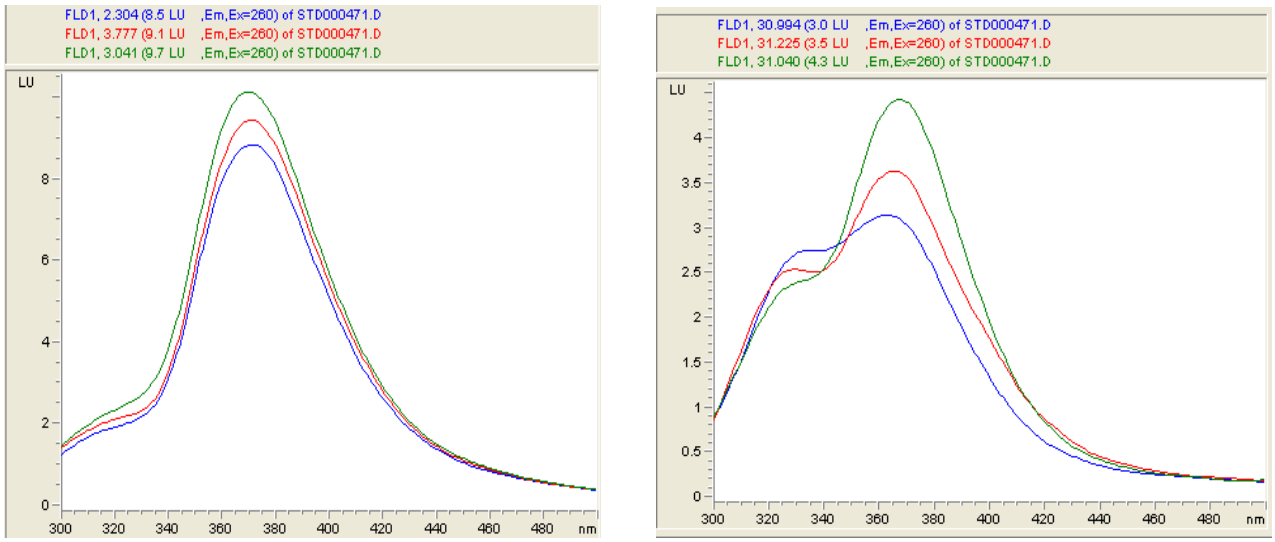


Figure 62: Emission spectra of March's peaks for point 4 in fluorescence detector.

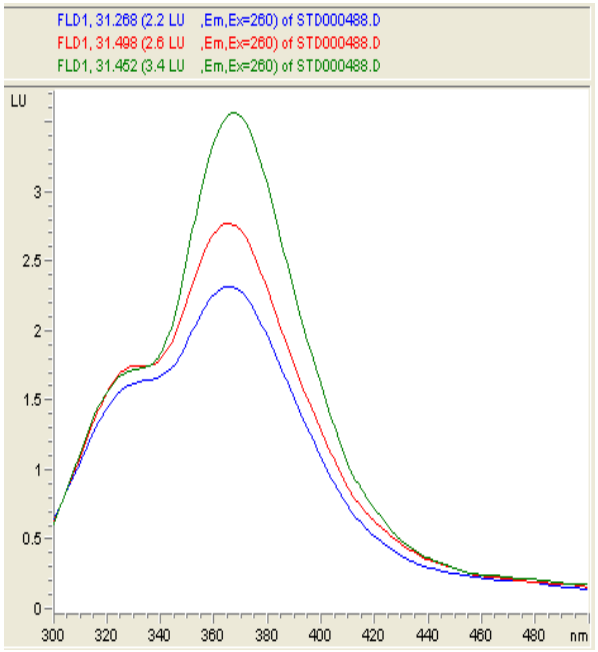


Figure 63: Emission spectrum of April's peaks for point 1 in fluorescence detector.

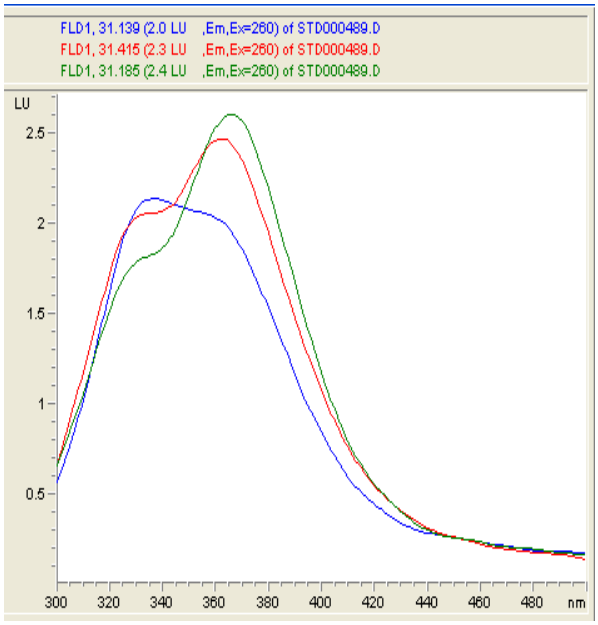


Figure 64: Emission spectrum of April's peaks for point 2 in fluorescence detector.

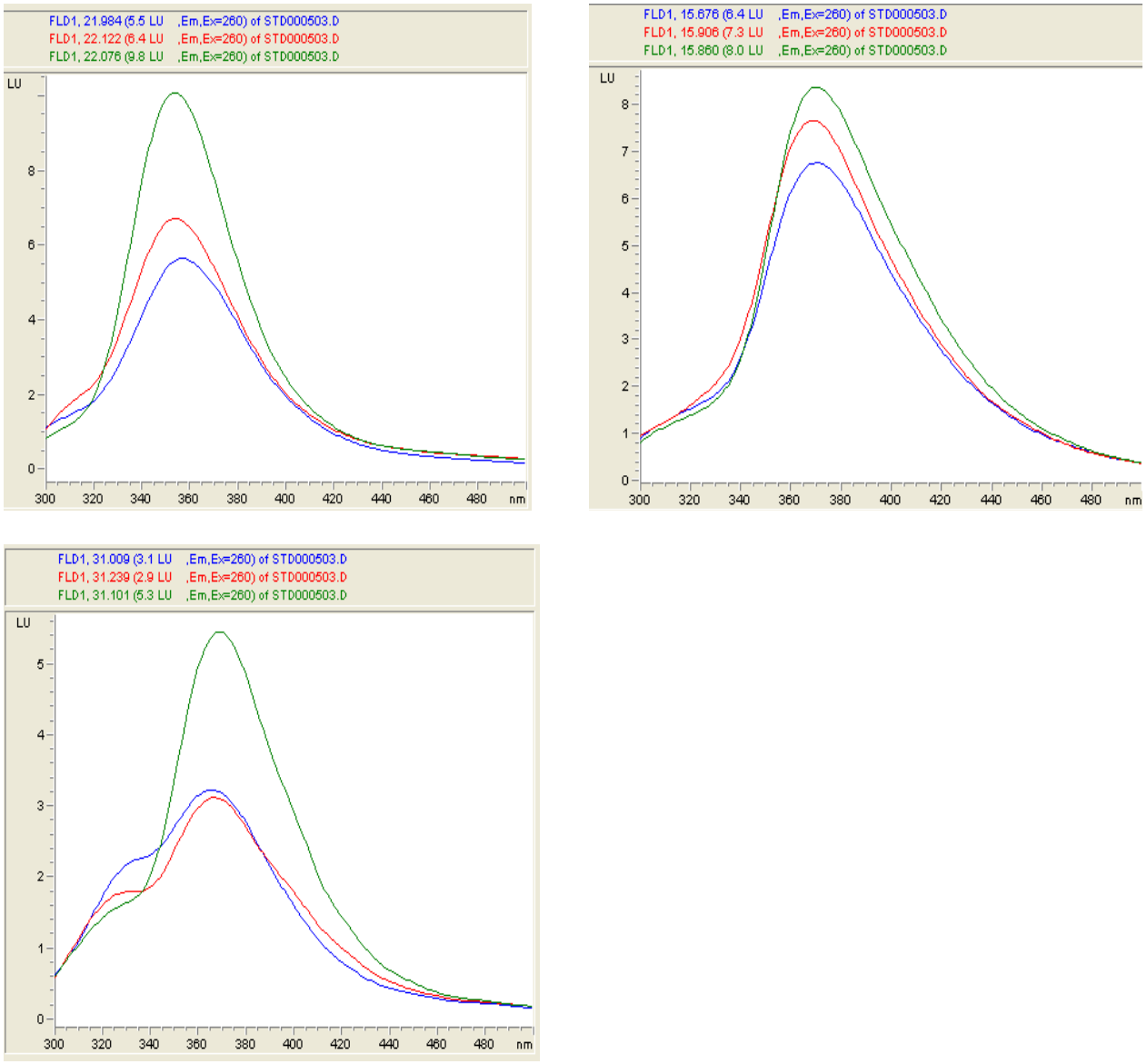


Figure 65: Emission spectrum of May's peaks for point 1 in fluorescence detector.

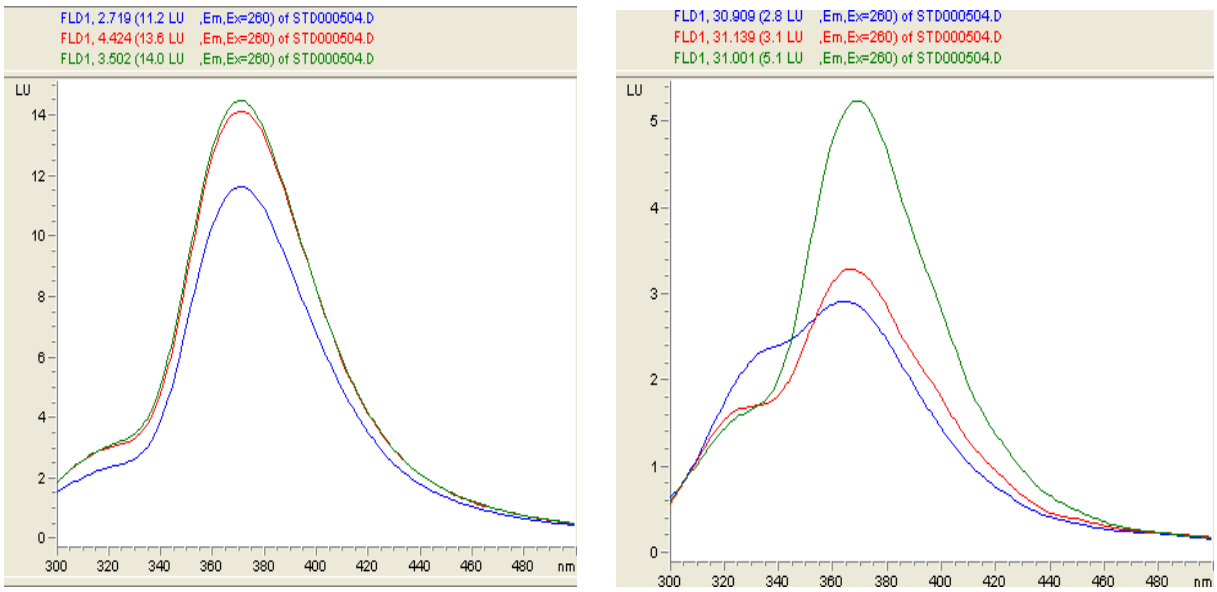


Figure 66: Emission spectrum of May's peaks for point 2 in fluorescence detector.

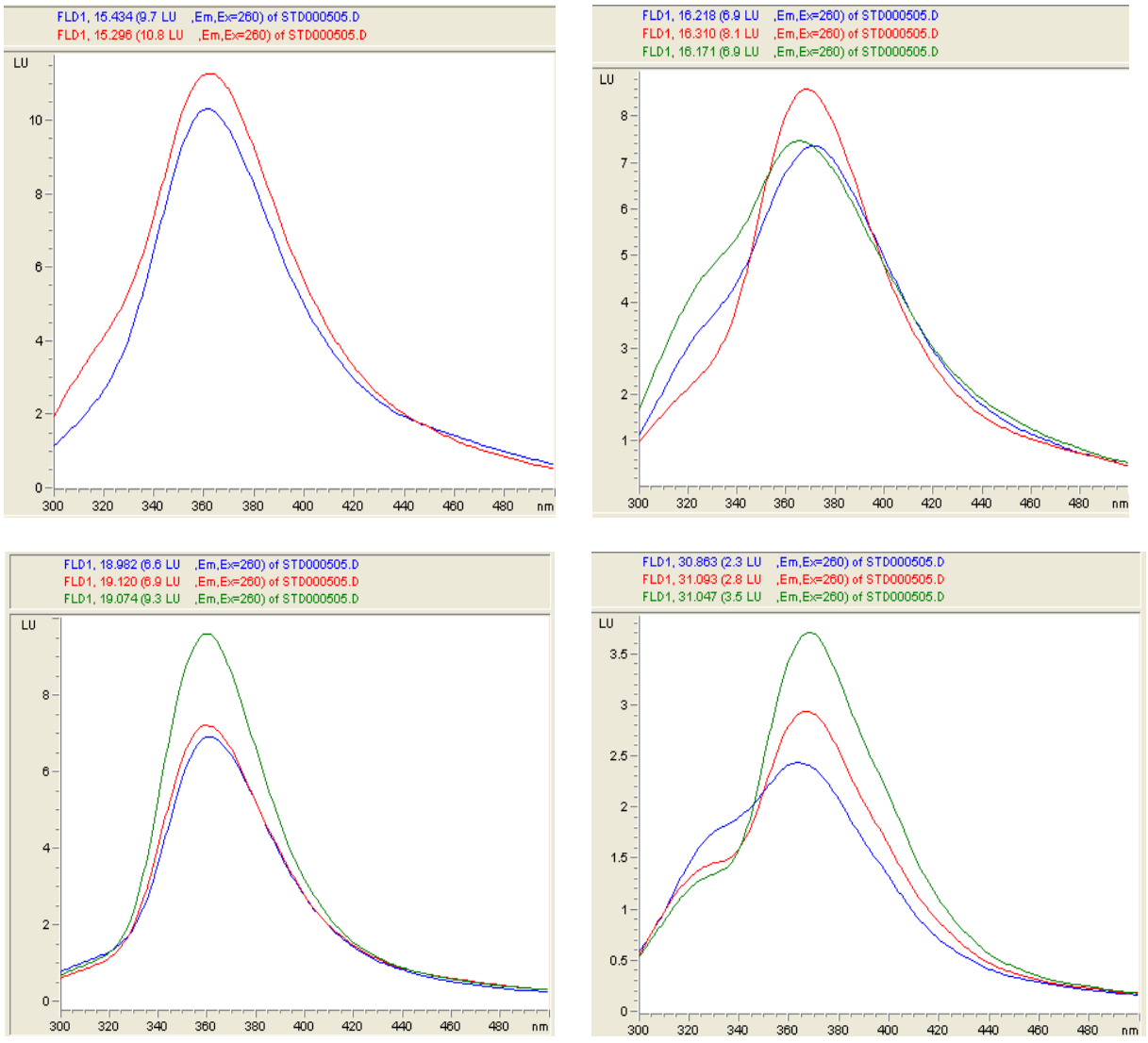


Figure 67: Emission spectrum of May's peaks for point 3 in fluorescence detector.

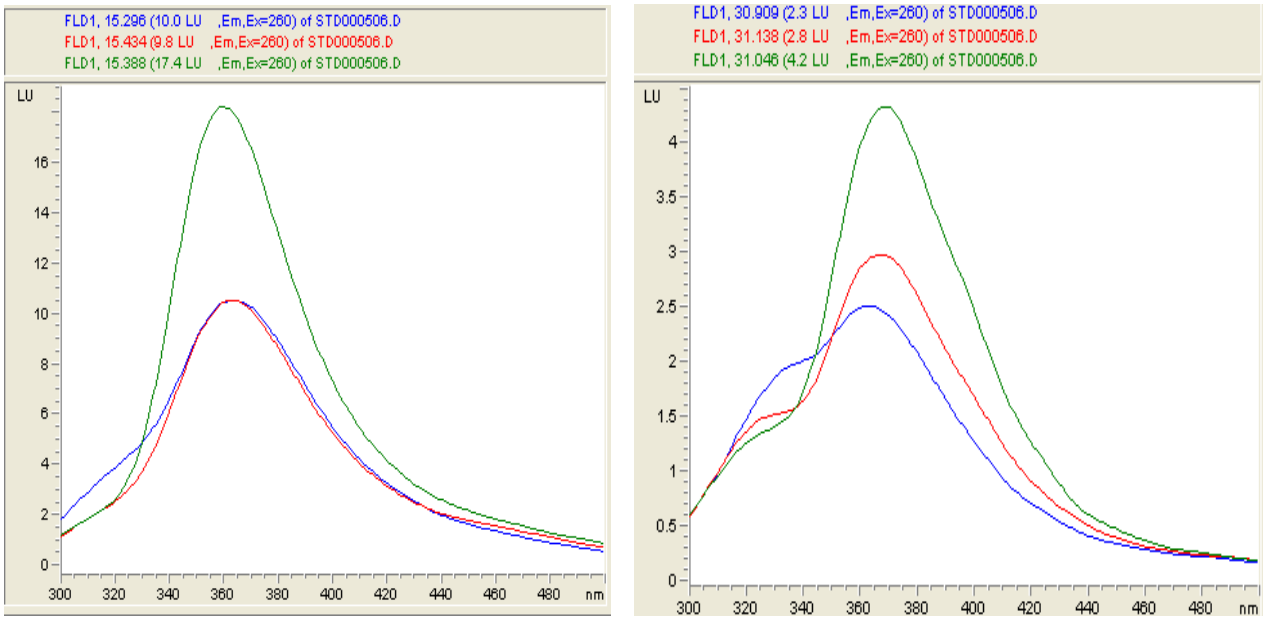
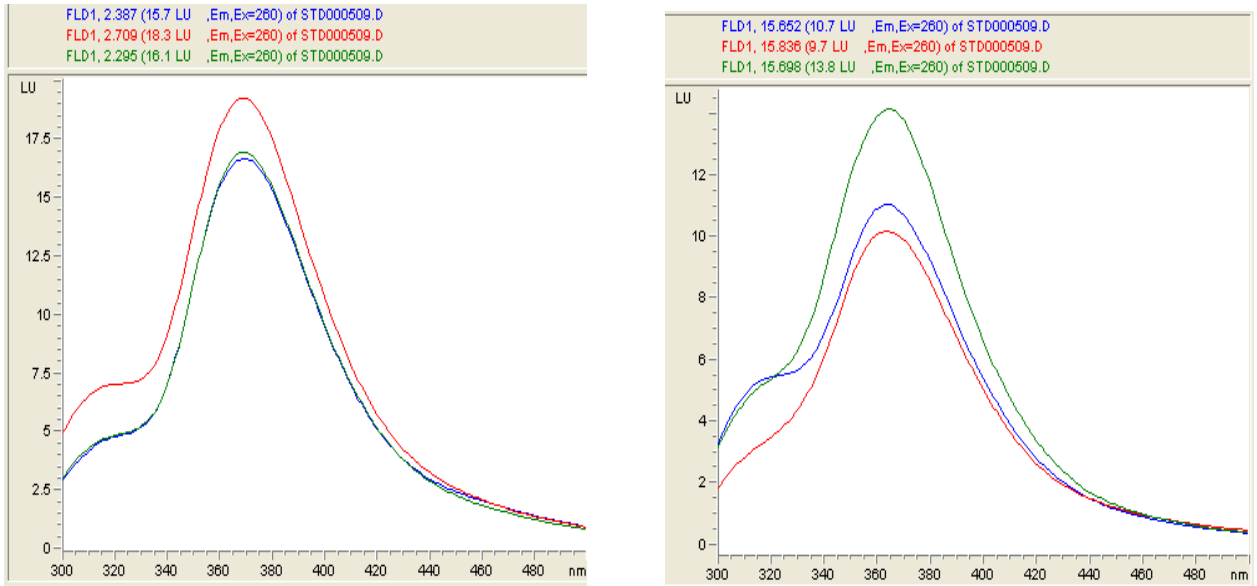


Figure 68: Emission spectrum of May's peaks for point 4 in fluorescence detector.



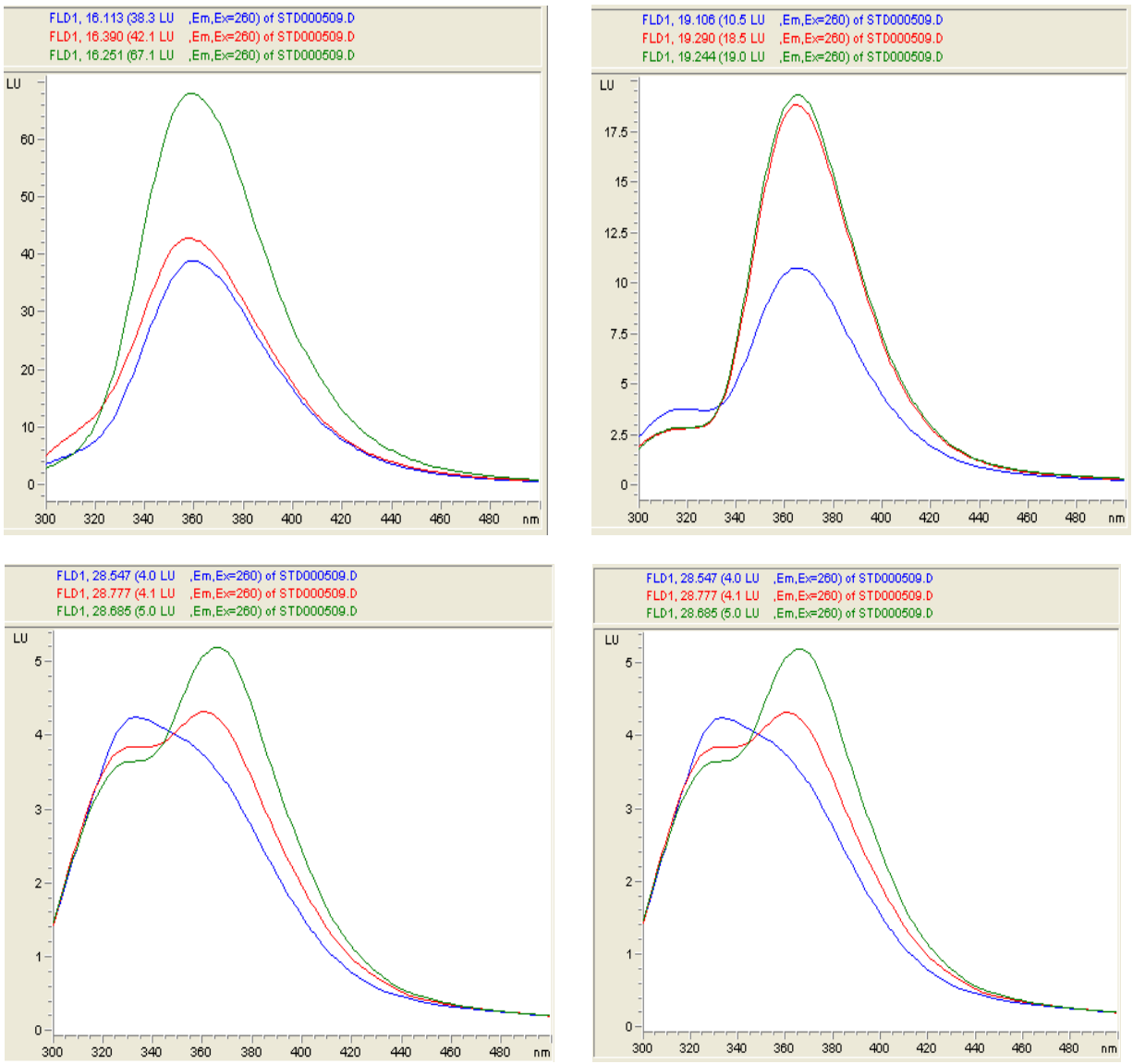
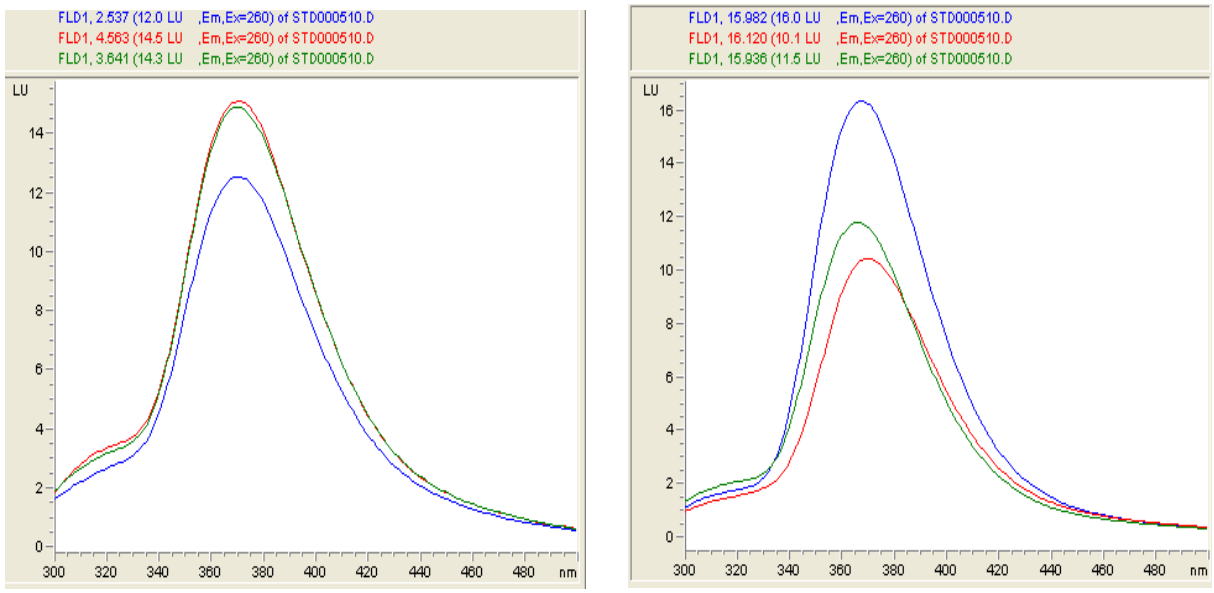


Figure 69: Emission spectra of June's peaks for point 1 in fluorescence detector.



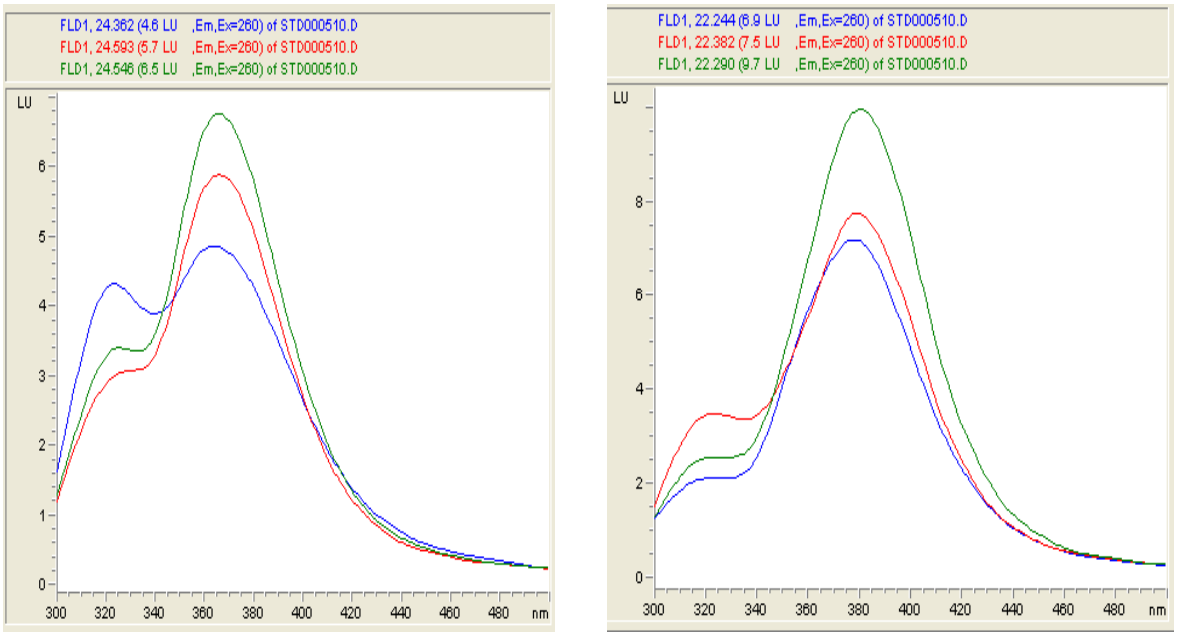
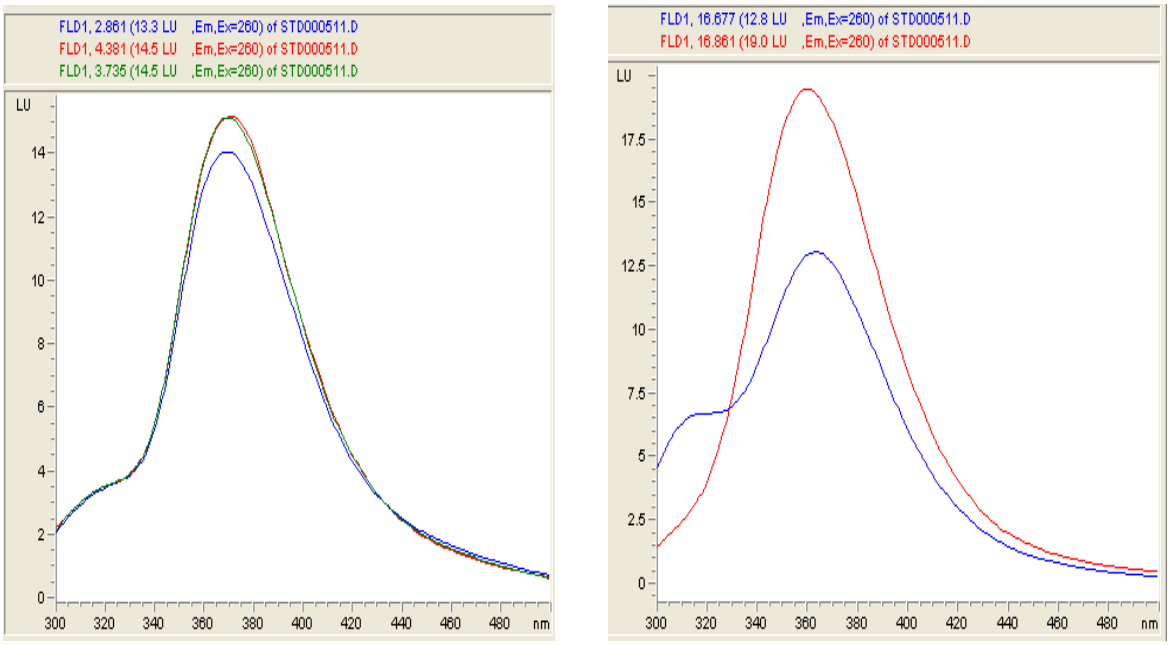


Figure 70: Emission spectra of June's peaks for point 2 in fluorescence detector.



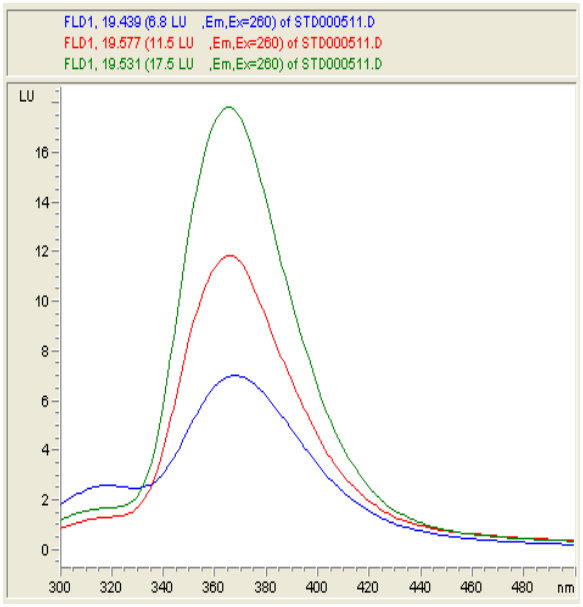
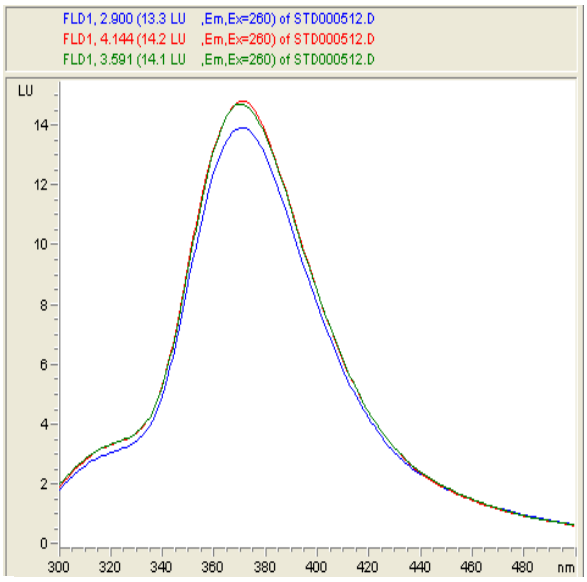
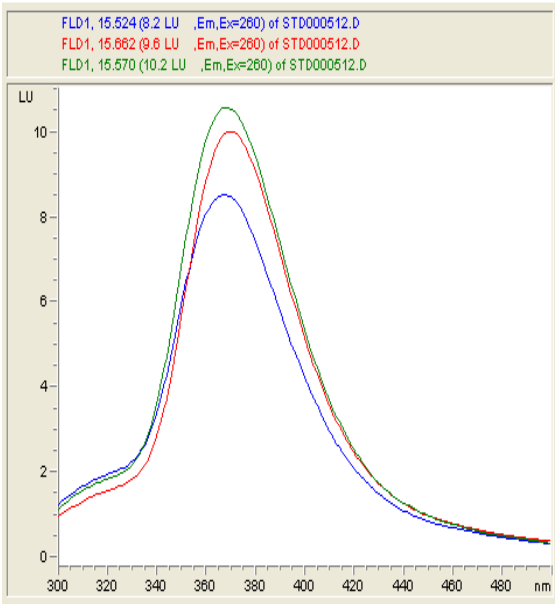


Figure 71: Emission spectra of June's peaks for point 3 in fluorescence detector.





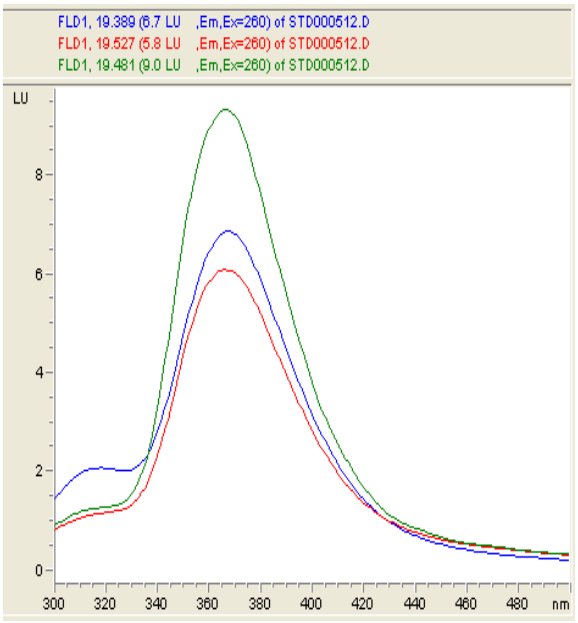


Figure72: Emission spectra of June's peaks for point 4 in fluorescence detector.

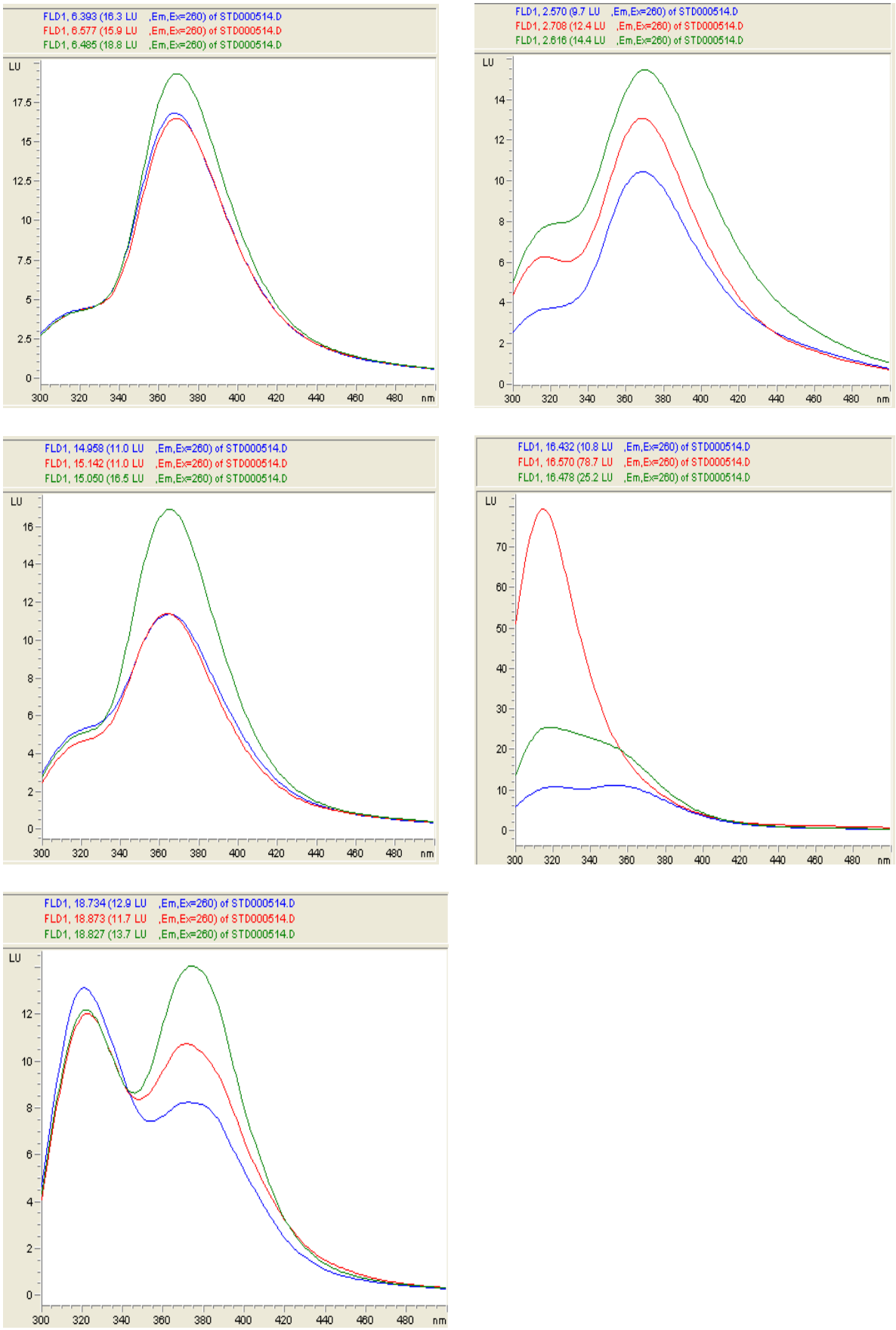


Figure 73: Emission spectra of July's peaks for point 1 in fluorescence detector.

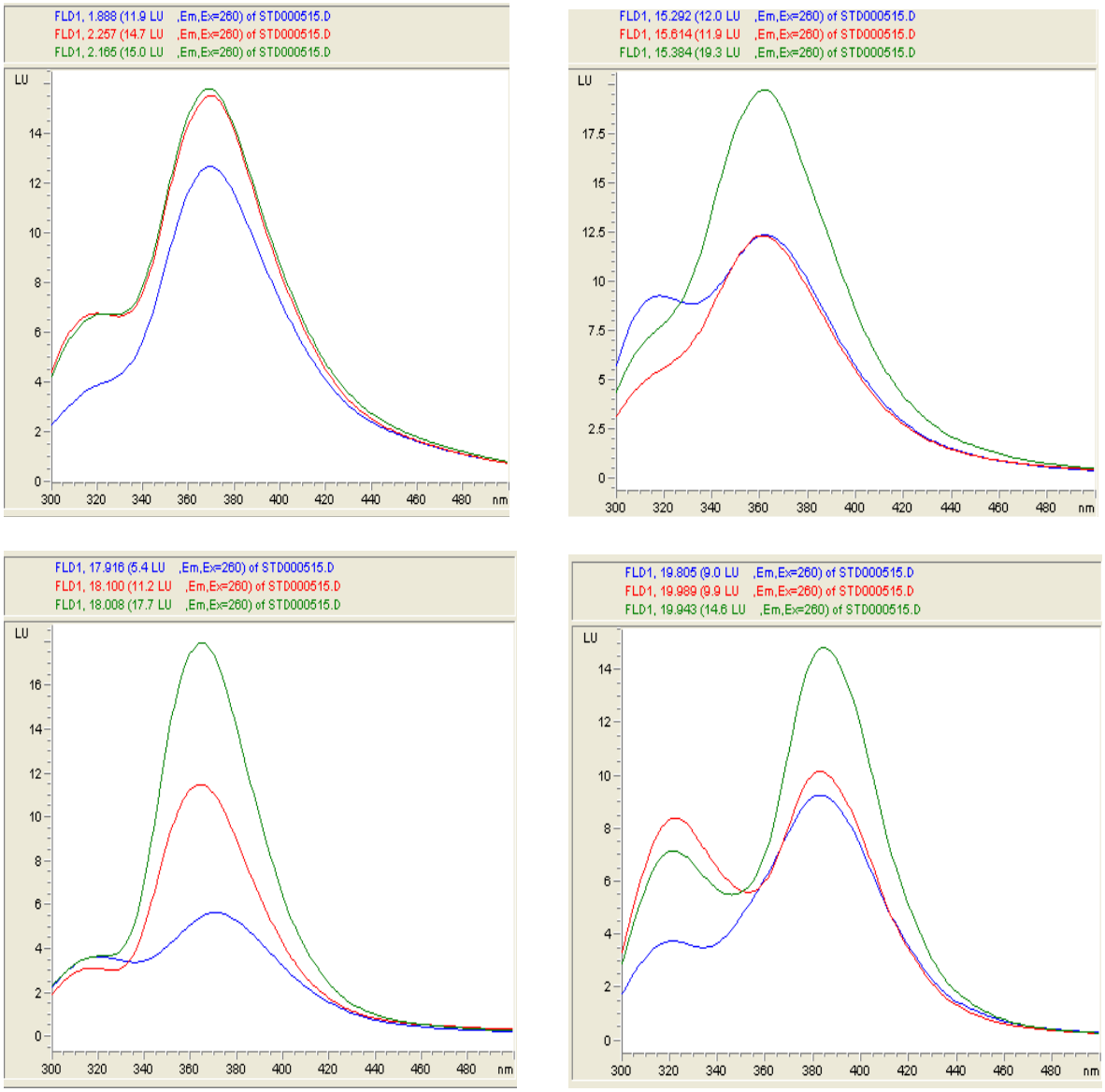


Figure 74: Emission spectra of July's peaks for point 2 in fluorescence detector.

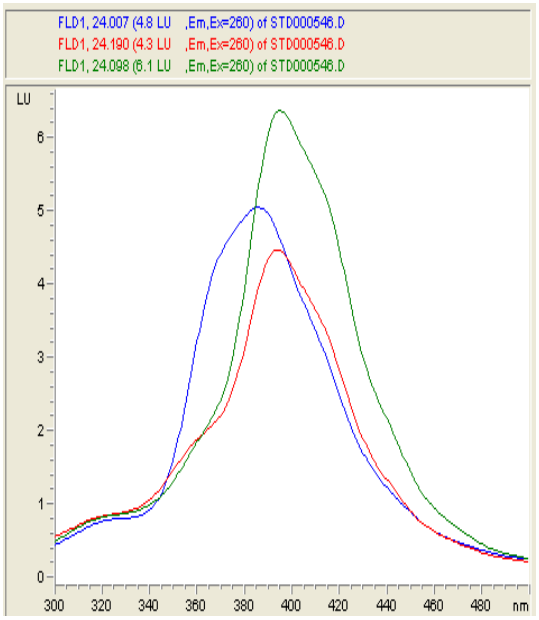
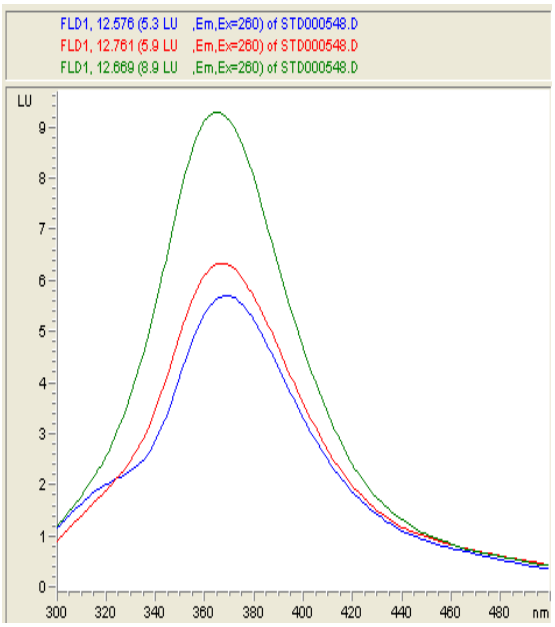
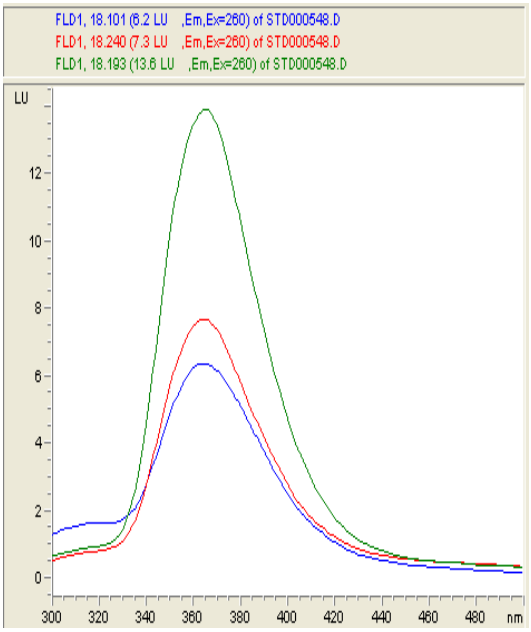


Figure 75: Emission spectrum of July's peaks for point 4 in fluorescence detector.





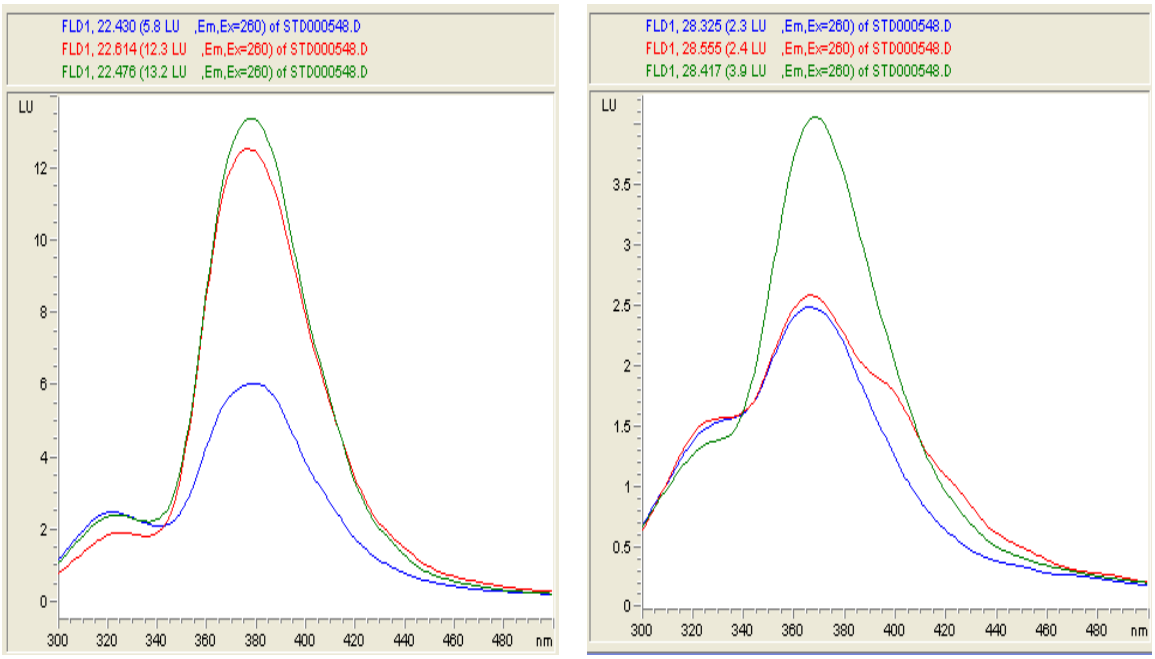


Figure 76: Emission spectra of August's peaks for point 1 in fluorescence detector.

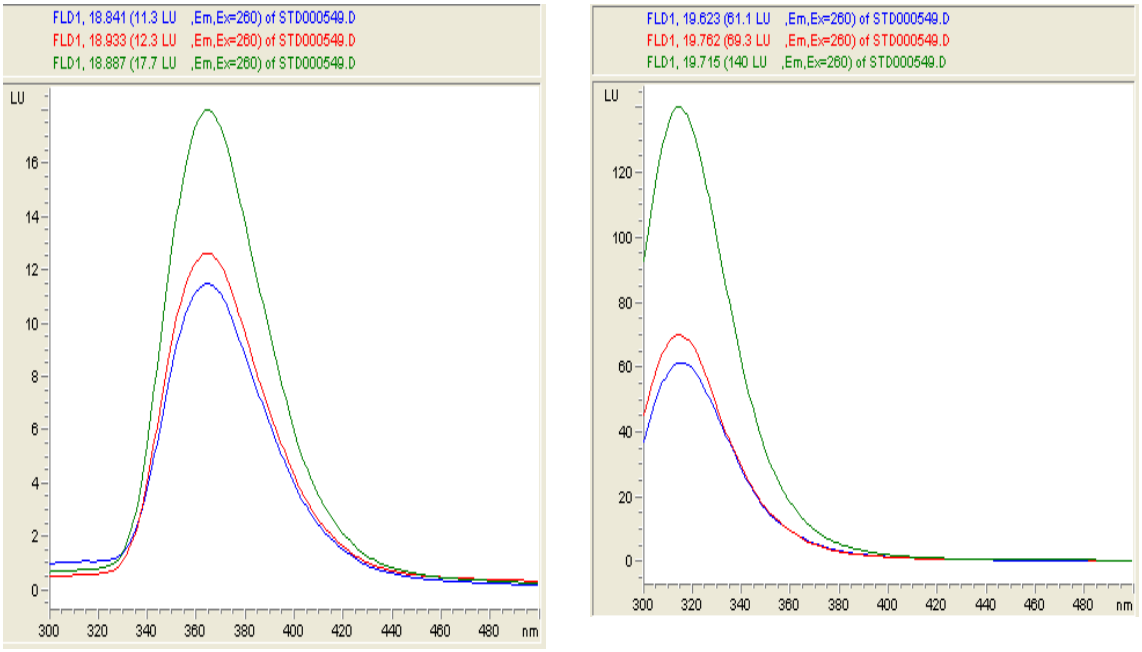


Figure 77: Emission spectra of August's peaks for point 2 in fluorescence detector.

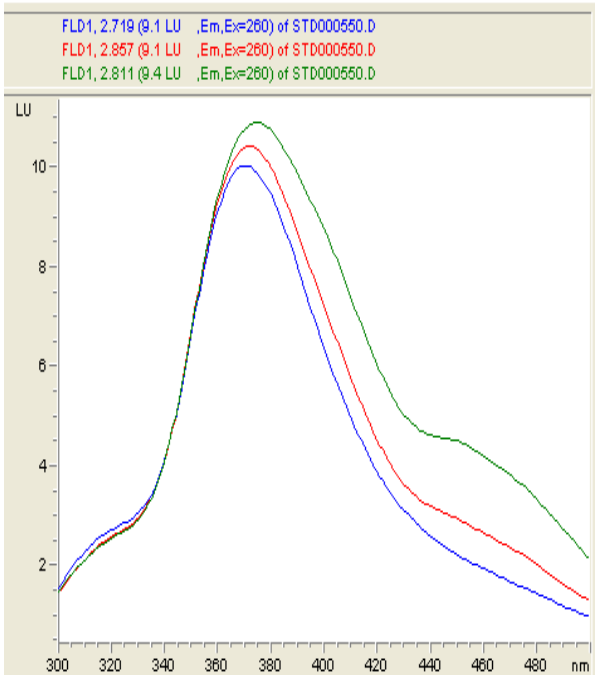
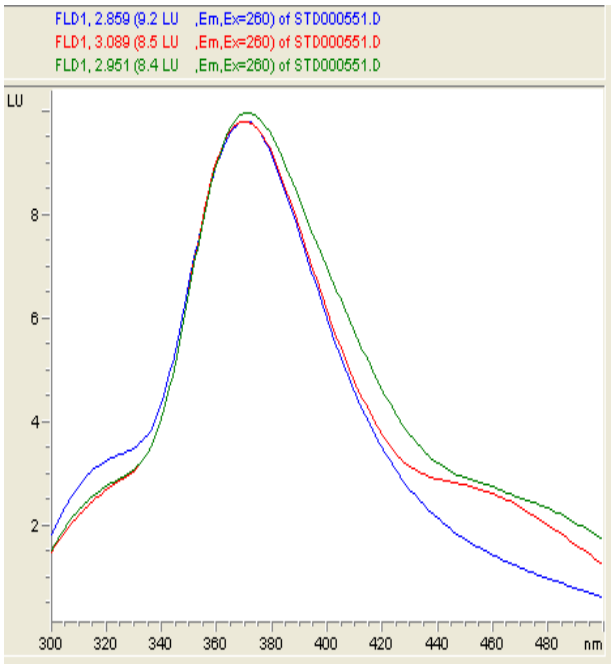
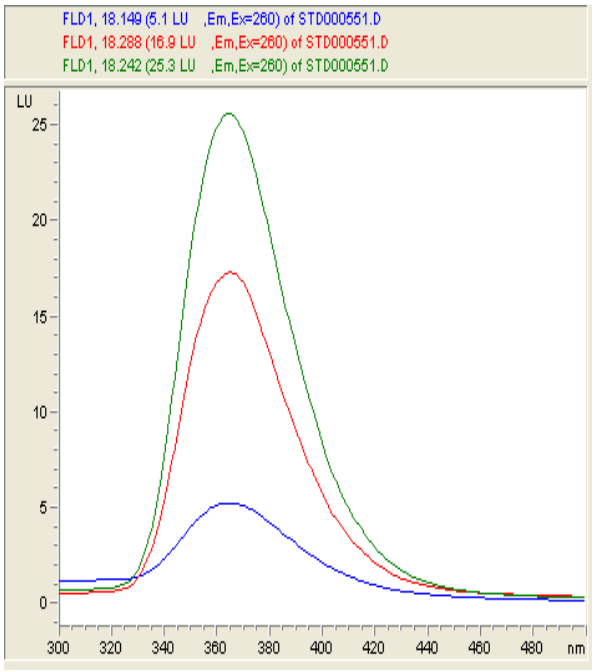


Figure 78: Emission spectra of August's peaks for point 3 in fluorescence detector.





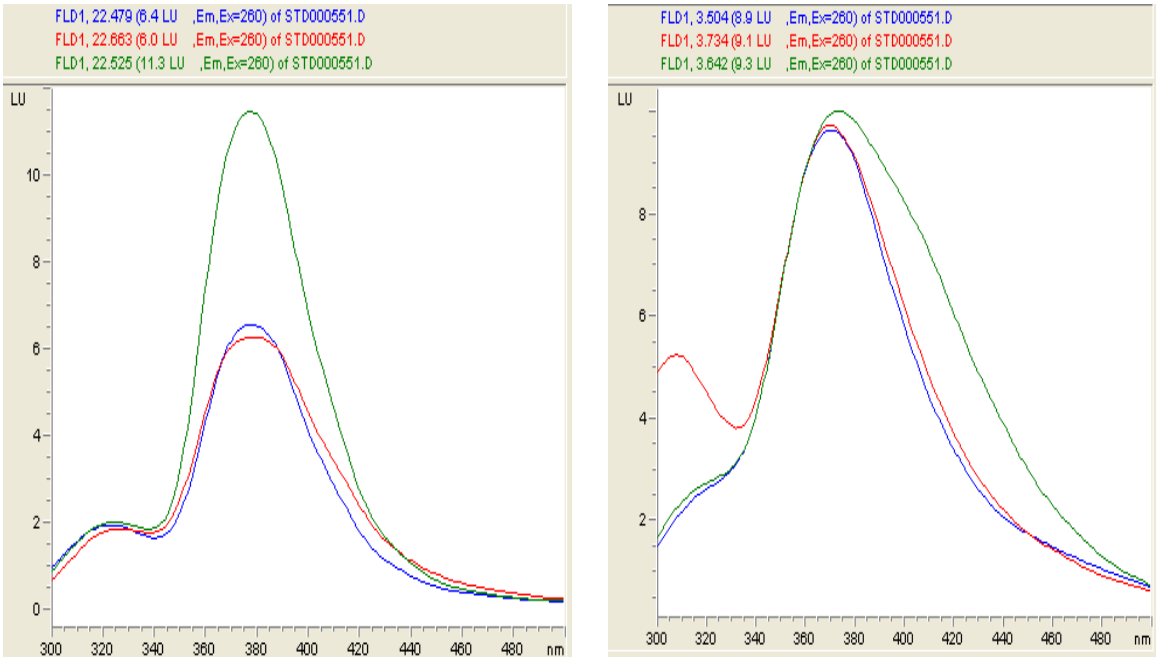
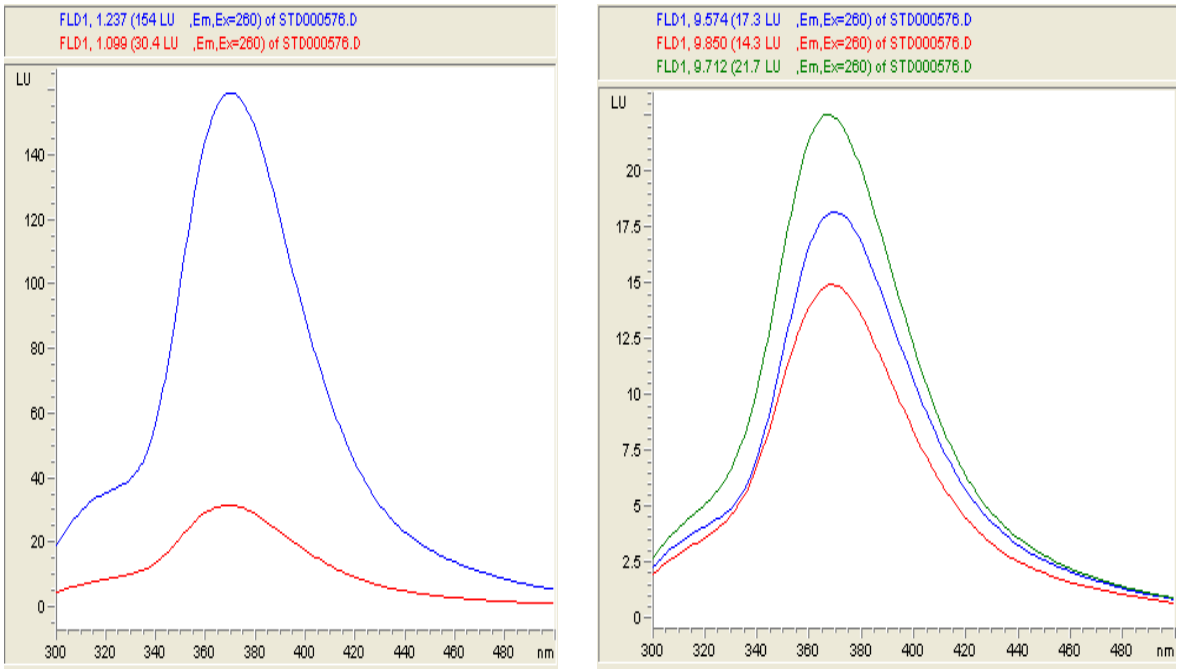


Figure 79: Emission spectra of Augusts' peaks for point 4 in fluorescence detector.



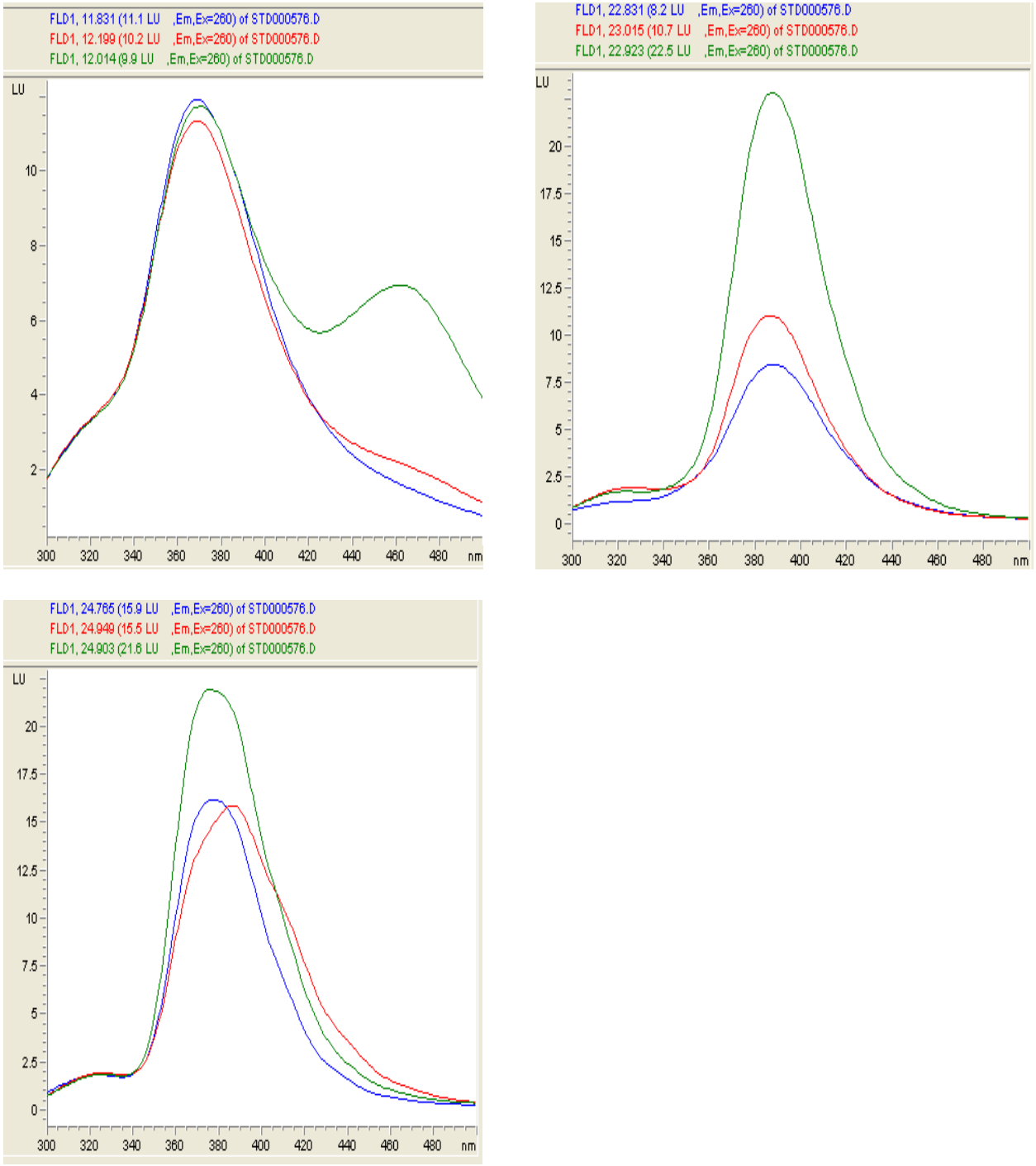
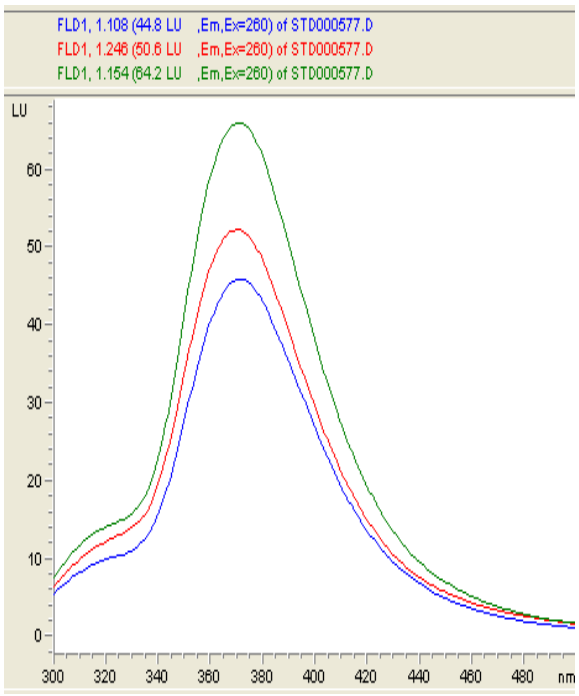
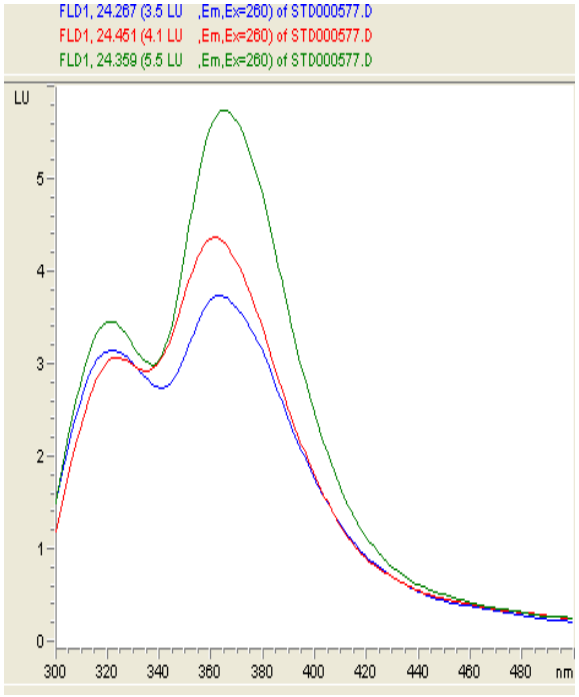
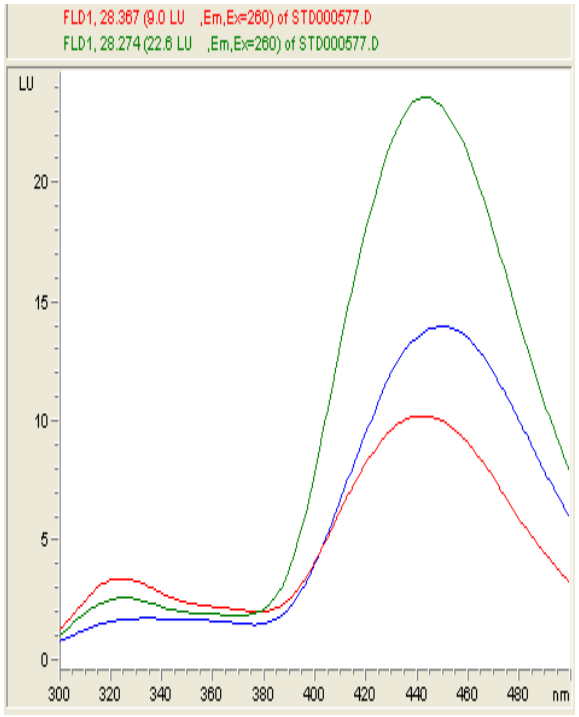
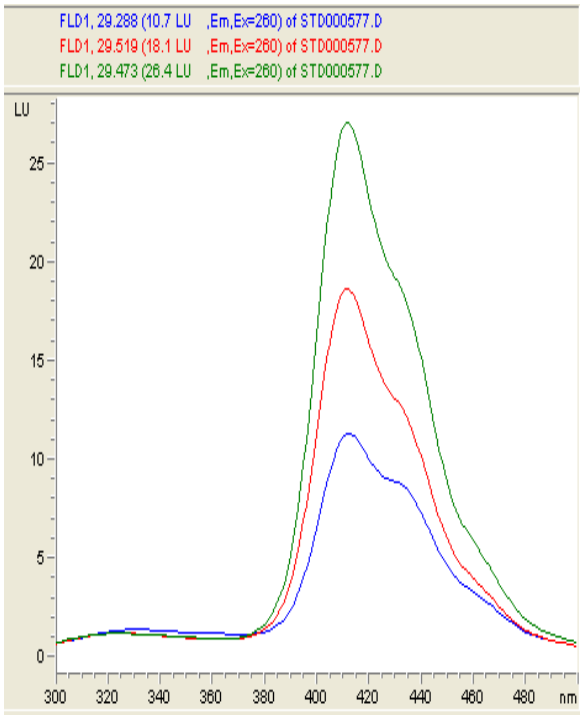


Figure 80: Emission spectra of October's peaks for point 1 in fluorescence detector.


FLD1, 1.108 (44.8 LU ,Em,Ex=260) of STD000577.D
FLD1, 1.246 (50.6 LU ,Em,Ex=260) of STD000577.D
FLD1, 1.154 (64.2 LU ,Em,Ex=260) of STD000577.D
LU
nm


FLD1, 24.267 (3.5 LU ,Em,Ex=260) of STD000577.D
FLD1, 24.451 (4.1 LU ,Em,Ex=260) of STD000577.D
FLD1, 24.359 (5.5 LU ,Em,Ex=260) of STD000577.D
LU
nm


FLD1, 28.367 (9.0 LU ,Em,Ex=260) of STD000577.D
FLD1, 28.274 (22.6 LU ,Em,Ex=260) of STD000577.D
LU
nm


FLD1, 29.288 (10.7 LU ,Em,Ex=260) of STD000577.D
FLD1, 29.519 (18.1 LU ,Em,Ex=260) of STD000577.D
FLD1, 29.473 (26.4 LU ,Em,Ex=260) of STD000577.D
LU
nm

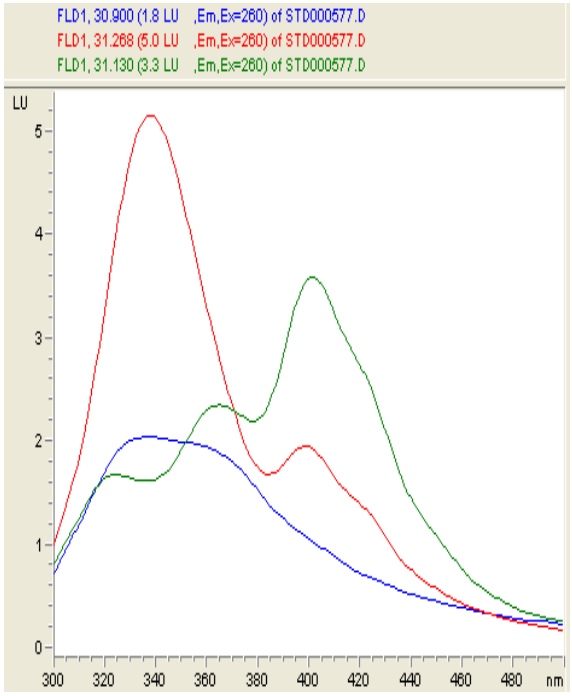
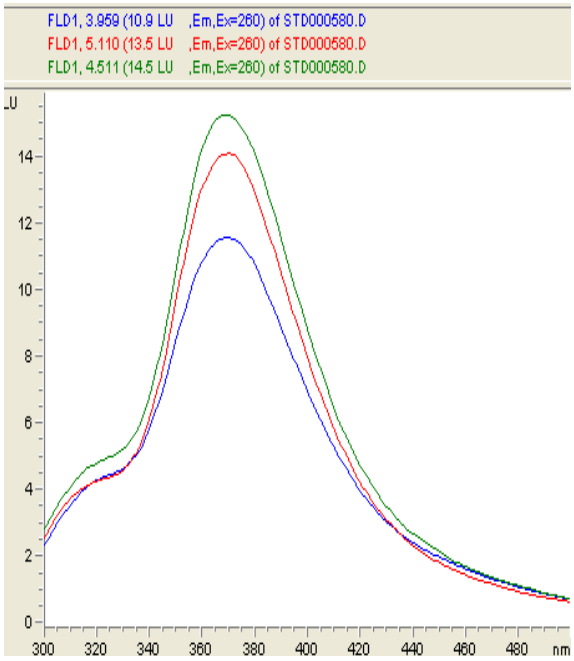
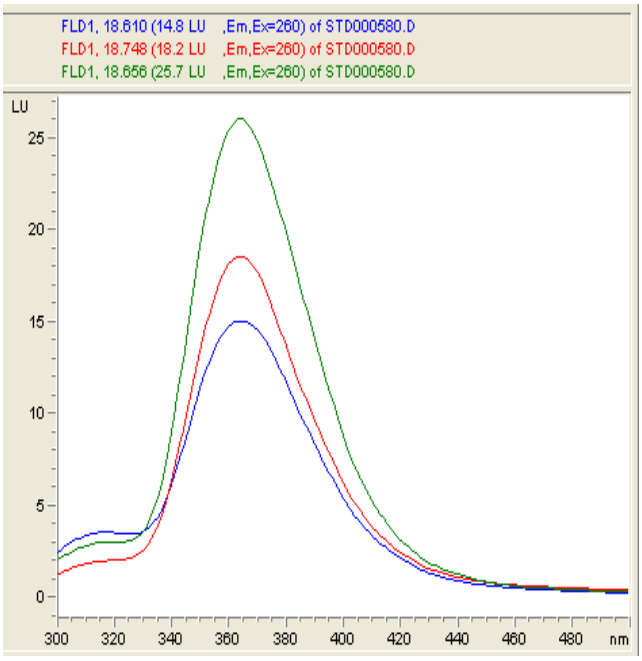


Figure 81: Emission spectra of October's peaks for point 2 in fluorescence detector.





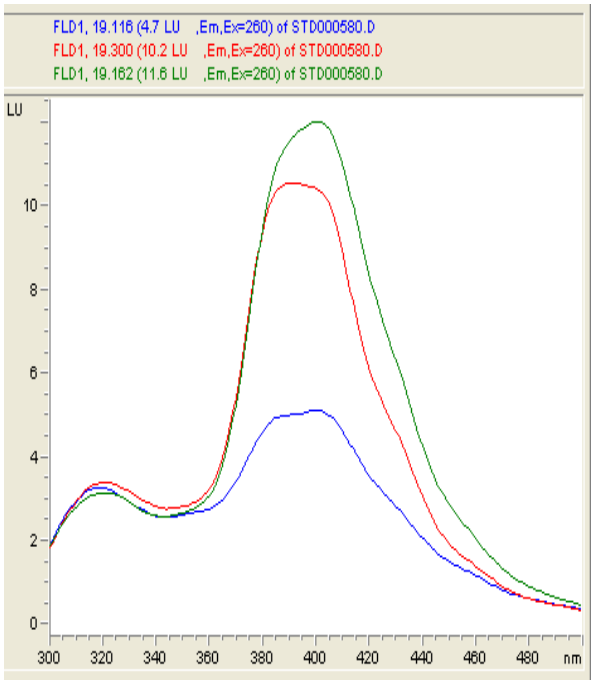


Figure 82: Emission spectra of October's peaks for point 3 in fluorescence detector.

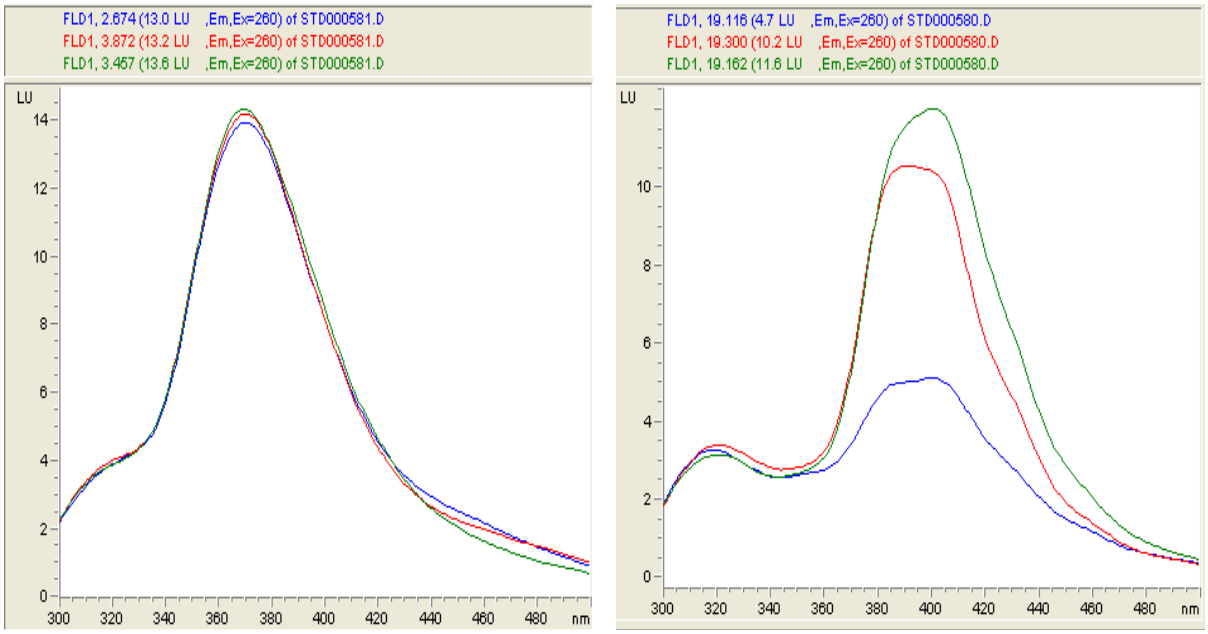


Figure 83: Emission spectra of October's peaks for point 4 in fluorescence detector.

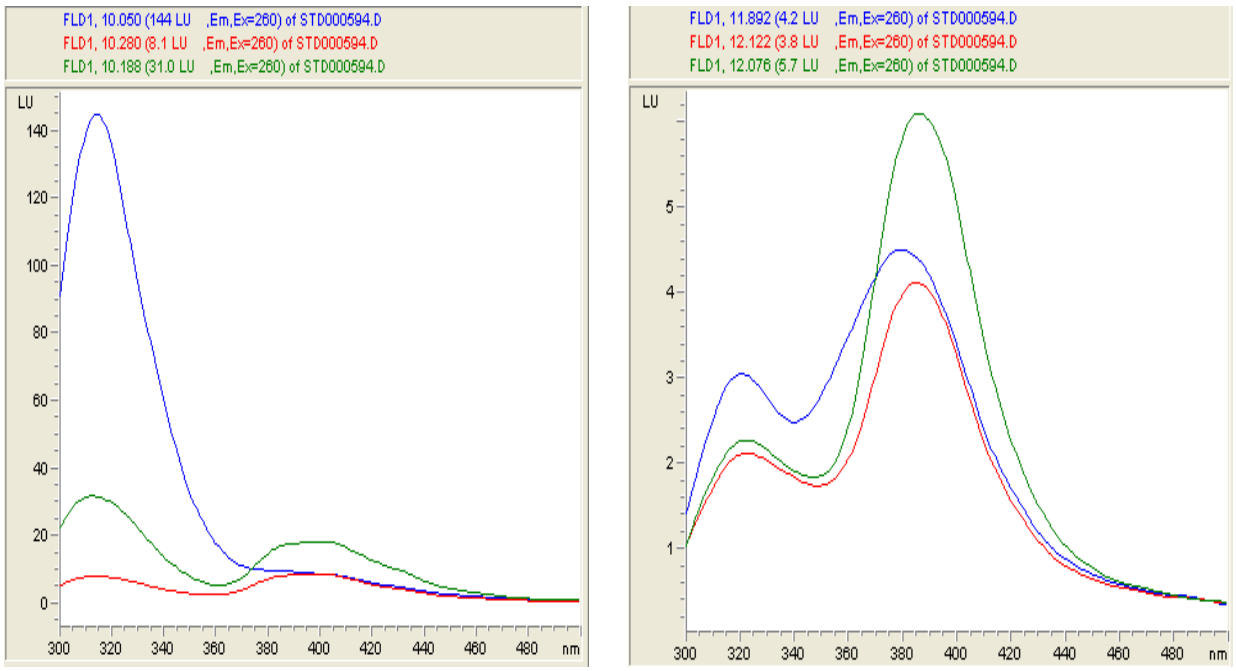
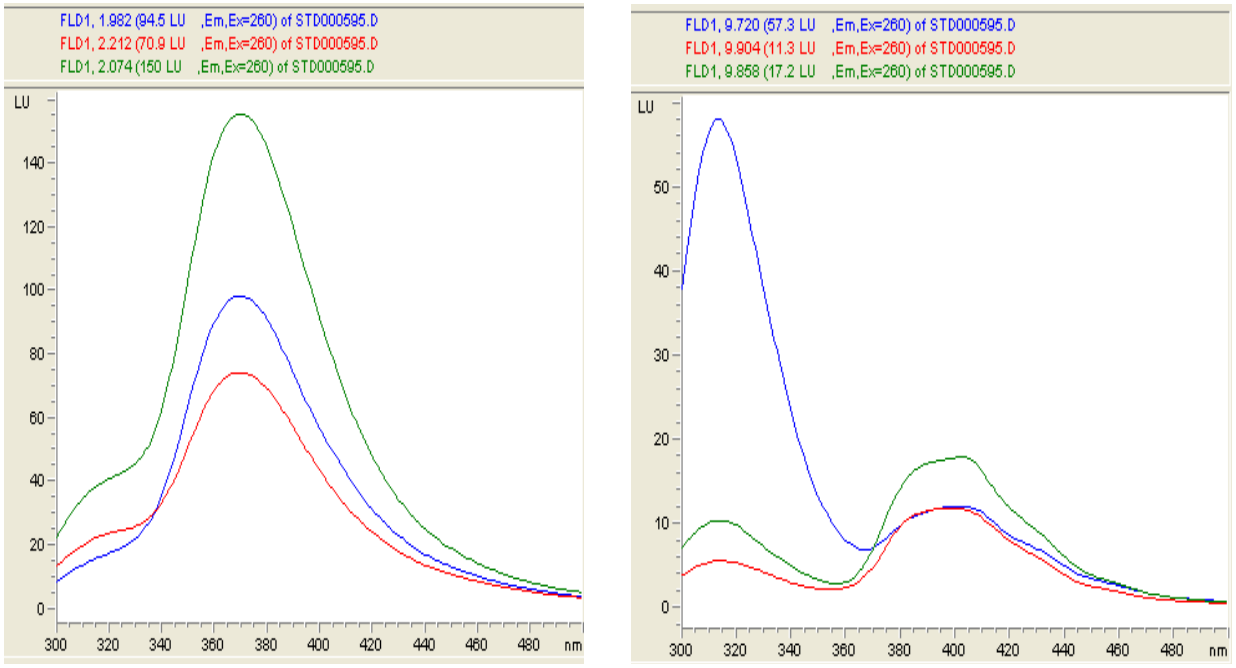


Figure 84: Emission spectra of November's peaks for point 1 in fluorescence detector.



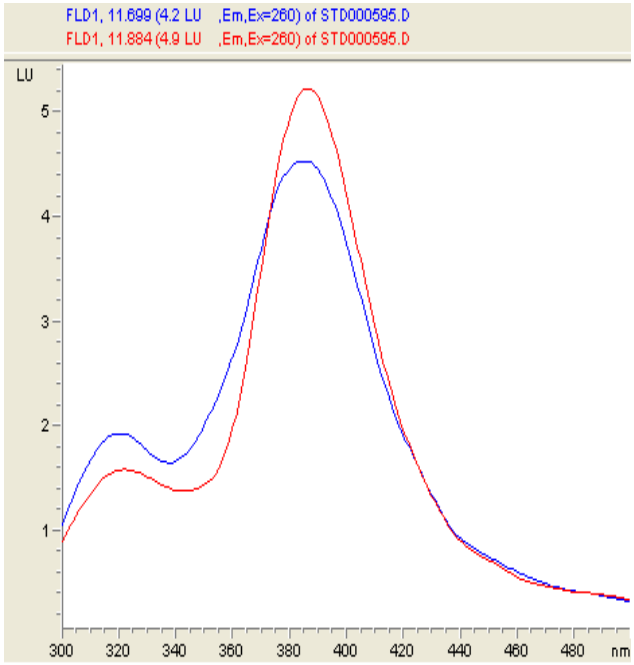
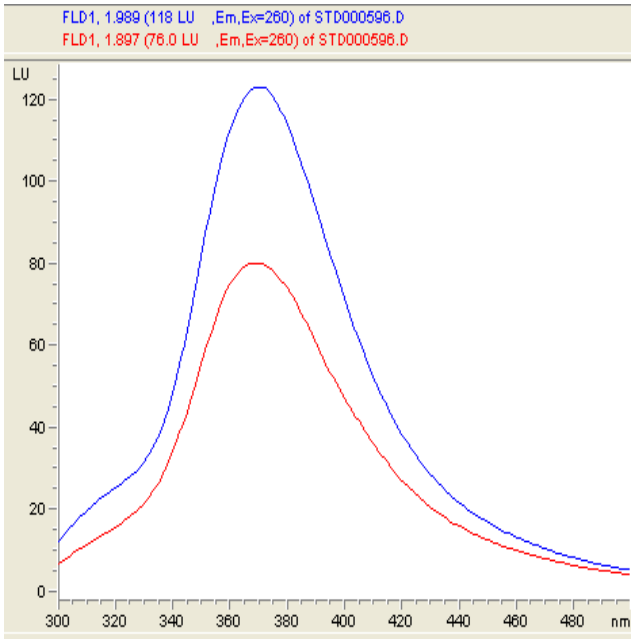
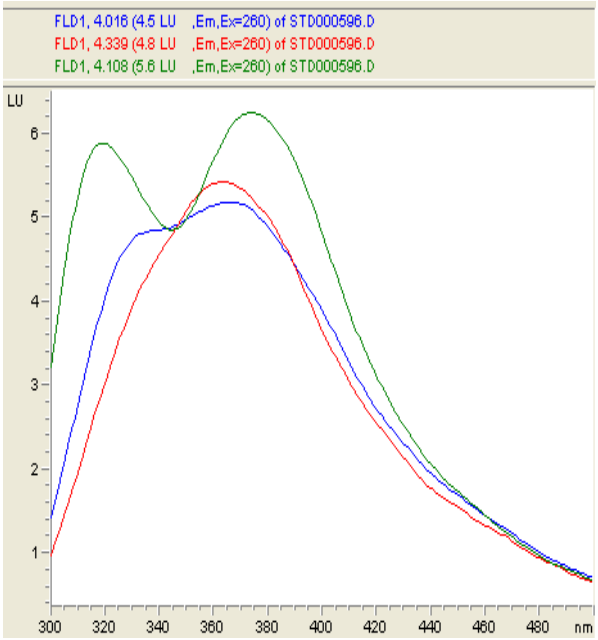


Figure 85: Emission spectra of November's peaks for point 2 in fluorescence detector.





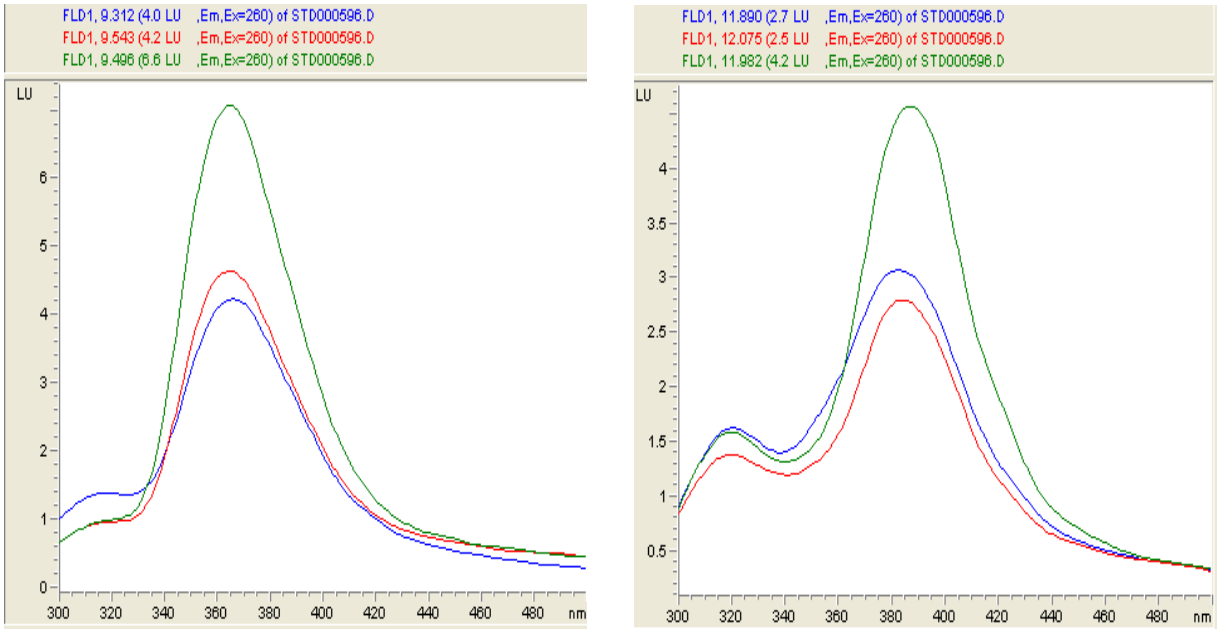
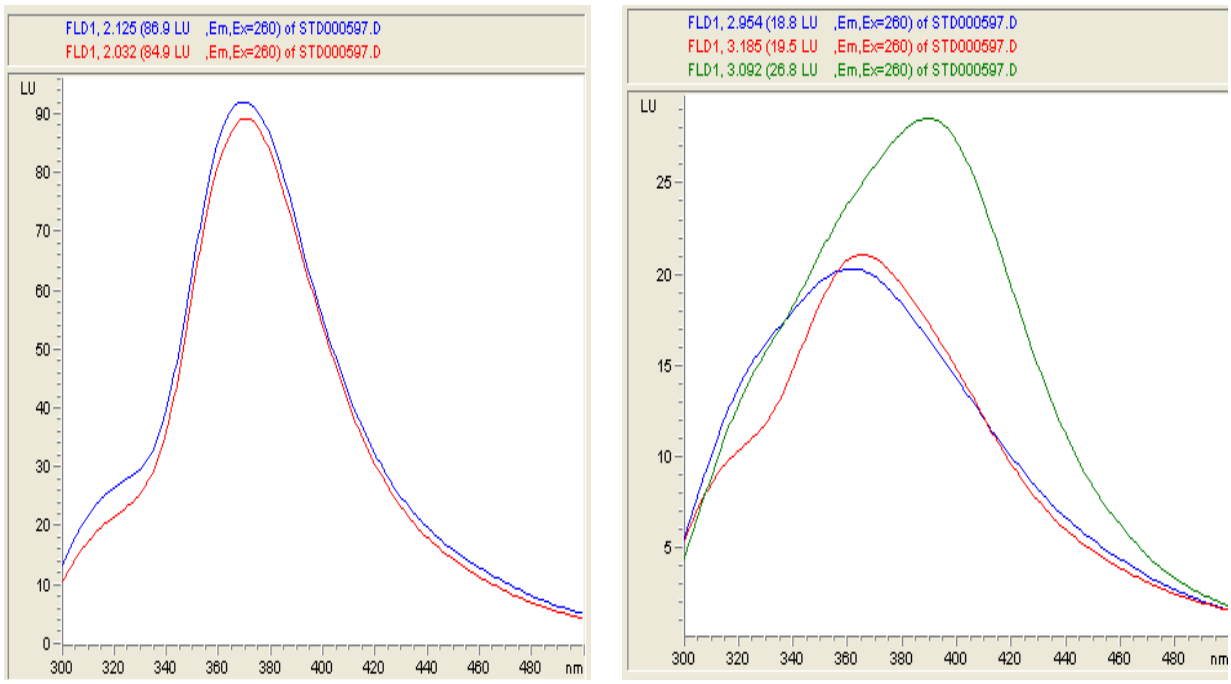
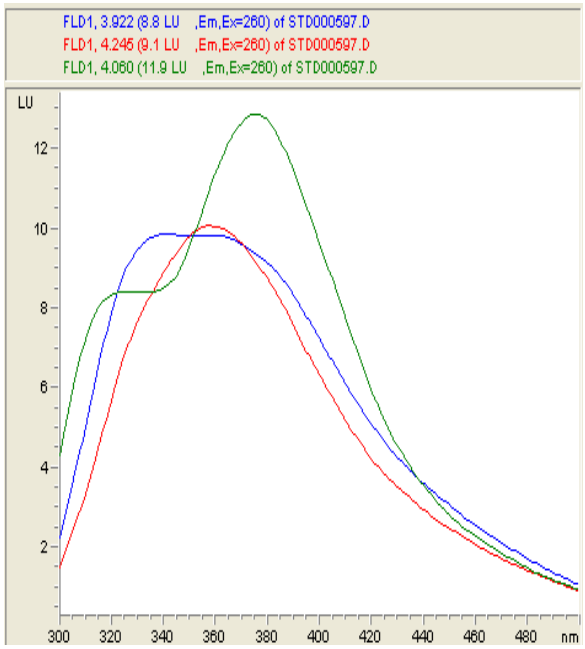
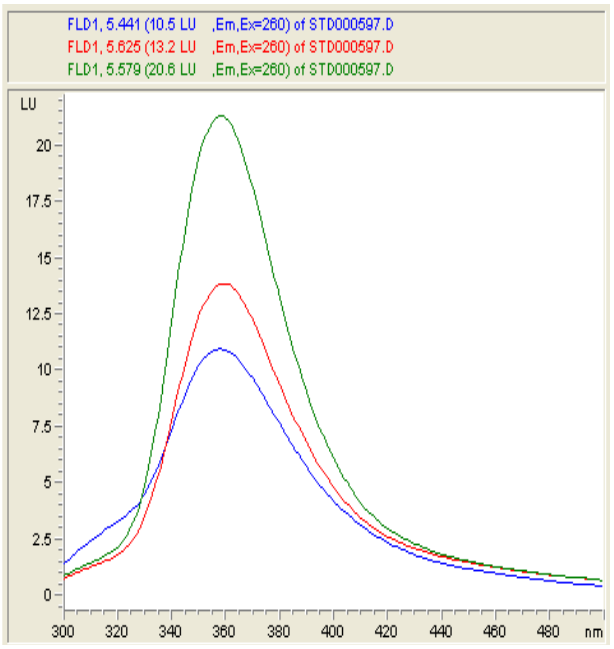
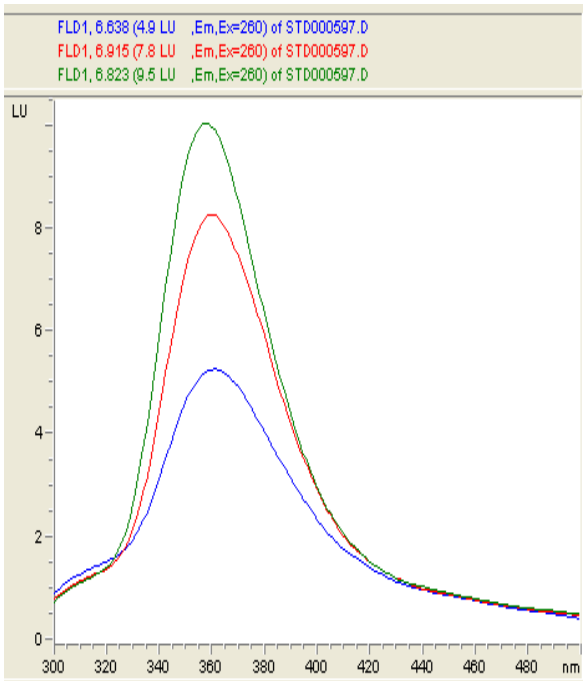


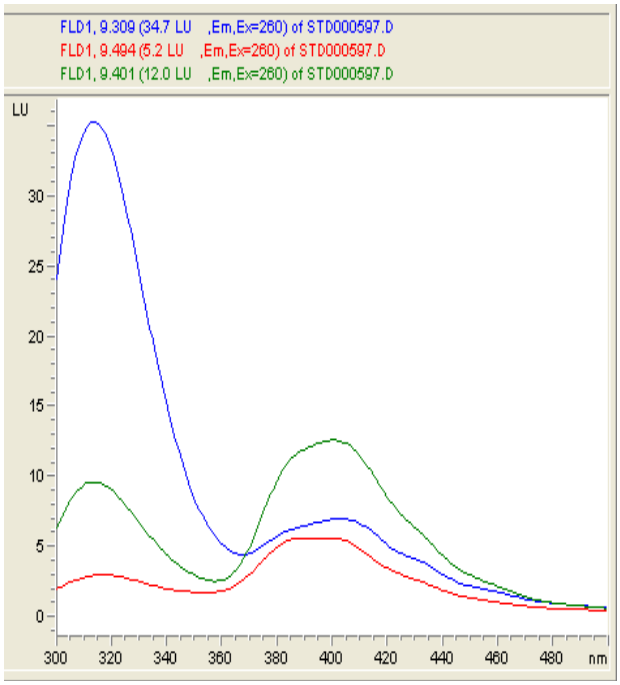
Figure 86: Emission spectra of November's peaks for point 3 in fluorescence detector.




FLD1, 3.922 (8.8 LU ,Em,Ex=260) of STD000597.D
FLD1, 4.245 (9.1 LU ,Em,Ex=260) of STD000597.D
FLD1, 4.060 (11.9 LU ,Em,Ex=260) of STD000597.D
LU
nm


FLD1, 5.441 (10.5 LU ,Em,Ex=260) of STD000597.D
FLD1, 5.625 (13.2 LU ,Em,Ex=260) of STD000597.D
FLD1, 5.579 (20.6 LU ,Em,Ex=260) of STD000597.D
LU
nm


FLD1, 6.638 (4.9 LU ,Em,Ex=260) of STD000597.D
FLD1, 6.915 (7.8 LU ,Em,Ex=260) of STD000597.D
FLD1, 6.823 (9.5 LU ,Em,Ex=260) of STD000597.D
LU
nm


FLD1, 9.309 (34.7 LU ,Em,Ex=260) of STD000597.D
FLD1, 9.494 (5.2 LU ,Em,Ex=260) of STD000597.D
FLD1, 9.401 (12.0 LU ,Em,Ex=260) of STD000597.D
LU
nm

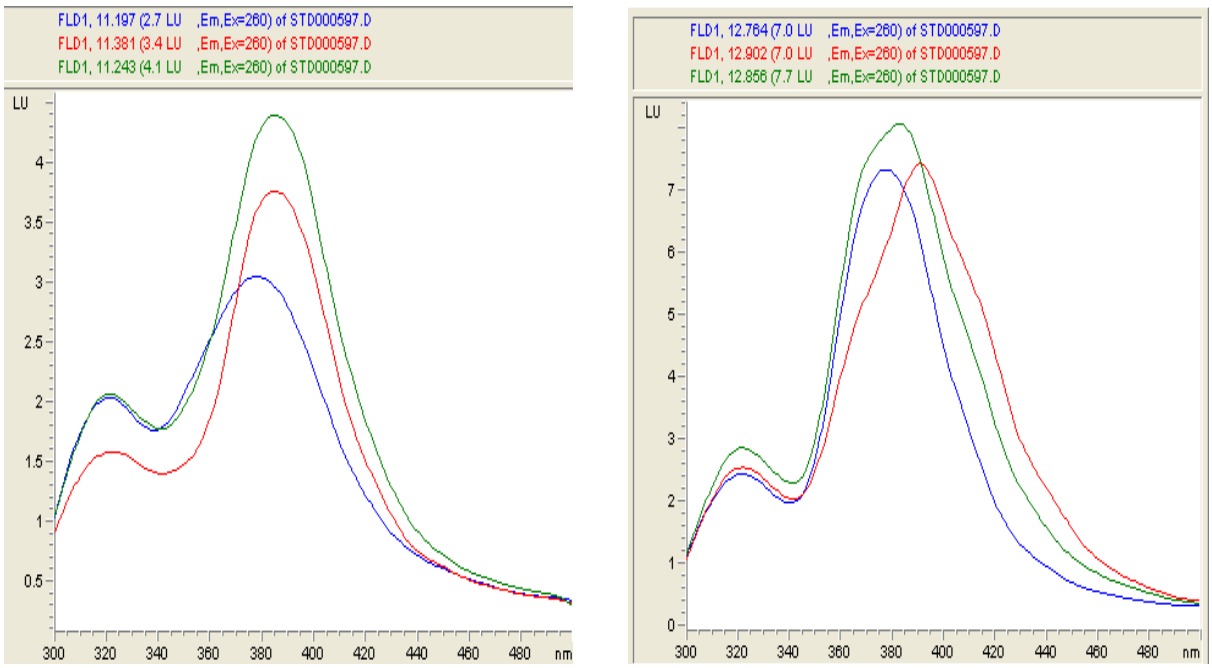


Figure 87: Emission spectra of November's peaks for point 4 in fluorescence detector.

SDBS chromatogram and UV spectra from DAD 225 nm

Figure 88 is shown a chromatogram, where SDBS are displayed in exact minutes. In figures 89 and 90, the UV spectra of SDBS are presented as they appear at 225 nm.

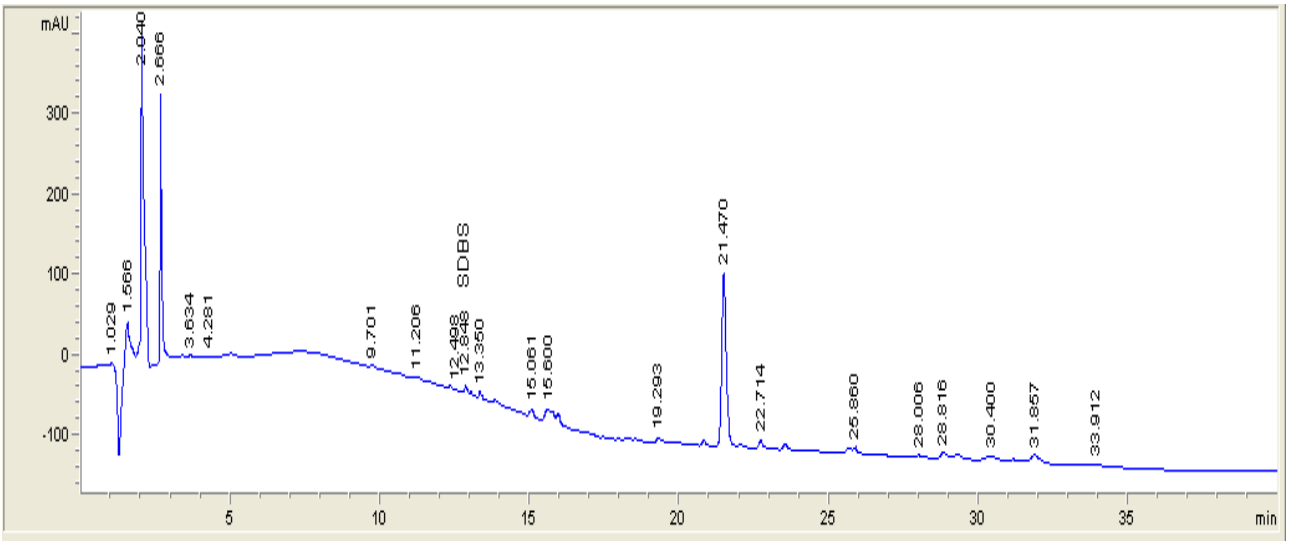


Figure 88: : Chromatogram of 10 ppb SDS (11.208 min till 15.051 min) at 225 nm.

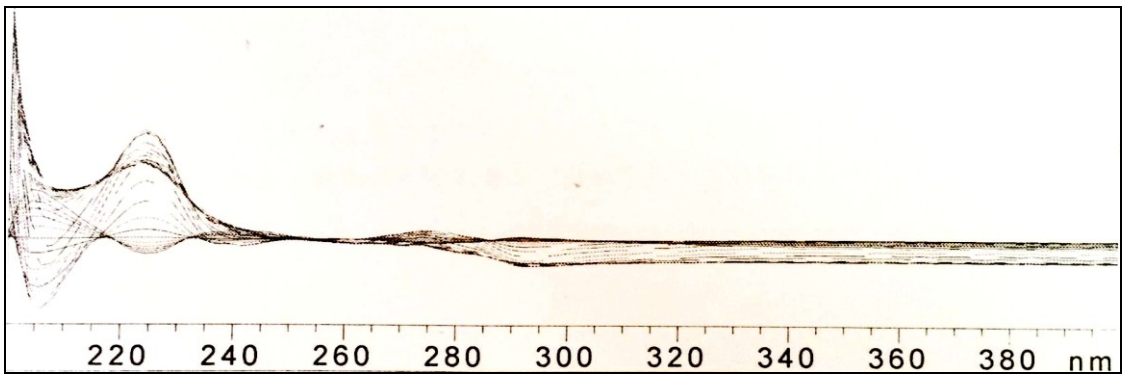


Figure 89: : UV spectrum of 10 ppb SDS (12.498 minutes).

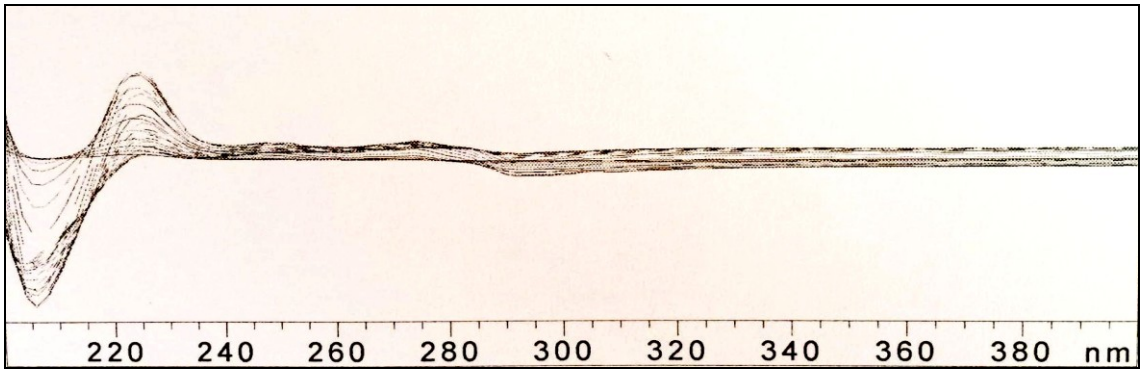


Figure 90: UV spectrum of 10 ppb SDS (12.848 minutes).

BIBLIOGRAPHY

[1]Christy Perrin (2011), Program Manager of Watershed Education for Communities and Officials Department of Agricultural and Resource Economics, Division of Water Quality North Carolina State University.
[2]Chris Woodford (2015). Water pollution: an introduction. UK Rivers Network.
[3]White Plains (2000). Water quality issues of electricity production: pollution of water bodies. Power Scorecard, Pace University, Pace energy and Climate center.
[4]Mark Sircus (2011). Water alkalinity and pH. IMVA Website.
[5]International humic substances society (2017). What are humic substances. IHSS, Natural organic matter research.
[6]Nova Scotia Environment (2008). Humic substances. (www.gov.ns.ca/nse/water/thm.asp).
[7]http://karnet.up.wroc.pl/~weber/kwasy2.htm. (Properties of humic substances).
[8]https://health-solution.eu/index.php/en/humic-fulvic-acid.
[9]Digambara Patra (2003). Applications and New Developments in Fluorescence Spectroscopic Techniques for the Analysis of Polycyclic Aromatic Hydrocarbons. Marcel Dekker Inc., Vol. 38, No. 2, pp. 155–185.
[10]Tsimeli Aikaterini (2011). Development of advanced analytical methods for the determination of polychlorinated hydrocarbons polycyclic aromatic hydrocarbons and cyanotoxins. Diploma thesis, NTUA.
[11]Agency for Toxic Substances and Disease Registry (ATSDR) (2009). Case Studies in Environmental Medicine,Toxicity of Polycyclic Aromatic Hydrocarbons (PAHs). Course: WB 1519.
[12]Regulations (1989). European communities (Quality of surface water intended for the abstraction of drinking water).
[13]Catharine Layton, Wilhad M. Reuter, PerkinElmer, Inc. Shelton, CT (2015). PAHs in Surface Water by PDA and Fluorescence Detection. PerkinElmer, Inc. 012102_01.
[14]Hussein I., Abdel-Shafy, Mona S.M. Mansour (2015). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egyptian Journal of petroleum, Volume 25, Issue 1, pages 107-123.
[15]Efstathios Stogiannidis and Remi Laane (2015). Source Characterization of Polycyclic Aromatic Hydrocarbons by Using Their Molecular Indices: an Overview of Possibilities. Springer International Publishing Switzerland 2015, D.M. Whitacre (ed.), Reviews of Environmental Contamination and Toxicology, Volume 234.
[16]Daniela M. Pampanin and Magne O. Sydnes (2013). Polycyclic Aromatic Hydrocarbons a Constituent of Petroleum: Presence and Influence in the Aquatic Environment. Hydrocarbon, Dr. Vladimir Kutcherov (Ed.), InTech, DOI: 10.5772/48176.
[17]German Federal Environment Agency (2012). Polycyclic Aromatic Hydrocarbons Harmful to the Environment, Toxic, Inevitable. Umvelt Bundes Amt For our environment, German Environment Agency.
[18]S. P. Sandoval, D. L. Pool and L. E. Schultze (1990). Effect of Nonionic Surfactants on Chalcopyrite Leaching Under Dump Chemical Conditions. Report of Investigations 9311, United States Department of the interior.
[19]M. Castillo, J. Riu, F. Ventura, R. Boleda , R. Scheding, H.Fr.Schroder, C Nistor J. Emneus, P. Eichhorn, Th.P. Knepper, C.C.A. Jonkers, P. de Voogt, E. Gonzalez-Mazo, V.M. Leon, D. Barcelo D. (2000). Inter-laboratory comparison of liquid chromatographic techniques and enzyme-linked immunosorbent assay for the determination of surfactants in wastewaters. Elsevier, Journal of Chromatography A, Volume 889, Issues 1-2, 11 August 2000, pages 195-209.
[20]Mérida-Venezuela Versión 2 (2002). Surfactants Types and Uses. Jean-Louis Salager, Laboratory of formulation, interfaces, rheology and processes. Firp booklet#E300-A.

[21]Arthur D. Little. Inc. Acom Park (1991).Anionic Surfactants Part 1, Linear Alkylbenzene Sulfonates Final Report To: The Soap and Detergent. Environmental and Human Safety of Major Surfactants, volume I. reference 65913.
[22]en.wikipedia.org/wiki/Sodium_dodecylbenzenesulfonate.
[23]New jersey department of health (2009). Hazardous substance fact sheet. (http://www.nj.gov/health/eoh/rtkweb/documents/fs/0164.pdf).
[24]Science lab (2013). Material Safety Data Sheet Dodecylbenzene sodium sulfonate MSDS. Chemical and laboratories equipment. (http://www.sciencelab.com/msds.php?msdsId=9923880).
[25]https:www.soinc.org/sites/default/files/uploaded_files/forensics/For_Chromatography3.pdf.
[26]Jim Clark (2007). High performance liquid chromatography (HPLC). (http://www.chemguide.co.uk/analysis/chromatography/hplc.html).
[27]Fundamentals of Liquid Chromatography (HPLC Basics). Courtesy of Agilent Technologies, Inc. (http://polymer.ustc.edu.cn/xwxx_20/xw/201109/P020110906263097048536.pdf).
[28]Waters - the science of what's possible (2017).
(http://www.waters.com/waters/en_SE/How-Does-High-Performance-Liquid-Chromatograph-Work%3F/nav.htm?cid=10049055&locale=en_SE).
[29]Agilent Technologies. Introduction to Chromatography. (http://www.team-cag.com/support/theory/chroma/hplc_bas_at/chromatography/retentionTime.html.)
[30]Taniya Biswas (2015). Detectors used in high performance liquid chromatography. (https://www.slideshare.net/arghasen90/detectors-used-in-hplc).
[31]Tony Taylor LCGC (2015). Important Aspects of UV Detection for HPLC. (http://www.chromatographyonline.com/important-aspects-uv-detection-hplc).
[32]Agilent Technologies. The DAD and MWD principle.
(http://www.team-cag.com/support/theory/chroma/hplc_bas_at/detectors/dadPrinciple.html).
[33]Adam Stelmaszewski (2012). Application o fluorescence in studies of marine petroleum pollutants. Akademia Morska w Gdyni.
[34]http://www.spectrophotometer.com/FAQ.htm.
[35]http://chem.libretexts.org/Core/Physical_and_Theoretical_Chemistry/Kinetics/Reaction_Rates/Experimental_Determination_of_Kinetcs/Spectrophotometry.
[36]Renee D. JiJi, Gary A. Cooper, Karl S. Booksh (1999). Excitation-emission matrix fluorescence based determination of carbamate pesticides and polycyclic aromatic hydrocarbons. Elsevier, Analytica Chimica Acta 397 (1999) 61 – 72.
[37]Fluorescence detectors. (http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_flur.html).
[38]Zoe Smith, Christina Roman (2016). Chemistry libre texts.
(https://chem.libretexts.org/Core/Physical_and_Theoretical_Chemistry/Spectroscopy/Electronic_Spectroscopy/Radiative_Decay/Fluorescence).
[39]Marion Egelkraut - Holtus, Shimadzu Europa GmbH (2016). Determination of PAHs or mineral oils in water using fluorescence spectroscopy, Simple analysis with high detection limits according to ASTM D 5412. New Analytic, 17 February 2016.
[40]Patrick D. McDonald (2011). A Sample Preparation Primer and Guide to Solid Phase Extraction Methods Development. Soli phase extraction Applications guide and bibliography, a resource of for sample preparation methods developmant, sixth edition.
[41]Guide to Solid Phase Extraction Bulletin 910 (1998) Sigma-Aldrich. (http://www.sigmaaldrich.com/Graphics/Supelco/objects/4600/4538.pdf).
[42]Waters the science of what's possible (2017). Beginners guide to SPE – solid phase extraction. (http://www.waters.com/waters/en_SE/Solid-Phase-Extraction-SPE-Guide/nav.htm?cid=134721476&locale=en_SE).
[43]B. Arfib, G. de Marsily, J. Ganoulis (2007) «Locating the zone of saline intrusion in a coastal karst aquifer using springflow data», Groundwater, 2007,45(1):28-35.
[44]N. Lambrakis, A. S. Andreou, P. Polydoropoulos, E. Georgopoulos and T. Bountis (2000) "Nonlinear Forecasting of a Brackish Karstic Spring", Water Resources Research, Vol. 36, No. 4, pp. 875-884.

[45]Giannarou Christina (2009). Hydrological investigation of the brackish karstic spring of Almiros in Heraklion Crete, Diploma thesis, NTUA.
[46]A. Maramathas, Z. Maroulis, D. Marinos-Kouris (2003) «Blocking sea intrusion in brackish Karstic Springs, the case of the Almiros Spring at Heraklion Crete, Greece», European Water 1 / 2:17-23.
[47]Melina Kotti, Eleni Kokinou and George Stavroulakis (2016). Classification of storm water and sea water samples by zero-, first- and second-order derivative UV spectra and pattern recognition methods. Int. Journal of Engineering Research and Applications, ISSN: 2248-9622, Vol. 6, Issue 3, (Part - 1) March 2016, pp.62-67.
[48]D. Voutsa, E. Manoli, C. Samara, M. Sofoniou and I. Stratis (1999). A quality study of surface waters of Macedonia, Greece: Specification of nitrogen and phosphorus, 6th International Conference on Environmental Science and Technology, Pythagorion, Samos, Greece 30/8 – 2/9/99.
[49]R. Saraswat, M. Kouthanker, S. Kurtarkar, R. Nigam, and V. N. Linshy (2011). Effect of salinity induced pH changes on benthic foraminifera: a laboratory culture experiment, Biogeosciences Discussions, 8, 8423-8450.
[50]Anastasiadis (2000). Unpublished data.
[51]Arfib B., De Marsily G., Ganoulis J (2007). Locating the zone of saline intrusion in a coastal karst aquifer using springflow data. Groundwater Volume 45, No 1, January–February (pages 28–35).
[52]Paolo Vineis, Queenie Chan, Aneire Khan (2011). Climate change impacts on water salinity and health. Journal of Epidemiology and Global Health (2011) 1, 5- 10.
[53]Sujitha P.C., Mitra Dev D., Sowmya P.K., Mini Priya R. (2012). Physico-chemical parameters of Karamana river water in Trivandrum district, Kerala, India. International Journal of Environmental sciences Volume 2, No3, 2012, ISSN 0976-4402.
[54]Kikuchi, M., A. Tokai and Y. Yoshida (1986). Determination of trace levels of linear alkylbenzenesulfonates in the marine environment by high-performance liquid chromatography. Water Resources 20(5):643-650.
[55]Arimoto, M., H. Tsujizawa, and T. Yokoyama, 1980. LAS (linear alkyl benzene sulfonate) phosphorus in Katsuura-Moriura Bays. Wakayama-ken Eisei Kenkyusho Nenp 27:39-42 (as cited in Painter and Zabel, 1988).
[56]Okamoto, T., and Y. Shirane, 1982. Pollution of linear alkylbenz;ene sUlphonate (LAS) in river and coastal area in Hiroshima Prefecture. Hiroshima-Ken Kankyo Senta Kenkyu Hokoku. 3:60-65 (as cited in Psinter and Zabel, 1988).
[57]Hon-ami, H. and T. Hanya (1980). Linear river estuary and bay water·gas spectrometry. Water Resources 14:1251-1256.
[58]Lever Brothers Company. Unpublished data.
[59]Okoro D (2008). Source determination of polynuclear aromatic hydrocarbons in water and sediment of a creek in the Niger Delta region. African Journal of Biotechnology Vol. 7 (3), pp. 282-285.
[60]Gree Ana, M.K.C.Sridhar and G.O Emerole (2011). Contamination of surface waters by polycyclic aromatic hydrocarbons in two Nigerian coastal communities. Journal of Environmental Health Research, Volume 11 Issue 2.
[61]Mohammad Hosein Habibi; Mohammad Reza Hadjmohammadi (2008). Determination of some polycyclic aromatic hydrocarbons in the Caspian seawater by HPLC following preconcentration with solid phase extraction. Iran, J. Chem. Chem. Eng, Vol. 27, No. 4, 2008.
[62]Dionex Corporation (2009). Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Tap Water Using On-Line Solid-Phase Extraction Followed by HPLC with UV and Fluorescence Detections. Application Note 213, LPN 2128 PDF 01/09.
[63]Hazim A. Walli (2015). Determination of PAHs in Surface Water of Al-Dalmaj Marsh, Al-Diwaniya Province, Iraq. Journal of Environment and Earth Science, ISSN 2224-3216(paper), ISSN 2225-0948 (online), Vol.5, No 12.

[64]Aniel da Costa Lima, Fernanda Fernandes Heleno, Robson José de Cássia Franco Afonso, Mauricio Xavier Coutrim (2015). Determination of PAHs in Surface Waters from the Doce and Piracicaba Rivers in Brazil. Journal of Water Resource and Protection,7,422-429.
[65]Joil José Celino, Henry Xavier Corseuil, Marilda Fernandes, Gisele Mara Hadlich (2012). Persistent toxic substances in surface water of Todos Os Santos Bay, Brazil. Resources and Environment 2012, 2(4): 141-149.
[66]Ywann Penru,F.Xavier Simon, Andrea R.Guastalli, Santiago Esplugas, Joan lorens, Sylvie Baig (2011). Characterization of natural organic matter from Mediterranean coastal. IWA Specialty Conference on Natural Organic Matter, Costa Mesa, CA, USA, July 27-29.
[67]Coble, P.G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Mar. Chem. 51:325-346.
[68]Senesi N. (1990). Molecular and quantitative aspects of the chemistry of fulvic acid and its interactions with metal ions and organic chemicals: Part II : The fluorescence spectroscopy approach. Analytica Chimica Acta 232:77-106.
[69]https://en.wikipedia.org/wiki/Alkalinity.