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Fluorescence Monitoring and Parallel Factor Analysis of Contaminant Mixtures in Water

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FLUORESCENCE MONITORING AND PARALLEL FACTOR ANALYSIS OF CONTAMINANT MIXTURES IN WATER

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DEDICATION

To my mother with unbreakable resolve and my father with immeasurable intelligence, thank you for your never ending support. As I get older I learn of more and more struggles you both went through, but I have always noticed one constant: you had a quiet dignity and grace through all uphill battles. Someday I will carry myself the same way, but for now I'll settle for making it through, as loud as I may be.

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ABSTRACT OF THE THESIS

FLUORESCENCE MONITORING AND PARALLEL FACTOR ANALYSIS OF SIMULATED CONTAMINANT MIXTURES IN WATER

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Professor Howard Patterson, Thesis Professor

Pharmaceuticals and Personal Care Products (PPCPs) pollute our water systems regularly by entering the environment through inefficient methods of water treatment, ineffective sanitation and unregulated industrial and agricultural runoff. Currently, the most common methods of identifying PCPPs are expensive, time consuming and often require the aid of a trained expert. This project aims to further the methodology of utilizing fluorescence spectroscopy as a means of pollutant detection with a focus on contaminant mixtures. Four non-regulated EPA contaminants were fluoresced at compound specific EEM parameters and then again in two component mixtures to determine their limits of detection (LOD). The results were analyzed using Parallel Factor (PARAFAC) analysis to decrease the LOD. The results showed that the LOD of the fluorescence spectrometer could be lowered to environmentally relevant concentrations when paired with PARAFAC analysis. Compound mixtures were detected with equivalent precision and accuracy to single compound solutions. This validates fluorescent spectroscopy paired with PARAFAC analysis as a means of contaminant detection with greater speed, ease and economy. This would allow for real-time decisions concerning water safety which would globally add to the overall health of communities. Further studies should be done to test collected water samples and greater mixture complexity.

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Chapter 1: Introduction

Annually, $260 billion is lost globally due to lack of safe water and sanitation. Universal access to clean water would save $18.5 billion in avoidance of preventable deaths, $32 billion in economic benefits from the reduction of health care costs and almost $1 trillion from avoidance of water crises from years to come.[1] Pharmaceuticals and Personal Care Products (PPCPs) pollute our water systems regularly by entering the environment through inefficient methods of water treatment, ineffective sanitation and unregulated runoff. The increasing rate of pollutant contamination is causing PPCPs to be detected regularly within our nation's bodies of water.[2] PPCPs are any chemical substance involved in the healthcare of one's body.[2] The Environmental Protection Agency (EPA) periodically releases the Contaminant Candidate List (CCL) which lists contaminants that are currently free of regulation and standards of concentration in water but are known to exist in public waterways. From this list, the EPA takes action to stabilize the increasing rate of a contaminant's presence in the environment and always among these pollutants are PPCPs.[3] Two studies conducted by the United States Geological Survey (USGS) in 2008 found that both groundwater and surface freshwater contained a large variety of PPCP mixtures. These results of contamination were consistent across test sites, confirming that PPCPs are widespread and pervasive in various watersheds[4,5]. This pollution occurs through incomplete removal of trace contaminants in human waste in water treatment, agricultural runoff, industrial effluents and point-source excretions.[6]

Left unchecked, PPCPs have the potential to adversely affect the environments where they eventually settle. Examples of adverse effects include, the release of antibiotics into the environment and the rise of superbugs from antibiotics, plankton and algae growth retardation from propranolol and ciprofloxacin, renal failure from diclofenac in the now endangered vultures

and exposure to17α-ethinylestradiol lessened fertility of the fathead minnow males as well as changed their sex.[6,7] These toxins exist in small concentrations in the environment, the doses are at far lower concentrations in water than levels of therapeutic treatment.[8] Despite this significant decrease in concentration, these non-lethal doses cause detrimental health effects in humans when paired with long term exposure. A majority of pharmaceuticals are non-lethal when administered at a dose of 50 mg or less. However, in the case of PPCPs, many collect in tissues and organs. This accumulation over time has been called bioaccumulation. Bioaccumulation is a chemical system outside of equilibrium resulting from high collection rates and low metabolic degradation. Thus PPCPs could accumulate to the point of lethality in target organs and tissues.[9] There is also great unknown risk concerning the "mixing" of trace contaminants intercellularly.[8] There are endless unknown and unpredictable interactions between the PPCP compounds.[10] A person may accumulate a harmless PPCP from one water source and then expose themselves to a different PPCP that will adversely interact with the former. There has yet to be extensive research on the detrimental health effects caused by PPCPs in humans.[2] It is reasonable to hypothesize the unwanted effects of PPCPs that have occurred in animals will also affect humans, thus making the successful detection of PPCP contaminants pertinent.

Detecting PPCPs is crucial to the health of our ecosystems and bodies and thus why improving upon the detection methodology is greatly beneficial to all water treatment. Currently the use of gas and liquid chromatography and mass spectrometry (GC/LC MS) are the most common methods of identifying PCPPs. Most detection systems involve a combination of all three methods. This allows for detection of each individual pharmaceutical during gas and liquid chromatography and determination of concentrations as low as parts per billion. However, these

methods are expensive and often require the aid of a trained expert. The tests are also time consuming and are limited to utilization in the laboratory.[8]

Fluorescence spectroscopy is currently used as an acceptable contaminates detection method due to its impressive sensitivity, reaching limits of detection of parts per billion.[10,11] The task of comparing fluorescence to more traditional GC/LC MS has already been performed by James Killarney of the University of Maine, proving the method has comparable limits of detection reaching parts per billion levels. Extensive work has continued at the University of Maine by the Patterson Research Group as well as several other research groups[12,13] to create a pre-treatment methodology that allows the utilization of fluorescence spectroscopy and parallel factor (PARAFAC) analysis as a means of cheap and efficient detection.[10] This project seeks to continue this work already done by the Patterson group with a focus on mixtures, specifically those of Quinoline (compound **1**), Permethrin (compound **2**), 1,2,3,4-Tetrahydroisoquinolin (compound **3**), and 6,7-Dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline (compound **4**).

Fluorescence measures the energy released as an electron goes from an excited state to a ground state. Electron relaxation from excited to ground state results in emission of photons of specific wavelengths. Conversely, electron excitation from ground state to various excited states can also be observed and measured. These emissions and excitations occur over unique wavelengths and with a variable intensity. When measured and plotted, the combined emission and excitation data creates a 3D mound or “peak” that is individual to every molecule and acts as an identifier. This allows for assertion of a molecule's presence.[11]

Chapter 2: Experimental Parameters

2.1 Materials

Molecules on the CCL with pi bond conjugation were chosen to be examined. Among these are molecules that are in the run off of manufacturing plants (compounds **3** and **4**), tapestry and dying procedures (compound **1**) and pesticides (compound **2**). Figure 1.1 shows the molecules utilized in these experiments. Each molecule was chosen from the EPA CCL. This means it does not have any environmental regulations, and specifically it does not have any maximum contaminant levels (MCLs) or maximum contaminant level goals (MCLGs).

2.1a Compound 1

(Quinoline) Compound **1** has a double ring, aromatic, heterocyclic molecule. The two rings are composed of a pyridine and a benzene ring joined at two adjacent carbons. The structure is almost entirely conjugated which makes it very fluorescent, the strongest luminescent intensity of all the compounds in this study. Compound **1** and its many derivatives are used in wood treatment and in as an additive in consumables but it is primarily used in the textile industry to create dyes for clothing.[14] Being a skin, eye, and liver irritant, a respiratory depressor[15] and suspected carcinogen, there is concern for remnants on clothing being so close in proximity to permeable skin thus a greater chance of absorption.[16,17,18] This concern also applies to ingestion when textile plant waste is disposed of improperly and pools in watersheds only to percolate into groundwater resources.[14]

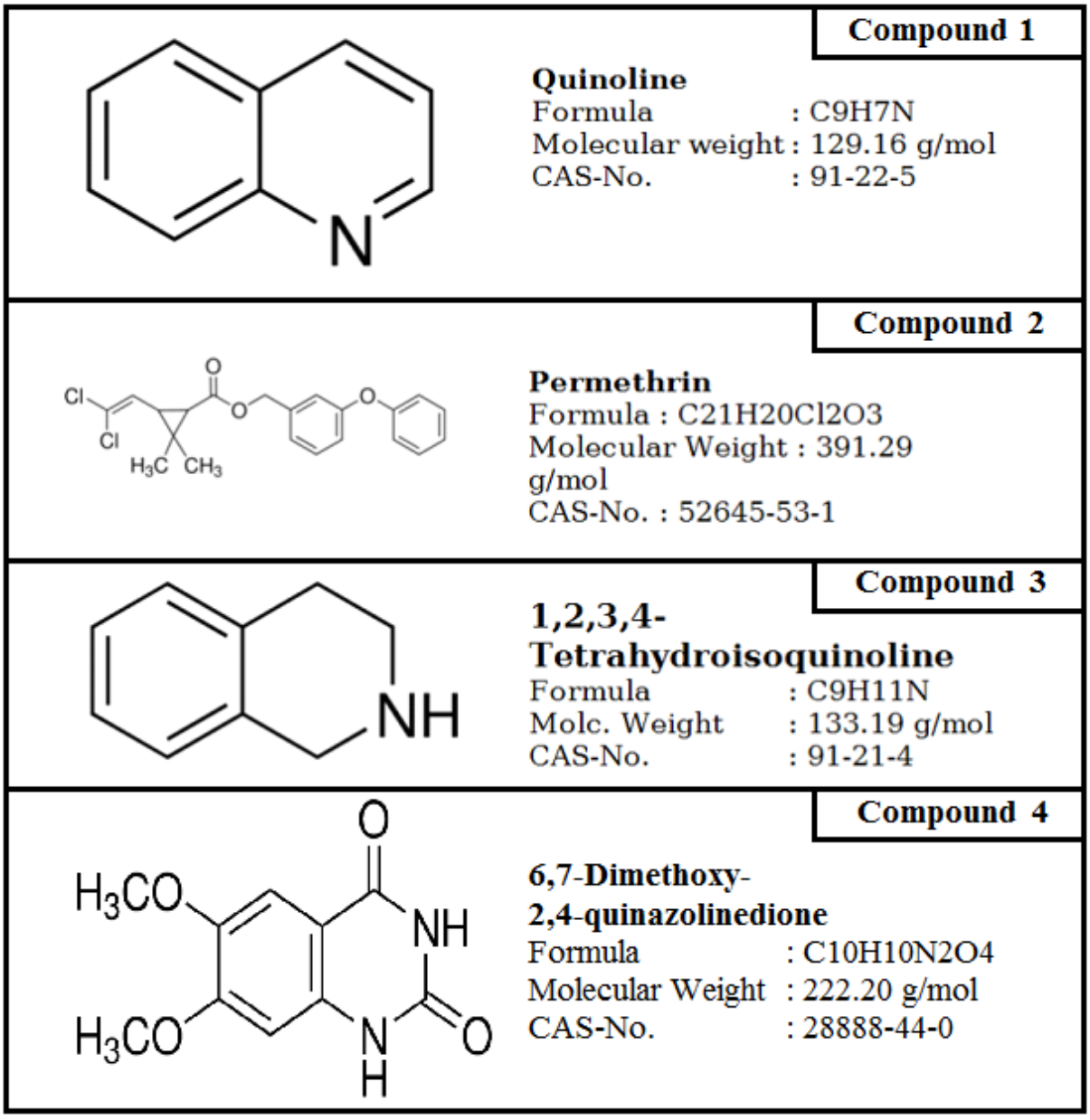

Figure 1.1: Properties and structures of compounds utilized in this study and their code names

2.1b Compound 2

(Permethrin) is composed of series of functional groups beginning with a double chlorinated ethene attached to a cyclic propane with two methyl groups followed by carboxylate and finally a phenoxy group with a phenyl group. Compound **2** is not fully conjugated, but gets its fluorescent properties from the last two functional groups (the phenoxy and phenyl) which are

both aromatic rings. Compound **2** is a pyrethoid, meaning it is a synthetic compound that targets the nervous system of any organic tissues, disrupting axonal communication. This is why it is a widely used pesticide known for its acute toxicity.[19] Compound **2** itself is not strongly toxic, however its presence creates high reactive oxygen species (ROS) which lead to re-writing of an organism's protein DNA. Aquatic species been shown to absorb compound **2** and experience changes to their protein synthesis such as crayfish, bivalve mollusks, nematodes, and riparian grasses.[19,20,21]

2.1c Compound 3

(1,2,3,4-Tetrahydroisoquinoline), being a derivative of compound 1, is very similar in structure: two rings, one benzene the other a heterocyclic ring of carbon and amine, joined at adjacent carbons. The benzene provides the only conjugation and thus the only source of fluorescent properties. This compound is also utilized in textile dyes and shares many of the same hazards as compound 1.[14]

2**.1d Compound 4**

(6,7-Dimethoxy-2,4-quinazolinedione) is part of the quinozoline family, similar to quinoline there is a benzene and heterocyclic pyridine ring joined at two adjacent carbons, however the pyridine ring contains two meta-nitrogen and two meta-ketones. Additionally, bonded to the heterocyclic ring are two adjacent ethers. However, it can also cause disorientation, nausea, unconsciousness, and pulmonary congestion and is easily absorbed through the skin. It is considered a neurotoxin, carcinogen and fertilization inhibitor.[22,23] All fluorescent properties come from the conjugated benzene ring. This compound can be used in treatment of Leukemia.[24]

2.2 Experimental Set-Up

2.2a Samples

Samples were created in-lab utilizing deionized water and methanol. The first solutions were spiked with only one compound and water to determine the ideal excitation and emission peaks of each compound. This rough data was followed by samples for each compound with known concentrations and a series of five halved dilutions from 1 ppm to 62.5 ppb (0.0625 ppm) in an effort to attain each compound's limit of detection (LOD). One compound, compound 4, required tenth dilutions from 62.5 ppb to 62.5 ppt. Finally, solutions were made with mixtures of two compounds in every combination, with one compound being held at a steady concentration of 1 ppm and the other diluted by half in five successive dilutions from 1 ppm to 62.5 ppb, only compound **4** then required three tenth dilutions. The reversal of this combination was also tested.

2.2a i Compound 1

The compound **1** solution is made through a series of half dilutions from 1 ppm to 62.5 ppb. The excitation range (285 to 335 nm) and emission range (361 to 475 nm) were determined using the optimized peak values of excitation and emission scans. The ranges were used to perform 3D scan resulting in one excitation scan, one emission scan and one PARAFAC output with EEM parameters summarized in Table 2.2a for the standardized values of compound **1**.

2.2a ii Compound 2

The compound **2** solution is made through a series of half dilutions from 1 ppm to 62.5 ppb. The excitation range (220 to 244 nm) and emission range (285 to 335 nm) were determined using the optimized peak values of excitation and emission scans. The ranges were used to perform 3D scan resulting in one excitation scan, one emission scan and one PARAFAC output with EEM parameters summarized in Table 2.2a for the standardized values of compound **2**.

2.2a iii Compound 3

The compound **3** solution is made through a series of half dilutions from 1 ppm to 62.5 ppb. The excitation range (240 to 271 nm) and emission range (286 to 325 nm) were determined using the optimized peak values of excitation and emission scans. The ranges were used to perform 3D scan resulting in one excitation scan, one emission scan and one PARAFAC output with EEM parameters summarized in Table 2.2a for the standardized values of compound **3**.

2.2a iv Compound 4

The compound **4** solution is made through a series of half dilutions from 1 ppm to 62.5 ppb. The excitation range (280 to 345 nm) and emission range (365 to 450 nm) were determined using the optimized peak values of excitation and emission scans. The ranges were used to perform 3D scan resulting in one excitation scan, one emission scan and one PARAFAC output with EEM parameters summarized in Table 2.2a for the standardized values of compound **4**.

2.2a v Mixture 1

Mixture **1** is combination of compounds **1** and **2**, with compound **1** being held at a steady concentration of 1 ppm while compound **2** underwent a series of half dilutions from 1 ppm to 62.5 ppb. The excitation range of compound **1** (285 to 335 nm) is in the emission range of compound **2** (285 to 335 nm), thus mixture **1** was scanned using the EEM parameters of compound **1** and compound **2** individually. This resulted in two excitation scans, two emission scans and two PARAFAC outputs with EEM parameters summarized in Table 2.2b for mixture **1.**

2.2a vi Mixture 2

Mixture **2** is combination of compounds **1** and **2**, with compound **2** being held at a steady concentration of 1 ppm while compound **1** underwent a series of half dilutions from 1 ppm to 62.5 ppb. The excitation range of compound **1 (**285 – 335 nm) is in the emission range of

compound **2** (285 – 335 nm), thus mixture **2** was scanned using the EEM parameters of compound **1** and compound **2** individually. This resulted in two excitation scans, two emission scans and two PARAFAC outputs with EEM parameters summarized in Table 2.2b for mixture **2**.

2.2a vii Mixture 3

Mixture **3** is combination of compounds **1** and **4**, with compound **1** being held at a steady concentration of 1 ppm while compound **4** underwent a series of half dilutions from 1 ppm to 62.5 ppb and tenth dilutions from 62.5 ppb to 62.5 ppt. Mixture **3** was scanned with the combined EEM parameters of compound **1** and compound **4** (An excitation range of 280 to 340 nm and an emission range of 360 to 476 nm), resulting in only one excitation scan, one emission scan and one PARAFAC output. The resulting EEM parameters can be seen in Table 2.2b for mixture **3**.

2.2a viii Mixture 4

Mixture **4** is combination of compounds **1** and **4**, with compound **4** being held at a steady concentration of 1 ppm while compound **1** underwent a series of half dilutions from 1 ppm to 62.5 ppb. Mixture **4** was scanned with the combined EEM parameters of compound **1** and compound **4** (An excitation range of 280 to 340 nm and an emission range of 360 to 476 nm), resulting in one excitation scan, one emission scan and one PARAFAC output. The resulting EEM parameters can be seen in Table 2.2b for mixture **4**.

2.2a ix Mixture 5

Mixture **5** is combination of compounds **1** and **3**, with compound **1** being held at a steady concentration of 1 ppm while compound **3** underwent a series of half dilutions from 1 ppm to 62.5 ppb. Mixture **5** was scanned with the combined EEM parameters of compound **1** and compound **3** (an excitation range of 220 to 304 nm and an emission range of 320 to 476 nm), resulting in one excitation scan, one emission scan and one PARAFAC output. The resulting EEM parameters can be seen in Table 2.2b for mixture **5**.

2.2a x Mixture 6

Mixture **6** is combination of compounds **1** and **3**, with compound **3** being held at a steady concentration of 1 ppm while compound **1** underwent a series of half dilutions from 1 ppm to 62.5 ppb. Mixture **6** was scanned with the combined EEM parameters of compound **1** and compound **3** (an excitation range from 220 to 304 nm and an emission range from 320 to 476 nm), resulting in one excitation scan, one emission scan and one PARAFAC output. The resulting EEM parameters can be seen in Table 2.2b for mixture **6**.

2.2a xi Mixture 7

Mixture **7** is combination of compounds **2** and **4**, with compound **2** being held at a steady concentration of 1 ppm while compound **4** underwent a series of half dilutions from 1 ppm to 62.5 ppb and tenth dilutions from 62.5 ppb to 62.5 ppt. The excitation range of compound **4** (280 to 345 nm) is in the emission range of compound **2** (285 to 335 nm**)**, thus Mixture **7** was scanned using the EEM parameters of compound **2** and compound **4** individually. This resulted in two excitation scans, two emission scans and two PARAFAC outputs with the EEM parameters summarized in Table 2.2b for mixture **7**.

2.2a xii Mixture 8

Mixture **8** is combination of compounds **2** and **4**, with compound **4** being held at a steady concentration of 1 ppm while compound **2** underwent a series of half dilutions from 1 ppm to 62.5 ppb. The excitation range of compound **4** (280 to 345 nm) is in the emission range of compound **2** (285 to 335 nm**)**, thus Mixture **8** was scanned using the EEM parameters of compound **2** and compound **4** individually. This resulted in two excitation scans, two emission scans and two PARAFAC outputs with the EEM parameters summarized in Table 2.2b for mixture **8**.

2.2a xiii Mixture 9

Mixture **9** is combination of compounds **2** and **3**, with compound **3** being held at a steady concentration of 1 ppm while compound **2** underwent a series of half dilutions from 1 ppm to 62.5 ppb. Mixture 9 was scanned using the combine EEM parameters of compound **2** and compound **3** (an excitation range from 220 to 270 nm and an emission range from 285 to 335 nm), resulting in one excitation scan, one emission scan and one PARAFAC output . The EEM parameters are summarized in Table 2.2b for mixture **9**.

2.2a xiv Mixture 10

Mixture **10** is combination of compounds **2** and **3**, with compound **2** being held at a steady concentration of 1 ppm while compound **3** underwent a series of five half dilutions. The results of Mixture 10 were statistically insignificant and will not be discussed in this thesis.

2.2a xv Mixture 11

Mixture **11** is combination of compounds **3** and **4**, with compound **3** being held at a steady concentration of 1 ppm while compound **4** underwent a series of half dilutions from 1 ppm to 62.5 ppb and a series of tenth dilutions from 62.5 ppb to 62.5 ppt. The excitation range of

compound **4** (280 to 345 nm) is in the emission range of compound **3** (285 to 335 nm), thus Mixture **11** was scanned using the EEM parameters of compound **3** and compound **4** individually. This resulted in two excitation scans, two emission scans and two PARAFAC outputs with the EEM parameters summarized in Table 2.2b for mixture **11**.

2.2a xvi Mixture 12

Mixture **12** is combination of compounds **3** and **4**, with compound **4** being held at a steady concentration of 1 ppm while compound **3** underwent a series of half dilutions from 1 ppm to 62.5 ppb. The excitation range of compound **4** (280 to 345 nm) is in the emission range of compound **3** (285 to 335 nm), thus Mixture **11** was scanned using the EEM parameters of compound **3** and compound **4** individually. This resulted in two excitation scans, two emission scans and two PARAFAC outputs with the EEM parameters summarized in Table 2.2b for mixture **12**.

Compound	**Excitation Range (nm)**	**Emission Range (nm)**	**Max Excitation (nm)**	**Maximum Emission (nm)**	**Slit Widths (nm)**	**Integration Time (s)**	**Step Size (nm)**
1	285 – 335	361 - 475	315	402	5	0.1	1
2	220 – 244	285 - 335	244	298	5	0.1	1
3	240 – 271	286 - 325	262	286	5	0.1	Ex: 1 Em: 2
4	280 – 345	365 - 450	328	373	5	0.1	2

Table 2.2a: The EEM parameters of each single compound. These values were used as a standard were used as a standard of comparison for the mixtures.

Mixture	Combined EEM ranges	Excitation Range (nm)	Emission Range (nm)	Slit Widths (nm)	Integration Time (s)	Step Size (nm)
Mixture 1	No	285 to 335 220 to 244	361 to 475 285 to 335	5	0.1	2
Mixture 2	No	285 to 335 220 to 244	261 to 475 285 to 335	5	0.1	2
Mixture 3	Yes	280 to 340	360 to 476	5	0.1	2
Mixture 4	Yes	280 to 340	360 to 476	5	0.1	2
Mixture 5	Yes	220 to 304	320 to 476	5	0.1	3
Mixture 6	Yes	220 to 304	320 to 476	5	0.1	3
Mixture 7	No	220 to 244 280 to 345	285 to 335 365 to 450	5	0.1	2
Mixture 8	No	220 to 244 280 to 345	285 to 335 365 to 450	5	0.1	2
Mixture 9	Yes	220 to 270	285 to 335	5	0.1	2
Mixture 10	Yes	220 to 270	285 to 335	5	0.1	2
Mixture 11	No	240 to 271 280 to 345	286 to 325 365 to 450	5	0.1	Ex: 1 Em: 2
Mixture 12	No	240 to 271 280 to 345	240 to 271 365 to 450	5	0.1	Ex: 1 Em: 2

Table 2.2b: The EEM parameters of each mixture.

2.2b Spectroscopy

All fluorescence measurements were made with Jobin Yvon and Optical Emission Spectrometer (JYS) with a xenon arc lamp. Solutions were held in a $1cm^2$ quartz rectangular cuvette.

Emission and excitation ranges are specific to each compound and each mixture. Single compound samples have emission and excitation ranges that include the maximum peak value for each range, combined compound samples use combined ranges to include the maximum

excitation and emission peaks for both compounds. Some compound mixtures have incompatible excitation and emission ranges and thus were scanned twice utilizing the specific EEM parameters of each single compound. Each scan was blanked with deionized water and normalized with the deionized water peak.

2.2c PARAFAC (Parallel Factor Analysis)

PARAFAC was accomplished utilizing the PLS_Toolbox 8.1® (Eigenvector Research Inc. Manson, WA).[25] This program is subroutine for MATLAB 8.4® (Mathworks Inc., Cambridge, MA). PARAFAC was analyzed as multi-way data given it had two independent variables (excitation and emission) and one dependent (intensity). Single value decomposition and A CORCONDIA analyses were performed to create the initial loadings and models.

Chapter 3: Background Information

3.1 Fluorescence

Luminescence is the emission of light (photons) from electrons that are falling from an excited state to a ground state. It can be classified into two categories, phosphorescence and fluorescence. Phosphorescence is when an electron in an excited state is returning to a ground state occupied by an electron with the opposite spin, this is called spin forbidden transition. This causes the electron to remain in the excited state for a relatively long time before it emits photons. This is a very long luminescence lifetime when compared to its counterpart Fluorescence. Fluorescence involves spin allowed transitions, an electron falls from an excited state to a ground state, but in this case experiencing minimal resistance and dipole allowed. Fluorescence luminescence lifetime is much faster than phosphorescence, usually occurring within 10 ns, and in some cases even shorter.[11]

3.1a Molecules that Fluoresce

Fluorescence usually occurs within aromatic materials due to the presence of conjugated pi bonds. The conjugation lessens the energy gap between the HOMO and LUMO energy levels and thus the energy needed to promote an electron from the ground state to an excited state is lessened and exists within the visible or low end of the UV spectrum.[10] A general name for molecules capable of the electron movement needed for excitation are called fluorochromes. when these molecules are bonded to large, organic macromolecules (such as nucleic acids, hormones, steroids, and proteins) they are called fluorophores.[26] Typical compounds of fluorescence are Pyridine 1, fluorescein and quinine all of which are utilized for their luminescent properties, the latter two most famously. Fluorescein glows green when in water and is used to locate individuals lost at sea. supplies are located on boat emergency kits. Quinine was

one of the first molecules to be observed fluorescing in 1845, producing a blue aura observed around the perimeter of the liquids.[11]

3.1b Core Fluorescence Concepts

Jablonski diagrams are a schematic of the excitation and emission of electrons. It depicts the ground, first and second excited singlet of an electron (referred to as S_0, S_1, and S_n respectively) and the existing levels within the singlet states. The arrows show the various transitions and pathways an electron can take.[11] Figure 3.1 is a typical rendition of electronic states. The vertical arrows are meant to illustrate the instantaneous nature of light absorption and emission. Internal conversion, which is a rapid non-radiative (no emission of light but rather the energy is released as heat) relaxation of an electron moving from S_n to S_1 as seen in blue and yellow wavy lines. the electron falls to the lowest vibrational energy level before emitting photons. It only occurs when the vibrational energy of an electron in a higher state is paired with that of a lower state. Also depicted is intersystem crossing, which is the flipping of spin in a photon emitting electron, now in a spin forbidden transition, which allows for phosphorescence.[26] These diagrams allow for a general understanding of fluorescence and thus an understanding of how it can be utilized. The varying wavelengths applied to the molecule will cause the electron to be excited to varying levels of excitation, perhaps only to the lowest S_1 level or to the highest S_n level. The absorption of photons for each wavelength will be different, and thus is a measurable method of changing fluorescence intensity that will be specific to each fluorophore, allowing with identification via emission spectra.[11]

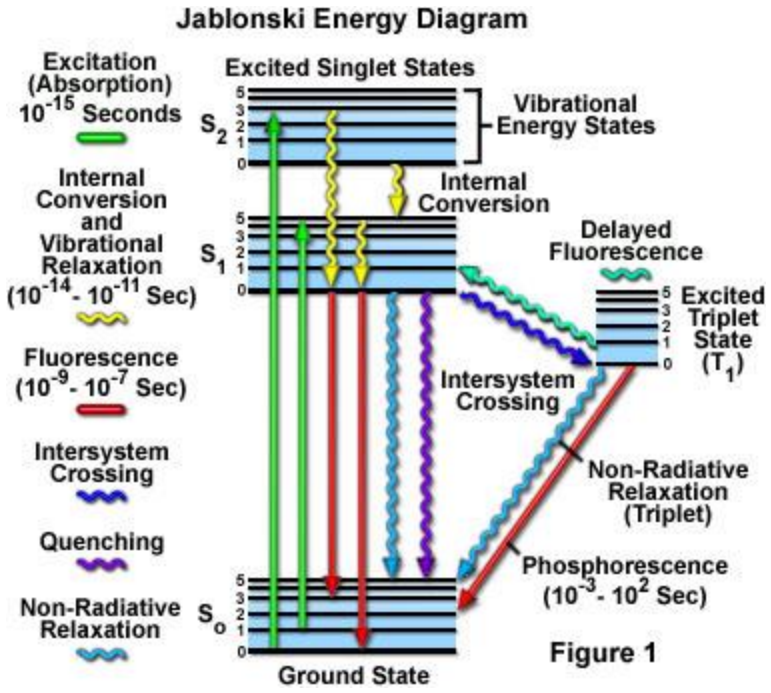

Figure 3.1: Jablonski diagram, Antonie J.W.G. Visser and Olaf J. Rolinski, http://www.photobiology.info/Visser-Rolinski.html

Kasha's Rule: An excitation spectrum shows wavelengths of light that resulted in emission of photons. Different wavelengths will excite electrons to different energy levels, some to S_2 or S_3 or even beyond, typically internal conversation occurs at a faster rate than reemission of photons. After non-radiative energy emissions allow the electron to fall to the lowest vibrational energy in S_1, then emission of photons will occur as it falls to the ground state.[11] Simplified, emission spectra are independent of excitation spectra. To better understand this, it can be noted an excitation spectrum has several peaks, each one showing an electron's transition from the ground state to a new energy level: From S_0 to S_1, S_0 to S_2, S_0 to S_3 and so on. The emission spectrum will only contain peaks that correspond to transitions from S_1 to S_0 (See Figure 3.2) To reiterate, this is because rapid internal conversions cause an electron to fall to S_1 before an emission occurs. No matter how many peaks an excitation spectra has, the emissions spectra will only correspond to peaks showing excitation transitions from S_1 to S_0.[26] However,

those peaks will be the mirror image of the excitation spectra's peaks showing the transitions from S_0 to S_1. This is called the *mirror image effect* and occurs because the most effective excitation paths are also the most effective emission paths.[11]

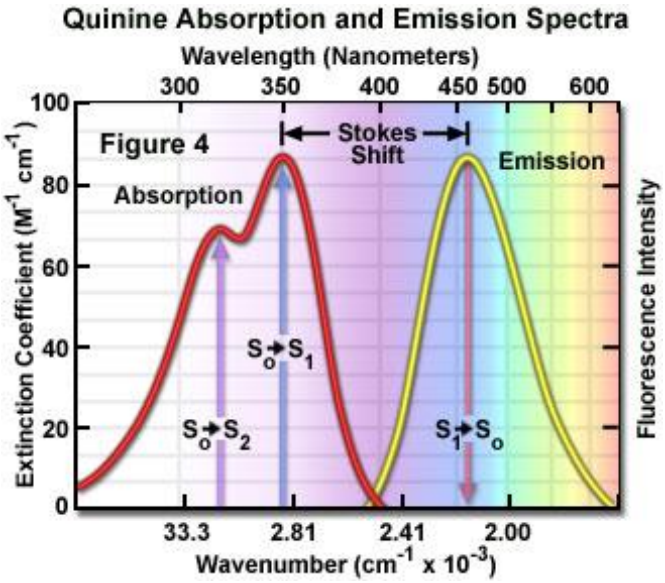

Figure 3.2: Excitation and Emission spectrum, http://www.olympusconfocal.com/theory/fluoroexciteemit.html

Stoke's Shift: Observing a Jablonski diagram (Figure 3.1), it can be seen excitation occurs along the light spectrum at shorter wavelengths than that of emission. The larger wavelength's of emission indicate a lower energy level, which can be explained by the emission's exclusivity of S_1 to S_0 transitions. This shift from shorter wavelengths to longer is termed "Stoke's shift."[26] The shift can actually be seen in the excitation and emission of quinine (Figure 3.2). The solution absorbs and excites in UV wavelengths, which explains the solution's clear appearance, but the surface emits a blue color. The absorbed UV wavelengths are shorter than those of visible blue light, thus indicating the shift. This example also demonstrates another important factor concerning solutions known as the inner filter effect.[11]

Inner Filter Effect: This principle was first noted by Dr. Fredrich Herschel when experimenting with quinine. As previously stated, Herschel observed a glowing blue aura from

the outer edges of a quinine solution, but not from within. When poured, the thinner stream radiated blue from the center and out. This demonstrated the inner filter effect. Light is "enfeebled" when a solution is highly concentrated with materials that absorb light. This means the light is entirely absorbed as it first passes through solution, never hitting the remaining molecules. When the solution is lessened in depth, the whole of it appears to fluoresce rather than just the edges because the light is able to penetrate the thinner thickness entirely, rather than just the surface[11]

3.2 Measurement of Fluorescence

Quantum Yields: Number of emitted photons relative to the number of absorbed photons, in other words, it measures the efficiency of photon transfer. It can be represented by the following equation, where the quantum yield, *Q*, is equal to the emissive rate of fluorescence, , over the emissive rate of fluorescence and the non-radiative decay.[11] Simplified this is the amount of photons emitted divided by photons absorbed.

Luminescence Lifetime: The average amount of time before a fluorophore emits photons from S_1 to S_0. This is usually around 10 ns. The average time is equivalent to the reciprocal of the emissive rate of fluorescence and the non-radiative decay.[11]

Graphic Representations – Steady State: This is the typical representation of fluorescence measurement, done with constant illumination and observation throughout the fluorescence. The sample is struck with a continuous beam of light in solid (optimally a single crystal) and the emission spectrum is recorded.[11]

3.3 Spectrometer Components

A spectrofluorometer measures both excitation and emission spectra of fluorescence. Excitation spectra show the wavelength range or distribution of an excitation measured at a

single emission wavelength, or in other words, it is the relative emission of a fluorophore at wavelengths that resulted in fluorescence. Emission spectra show the wavelength range or distribution of emission measured for a single excitation wavelength, in other words the photon emission rate at a single wavelength interval determined by the width in the slits of the spectrometer. In order to understand this further it is important to understand the layout of a spectrometer.[27] A schematic is presented in Figure 3.3.

The light source is for the spectrometer utilized was a xenon arc lamp with a 450 watt bulb. The light needs to be encased because it is so bright, but the intensity is necessary in order to provide enough energy for excitation.[27] The shutters and slits around the light control the intensity of the light.[27] Opening them wider will allow a fluorochrome to fluoresce more brightly, but lessens the resolution of output data. All experiments were done with a 5nm slit width.

The excitation monochromator allows for selection of the excitation wavelength that will interact with the sample during emission scans, as well as the selection of the excitation wavelength when emission is fixed in excitation scans. It uses diffraction gratings to break down the light into its spectral components, thus allowing for specific selection of a certain wavelength.[28] The beam splitter takes some of the light beam and focuses it on the reference detector, a fluorophore of known excitation and emission wavelengths, to compensate for the changing intensities of the lamp.[28] The polarizer selects light with its electric vector oriented only in a particular direction[27] thus allowing for a single beam of light. The emission monochromator selects the emission wavelength that may be emitted by the sample, as well as the selection of emission wavelengths when excitation is fixed.[28]

The photo multiplier tube (PMT) is a photo-sensitive plate that is senses many wavelengths along the light spectrum, including ultraviolet, visible and infrared.[28] It acts as a vacuum tube that multiplies the electric current up to 100 million times and enables the detection of photons, even when the light is very faint.[27]

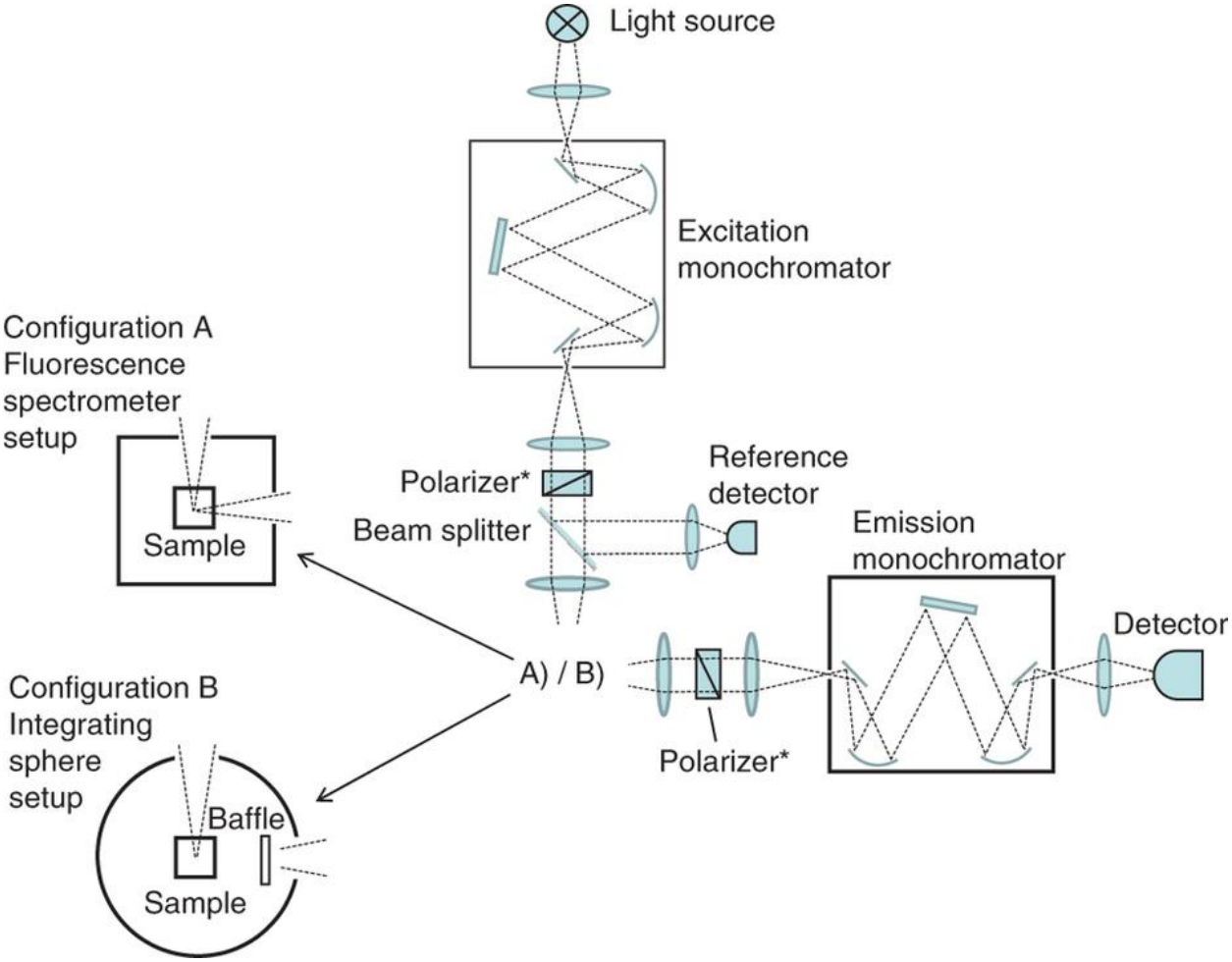

Figure 3.3: Schematic of spectrometer, http://www.nature.com/nprot/journal/v8/n8/images/nprot.2013.087-F3.jpg

3.4 Parallel Factor Analysis

Parallel Factor Analysis (PARAFAC) is a multi-variable model generation system that will allow for the analysis multi-way data, or data with more than two variables. It is a bilinear decomposition method, meaning the analysis breaks down the data into sets of scores and

loadings.[29] A score is the magnitude of effect a factor will have on an outcome, or the value it has in determining the end result.[30] A loading measures what factors affect the magnitude of the score.[31] For example, a score in a EEM would be how influential a particular data point was in determining the intensity. The data point that represents the peak excitation and emission values has a greater score than the data point on the outskirts of scan. A loading in this same scenario would be that a maximum peak occurred at emission Y from excitation X: emission Y loads on excitation X. Essentially, PARAFAC will determine which data points are the most indicative of trend, what affects that trend and then projects output data if that trend were to continue.

PARAFAC datasets are made by creating a 3D set, often done by layering 2D arrays. Although these 2D arrays can be analyzed individually utilizing principal component analysis, this does not allow for three dimensional analysis that finds correlation between data and variables that would otherwise not be associated.[29] For example fluorescent intensity of a compound at excitation X and emission Y at varying concentrations. Traditional two-dimensional analysis is adequate for solutions with single fluorophores, multi-way analysis becomes necessary with multiple components.

The general equation for PARAFAC is as follows:

$$X_{ijk}=\Sigma a_{ij}b_{jf}c_{kf}+e_{ijk}$$

Where *F* is the number of components extracted from the sample, are the excitation, emission and intensity of a scan respectively, *E* is the residual error, and *X* is the intensity of the sample, the excitation and the emission.[10] X is the cumulative data. Each component (a,b,c and e) is a vector called a "loading." The spectral loadings can be interpreted as the excitation and emission spectra of those compounds or as a group of co-varying compounds giving rise to that florescent signal. Figure 3.4 gives a visual aid to this process. It can be used to analyze the kinetic energy

that arises through increased concentration and interaction in mixtures.[32] This equation interprets data by holding two of the three variables (emission, excitation, and intensity) constant while the third is interpreted. It is also assumed that the fluorophores do not interact with each other and follow Beer's Law that the molecules will absorb and fluoresce in proportion to their concentration and the length of the light path.[32]

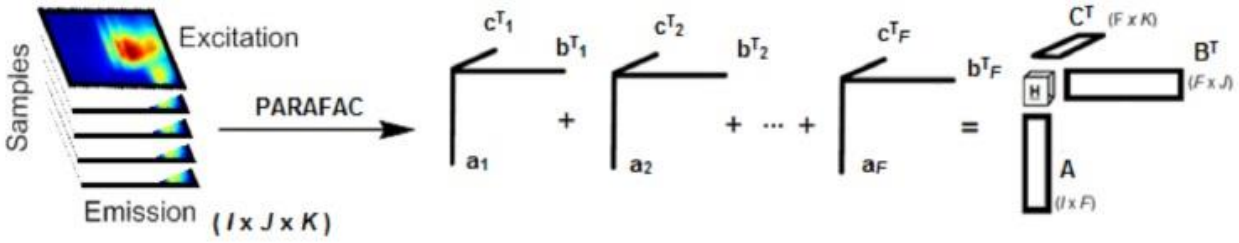

Figure 3.4: PARAFAC visual aid, http://www.scielo.br/img/revistas/jbchs/2012nahead/aop132_12fig04.jpg

Chapter 4: Results

Compound	Limit of Detection before PARAFAC Analysis	Limit of Detection after PARAFAC Analysis	Percent Improvement
Compound 1	125 ppb	62.5 ppb	50.0%
Compound 2	250 ppb	62.5 ppb	75.0%
Compound 3	250 ppb	62.5 ppb	75.0%
Compound 4	625 ppt	62.5 ppt	90.0%

Table 4.1a: PARAFAC results for single compound solutions.

Mixture	Limit of Detection before PARAFAC Analysis	Limit of Detection after PARAFAC Analysis	Percent Improvement
Mixture 1	125 ppb	< 62.5 ppb	50.0%
Mixture 2	500 ppb	< 62.5 ppb	87.5%
Mixture 3	6.25 ppb	< 62.5 ppt	99.9%
Mixture 4	1 ppm	< 62.5 ppb	93.75%
Mixture 5	250 ppb	< 62.5 ppb	75.0%
Mixture 6	125 ppb	< 62.5 ppb	50.0%
Mixture 7	6.25 ppb	< 62.5 ppt	99.90%
Mixture 8	500 ppb	< 62.5 ppb	87.50%
Mixture 9	250 ppb	< 62.5 ppb	75.0%
Mixture 11	1 ppm	< 62.5 ppb	93.75%
Mixture 12	250 ppb	< 62.5 ppb	75.0%

Table 4.1b: PARAFAC results for mixture solutions. Limit of Detection for PARAFAC analysis have been marked with a “less than” symbol to denote the limit of detection could be even less than this, but scans were not done to determine the actual limit.

4.1 Compound 1

The excitation range of compound **1** is 285 nm to 335 nm and the emission range is 361 nm to 475 nm seen in Figure 4.1. The maximum peak is at excitation 315 nm (Figure 4.2) and emission 402 nm (Figure 4.3). It is moderately intense with a narrow excitation spectrum and long emission spectrum. For single compound solutions the JYS limit of detection is 125 ppb, PARAFAC analysis decreases this to <62.5 ppb (Figure 4.4).

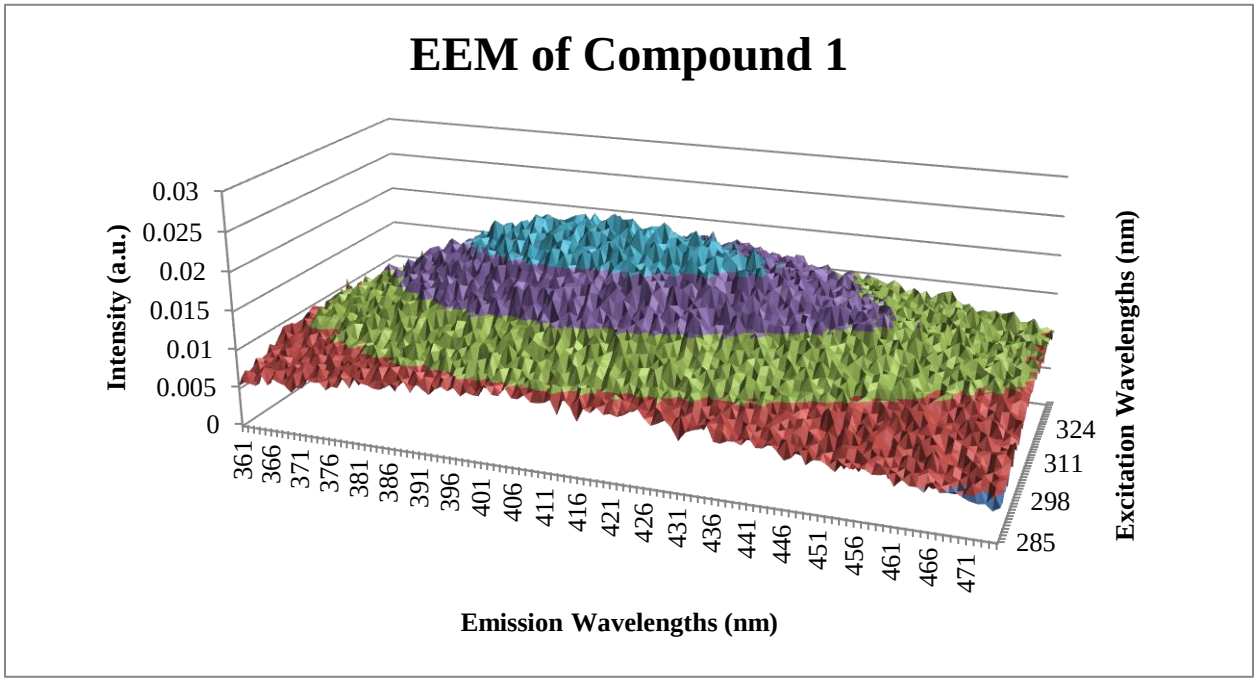

Figure 4.1: Compound 1 JYS EEM with a maximum excitation at 315 nm and maximum emission at 402 nm at a concentration of 1 ppm.

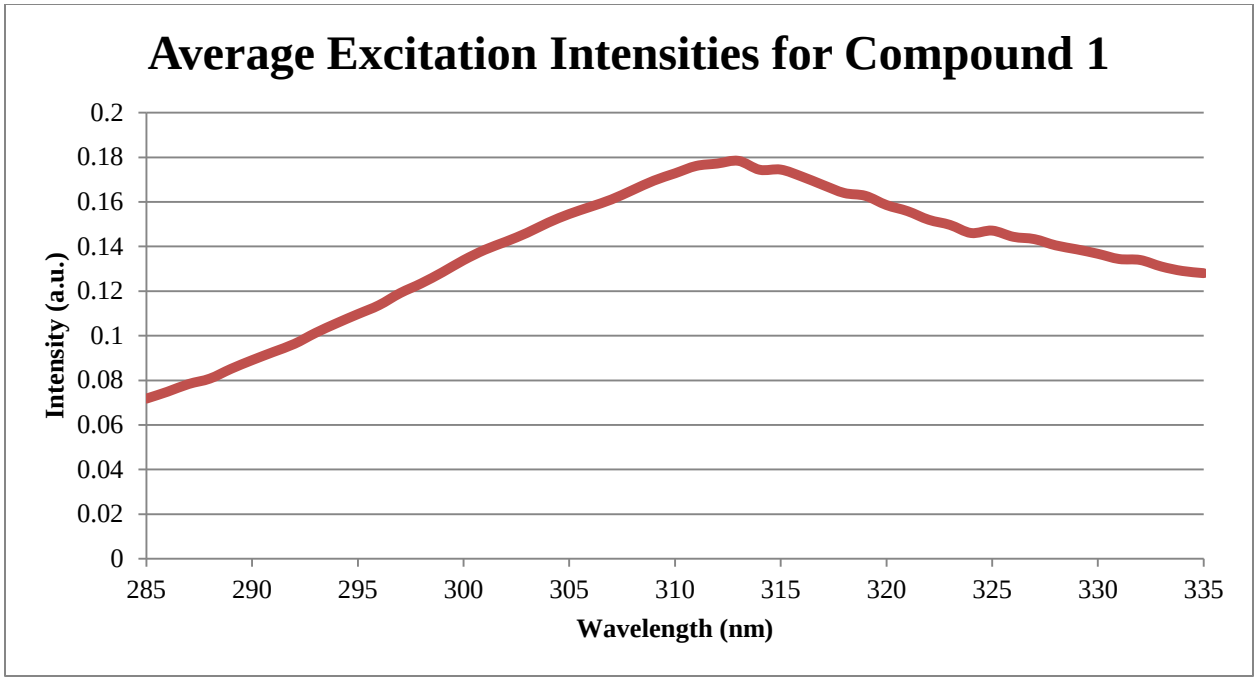

Figure 4.2: Average excitation scan of compound 1 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shape of compound 1 for any given concentration. The maximum excitation appears at 313 nm.

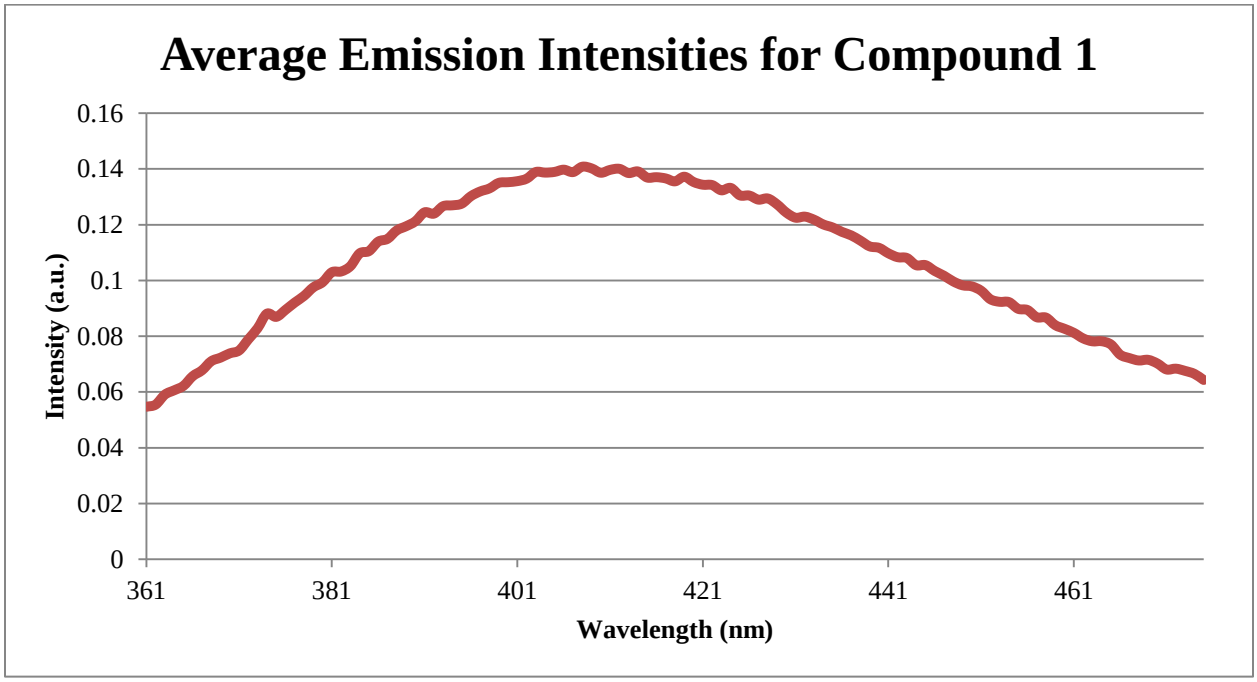

Figure 4.3: Average emission scan of compound 1 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of compound 1 for any given concentration. The maximum emission appears at 407 nm.

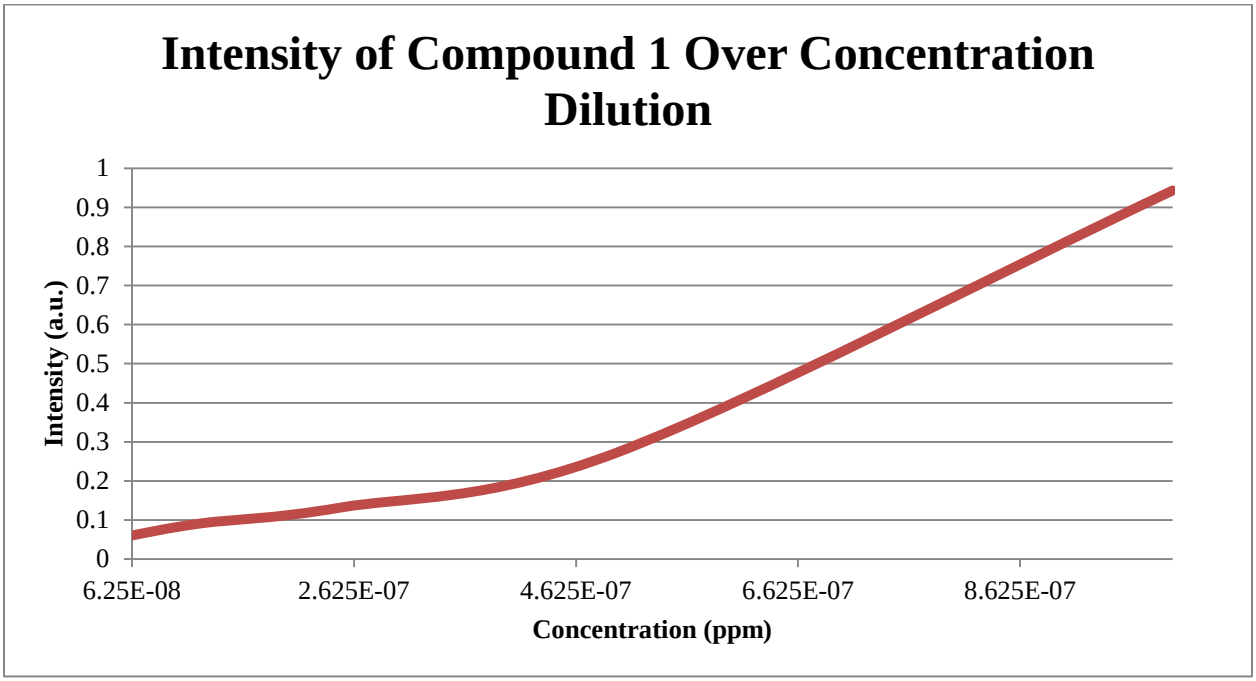

Figure 4.4: Concentration intensities for compound 1 after PARAFAC analysis showing a nearly linear relationship between intensity and concentration.

4.2 Compound 2

The excitation range of compound **2** is 220 nm to 244 nm and the emission range is 285 nm to 335 nm seen in Figure 4.5. The maximum peak is at excitation 244 nm (Figure 4.6) and emission 289 nm (Figure 4.7). It is moderately intense with a sloped excitation spectrum and a plateaued emission spectrum. Most of the peak is not viewed due to Raman scattering. For single compound solutions the JYS limit of detection is 250ppb, PARAFAC analysis decreases this to <62.5ppb (Figure 4.8).

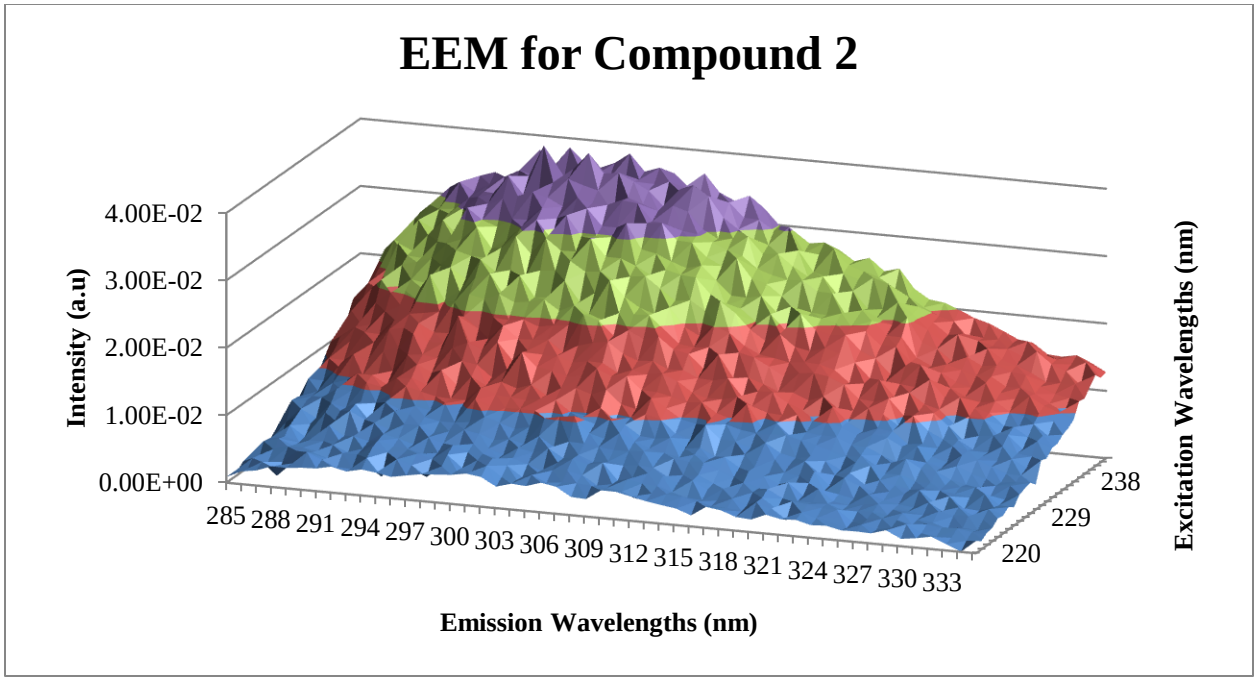

Figure 4.5: Compound 2 JYS EEM with a maximum excitation at 244 nm and maximum emission at 289 nm at a concentration of 1 ppm.

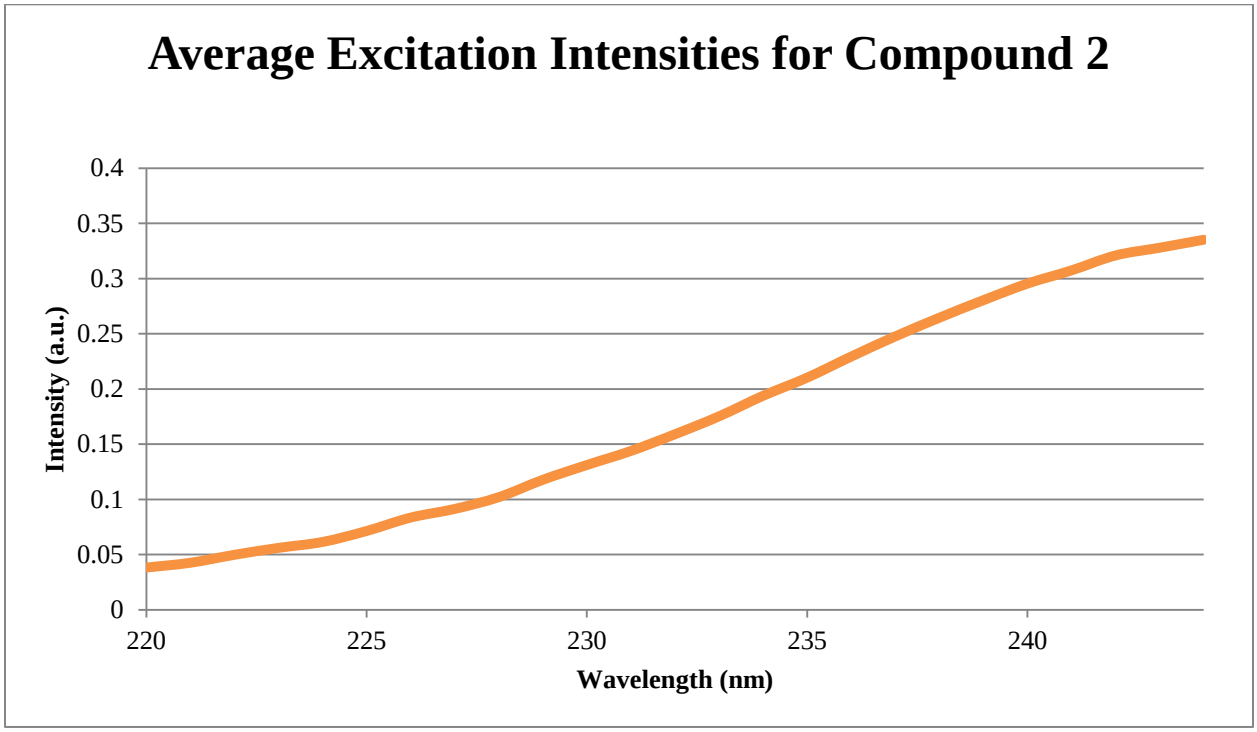

Figure 4.6: Average excitation scan of compound 2 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shape of compound 1 for any given concentration. The maximum excitation appears at 244 nm.

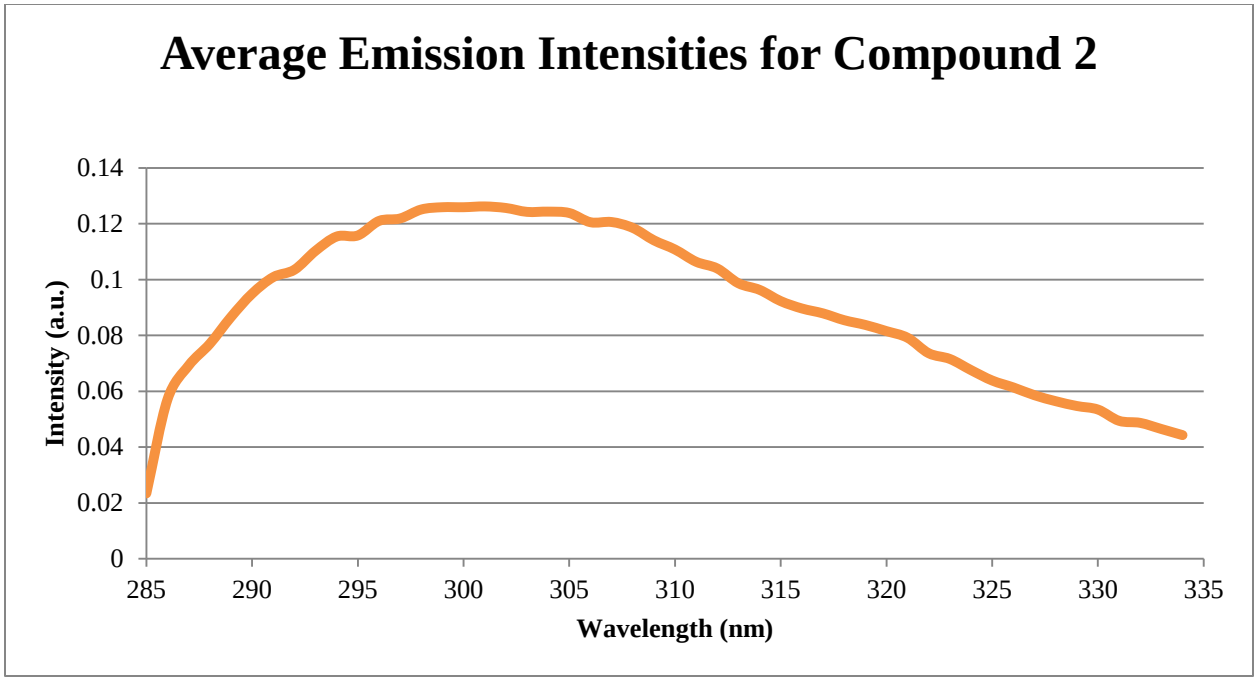

Figure 4.7: Average emission scan of compound 1 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of compound 1 for any given concentration. The maximum emission appears at 301 nm.

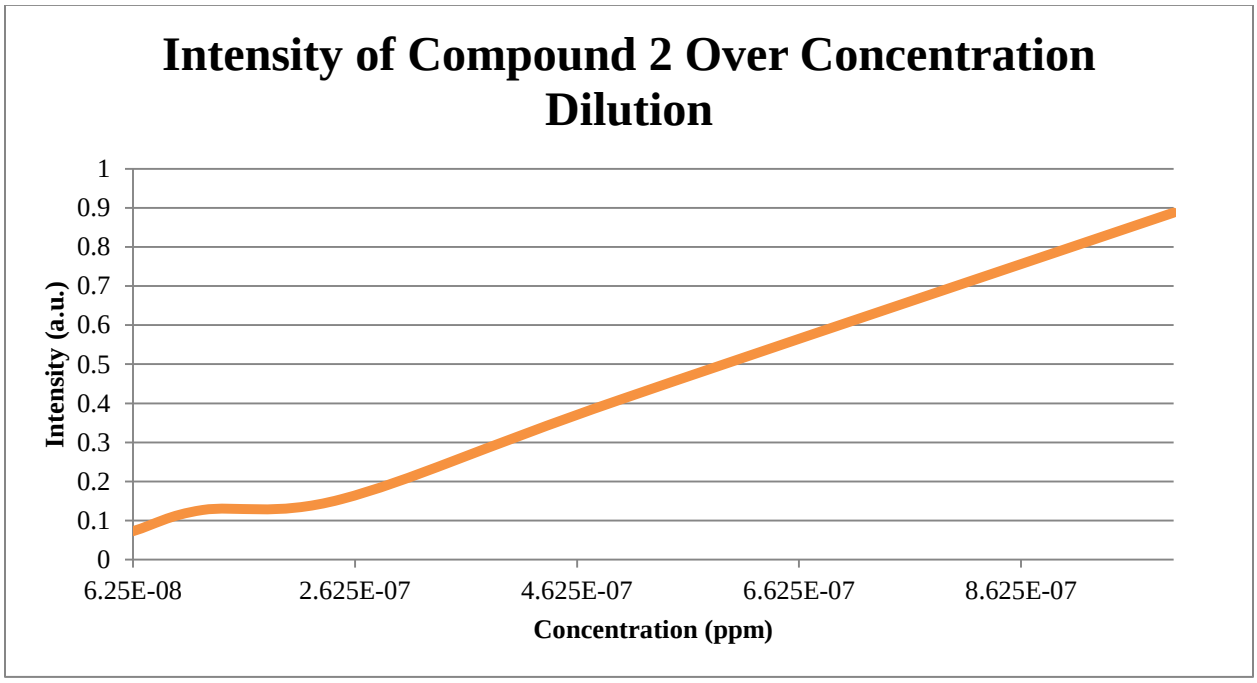

Figure 4.8: Concentration intensities for compound 2 after PARAFAC analysis showing a nearly linear relationship between intensity and concentration.

4.3 Compound 3

The excitation range of compound **3** is 240 nm to 271 nm and the emission range is 286 nm to 325 nm in Figure 4.9. The maximum peak is at excitation 262 nm (Figure 4.10) and emission 286 nm (Figure 4.11). It is the least intense with a broad excitation spectrum and sloped emission spectrum, most of which is not viewed due to Raman scattering. For single compound solutions the JYS limit of detection is 250ppb, PARAFAC analysis decreases this to <62.5ppb (Figure 4.12).

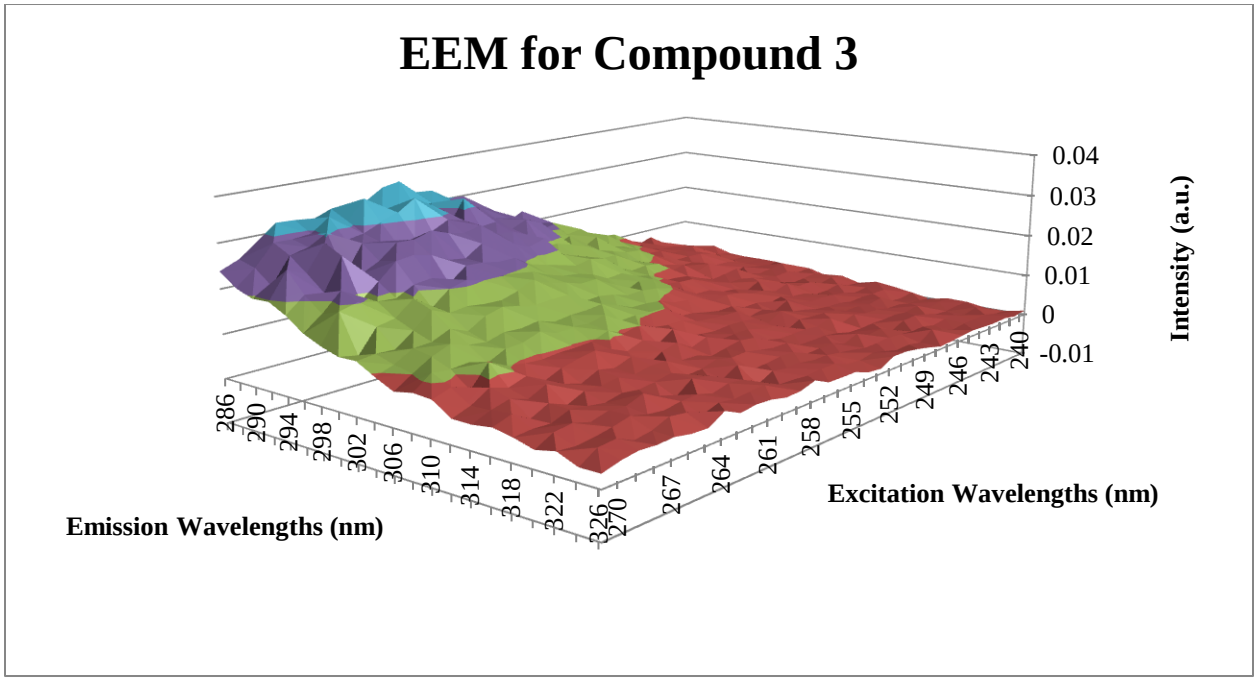

Figure 4.9: Compound 3 JYS EEM with a maximum excitation at 262 nm and maximum emission at 286 nm at a concentration of 1 ppm.

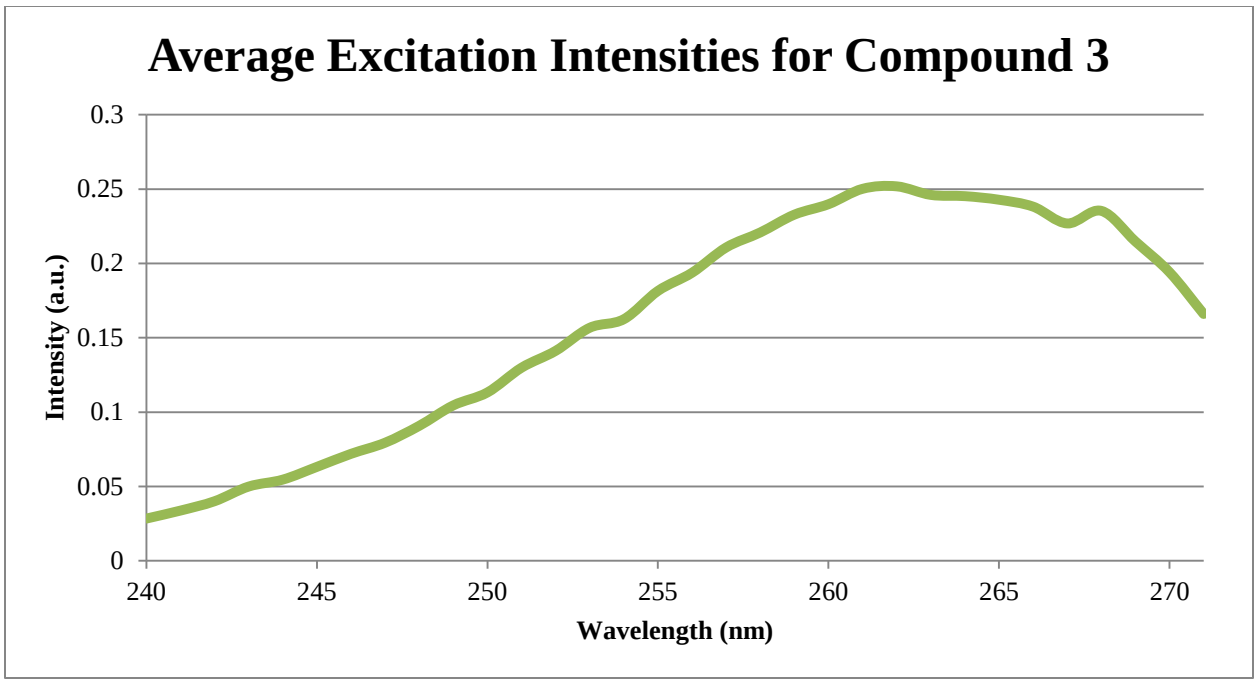

Figure 4.10: Average excitation scan of compound 3 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shape of compound 3 for any given concentration. The maximum excitation appears at 262 nm.

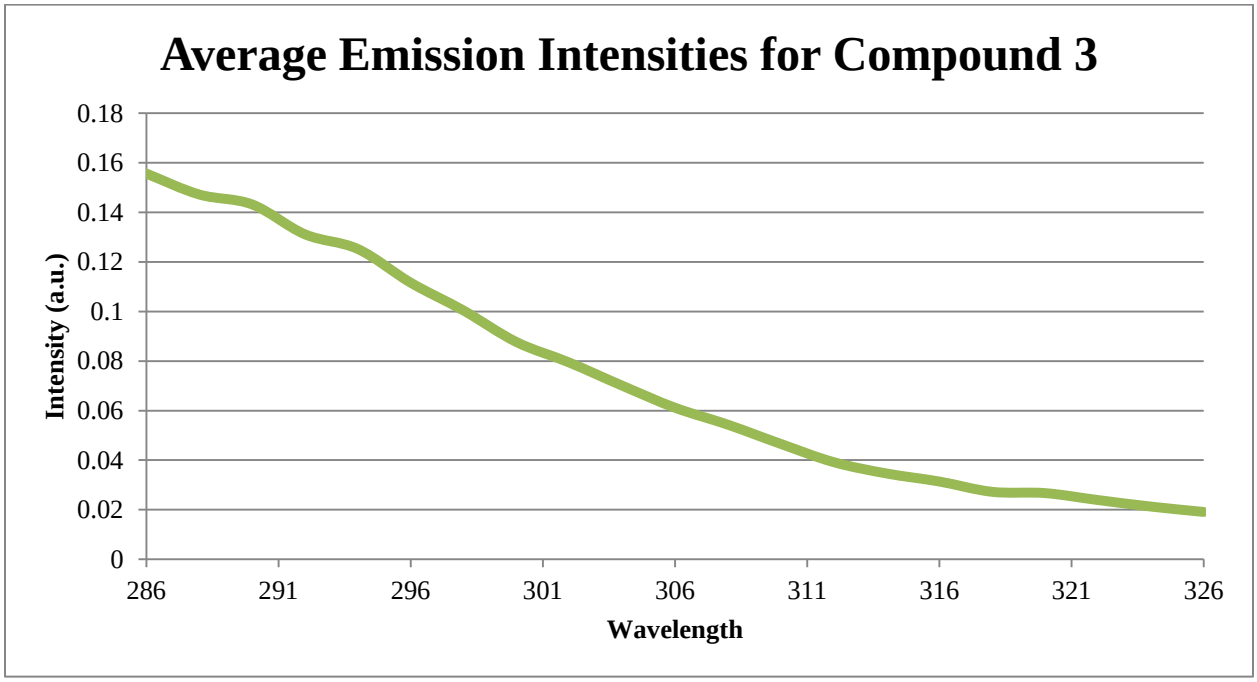

Figure 4.11: Average emission scan of compound 3 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of compound 3 for any given concentration. The maximum emission appears at 286 nm.

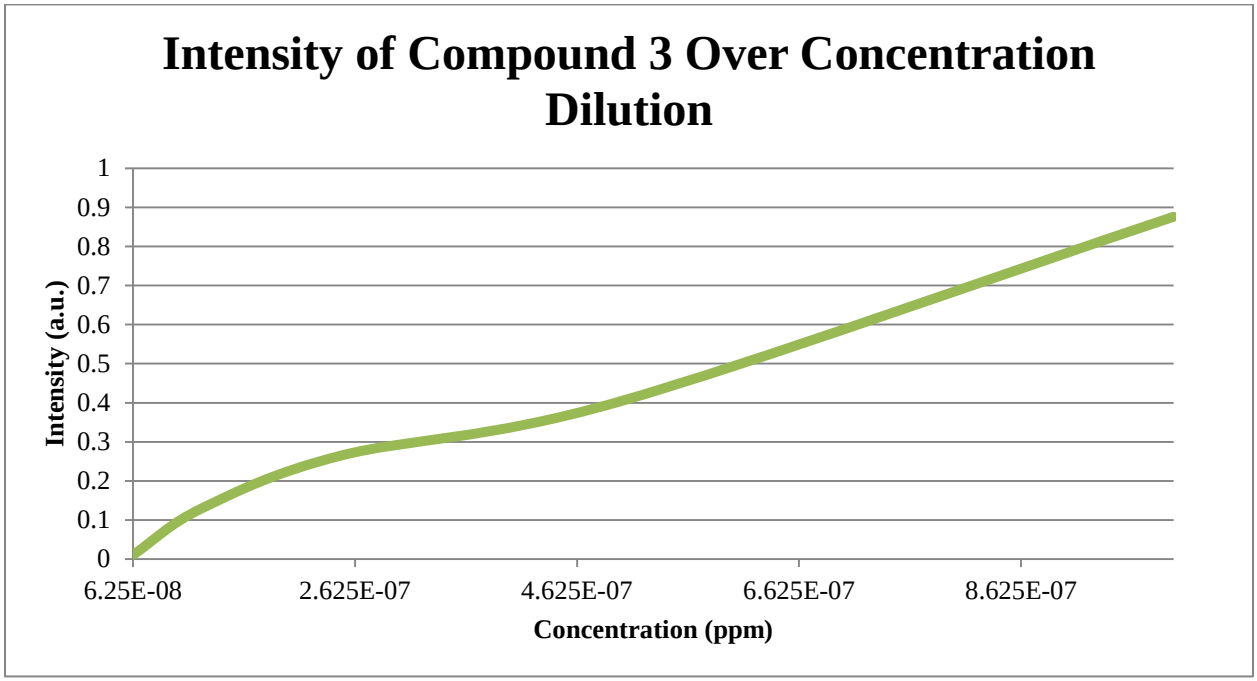

Figure 4.12: Concentration intensities for compound 3 after PARAFAC analysis showing a nearly linear relationship between intensity and concentration.

4.4 Compound 4

The excitation range of compound **4** is 280 nm to 345 nm and the emission range is 365 nm to 450 nm seen in Figure 4.13. The maximum peak is at excitation 328 nm (Figure 4.14) and emission 373 nm (Figure 4.15). It is the most intense, 300 times the intensity of the others, with a bell-curve peak shape, half of the peak is not viewed due to Raman scattering. For single compound solutions the JYS limit of detection is 250ppb, PARAFAC analysis decreases this to <62.5ppb (Figure 4.16).

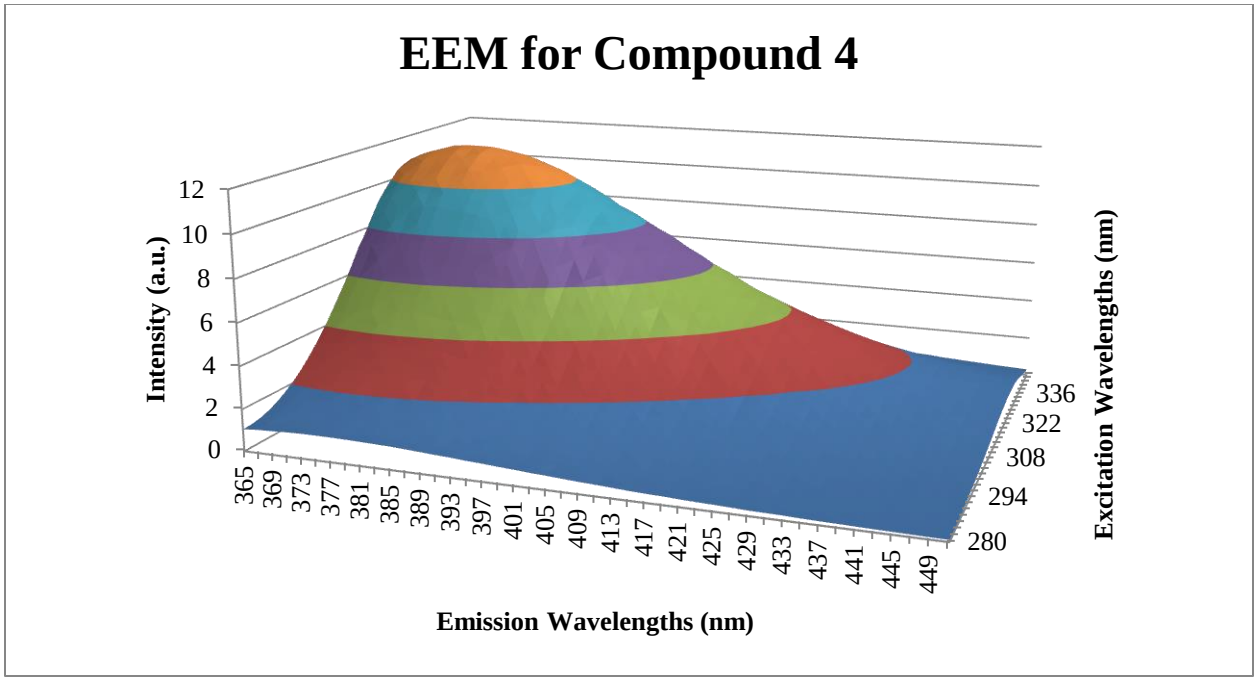

Figure 4.13: Compound 4 JYS EEM with a maximum excitation at 328 nm and maximum emission at 373 nm at a concentration of 1 ppm.

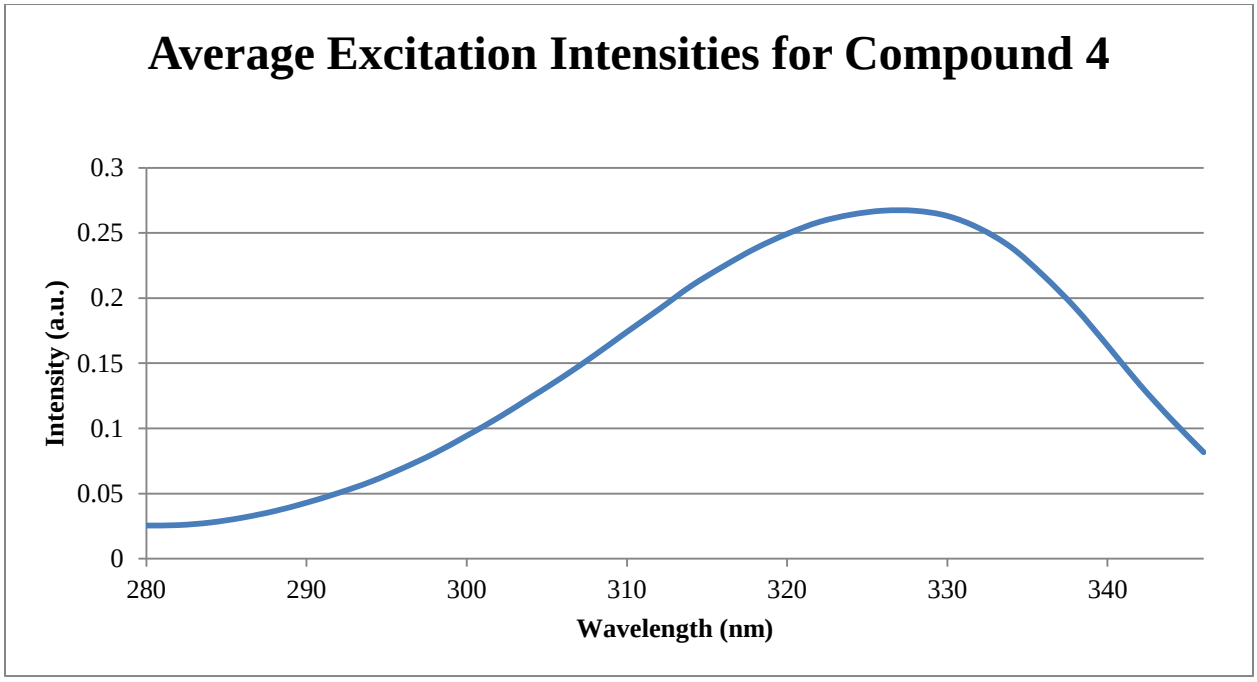

Figure 4.14: Average excitation scan of compound 4 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shape of compound 4 for any given concentration. The maximum excitation appears at 328 nm.

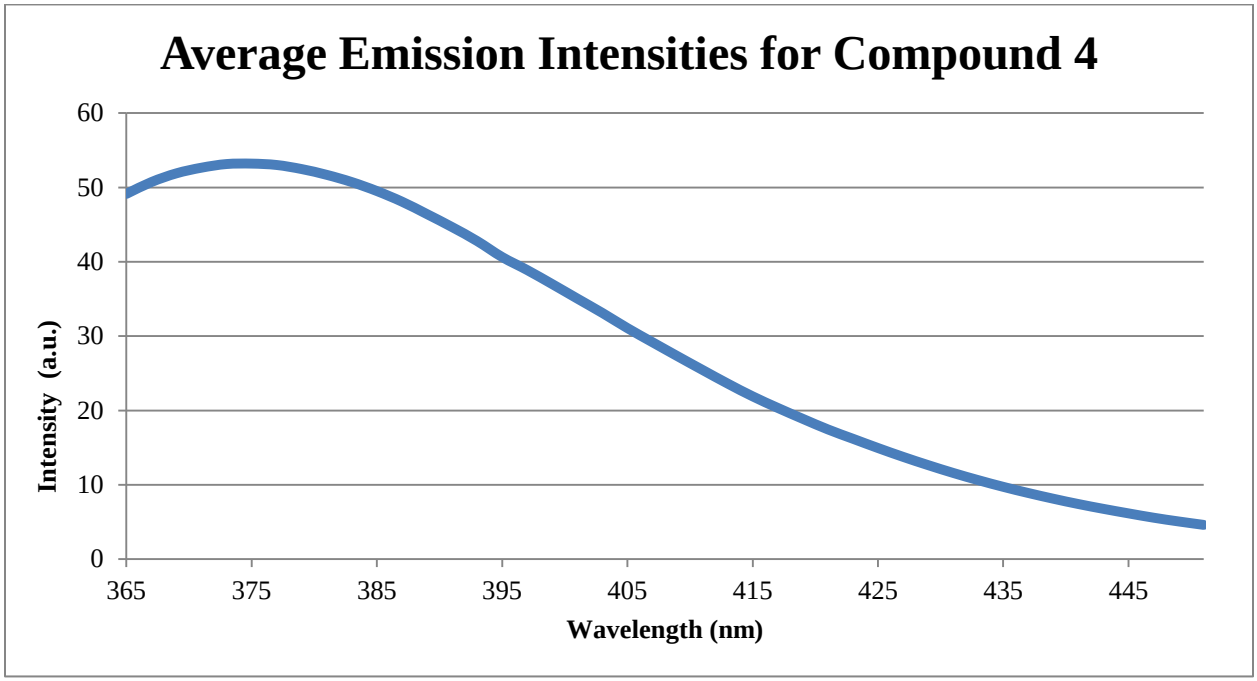

Figure 4.15: Average emission scan of compound 4 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of compound 4 for any given concentration. The maximum emission appears at 375 nm.

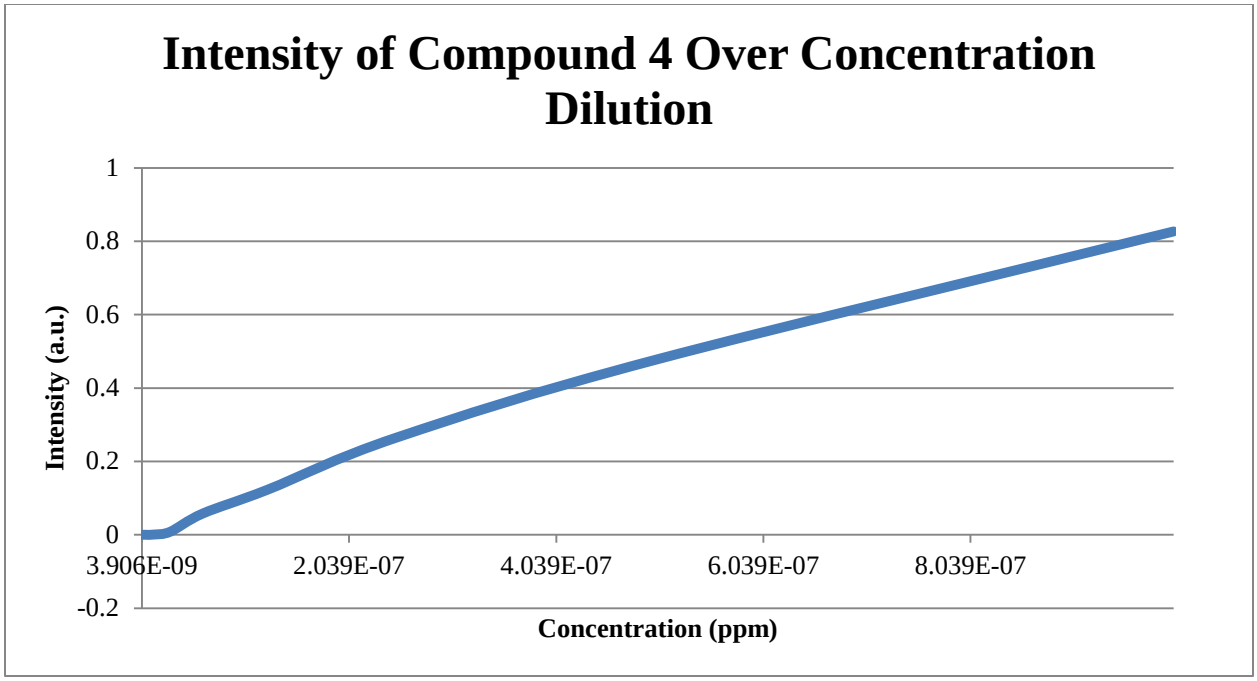

Figure 4.16: Concentration intensities for compound 4 after PARAFAC analysis showing a nearly linear relationship between intensity and concentration.

4.5 Mixture 1

PARAFAC analyzed excitation scans of mixture **1** with compound **1** parameters show a maximized peak at 312 nm for compound **1** shifted down from the standardized compound peak by 3 nm (Figure 4.17). The compound **2** excitation scan exhibited a maximized peak at 244 nm for compound **2** (Figure 4.18). The compound **1** emission scan exhibited a maximized peak at 402 nm for compound **1** (Figure 4.19). The compound **2** scan exhibited a maximized peak for compound **2** at 295 nm shifted down from the standardized compound peak by 3 nm and an exhibition of fluorescent noise for compound **1** (Figure 4.20). Some of the data had compound signals removed due to interference (Figures 4.17, 4.18, 4.19, 4.21 and 4.22).

The PARAFAC analyses for compound **1** parameter scans exhibit a trend of consistent intensity for compound **1** and shows a spike in intensity at the lowest concentration (Figure

4.21). The PARAFAC analysis of compound **2** parameter scans exhibit a linear trend for compound **2**, showing the direct relationship between intensity and concentration (Figure 4.22). For mixture **1** solutions the JYS limit of detection is 125ppb, PARAFAC analysis decreases this to less than 62.5ppb improving the detection by 50%.

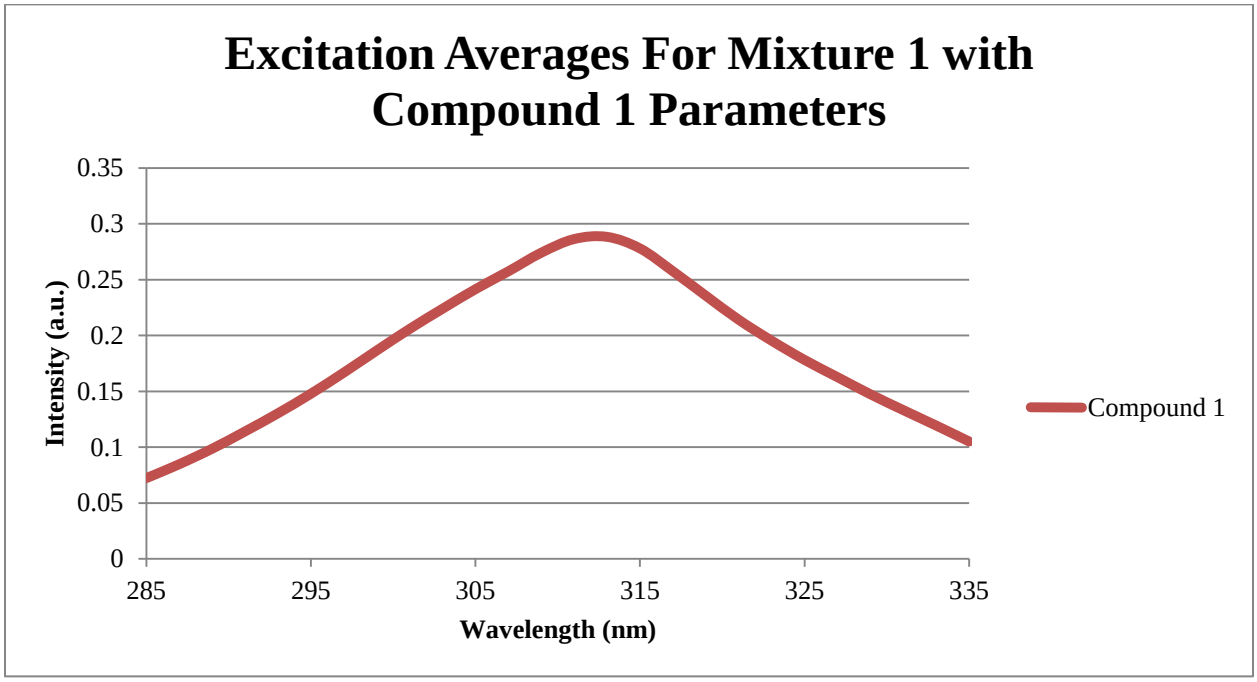

Figure 4.17 Average excitation scan with compound 1 parameters of mixture 1 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 1 for any given concentration. Maximum excitation appears at 313 nm.

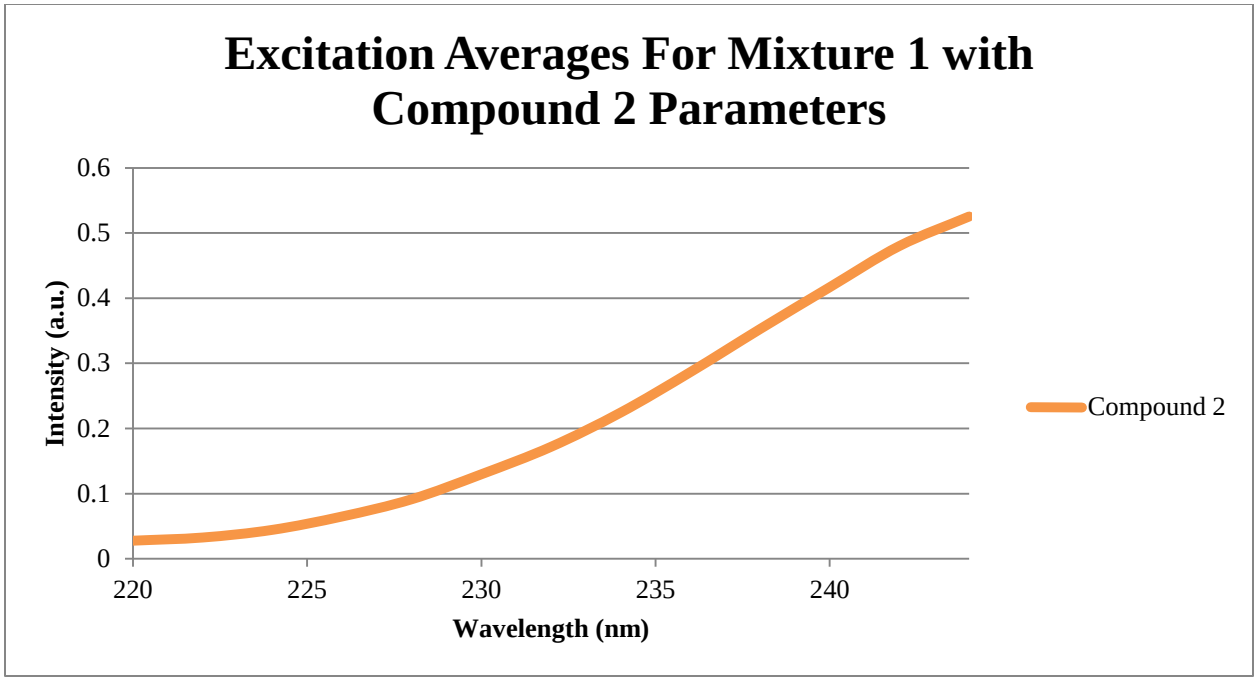

Figure 4.18: Average excitation scan with compound 1 parameters of mixture 1 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 1 with compound 2 parameters for any given concentration. Maximum excitation appears at 244 nm.

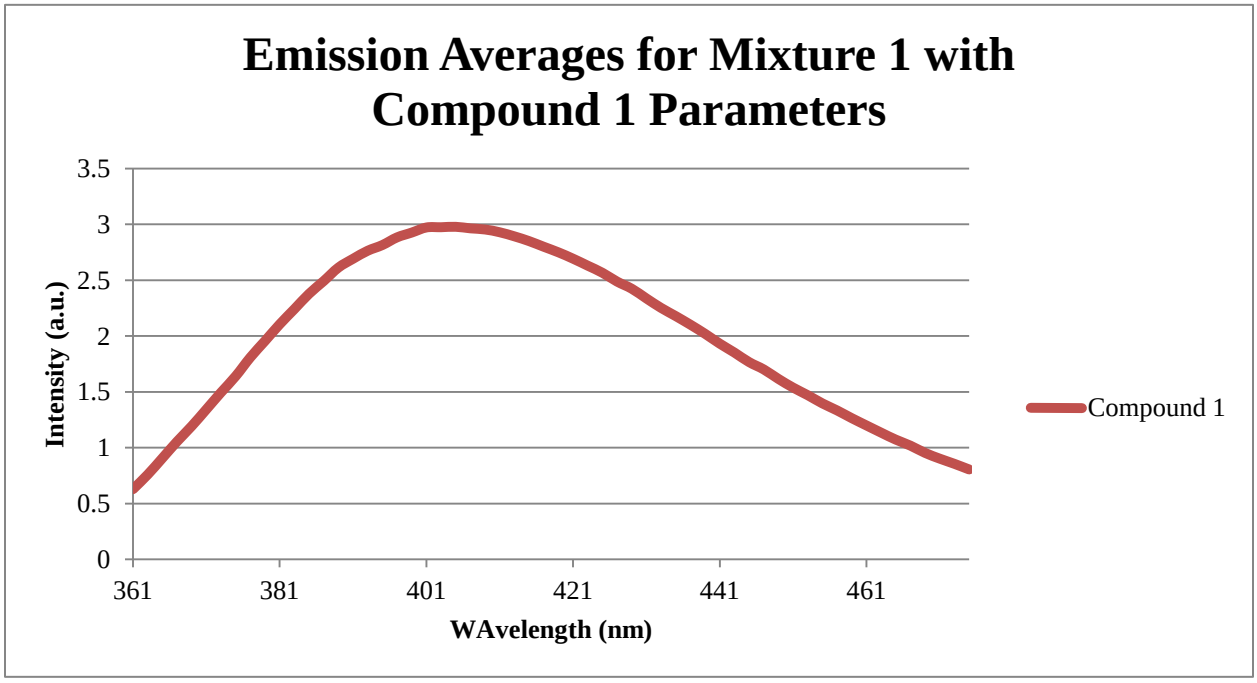

Figure 4.19: Average emission scan of Mixture 1 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 1 with compound 1 parameters for any given concentration. The maximum emissions appear at 403 nm.

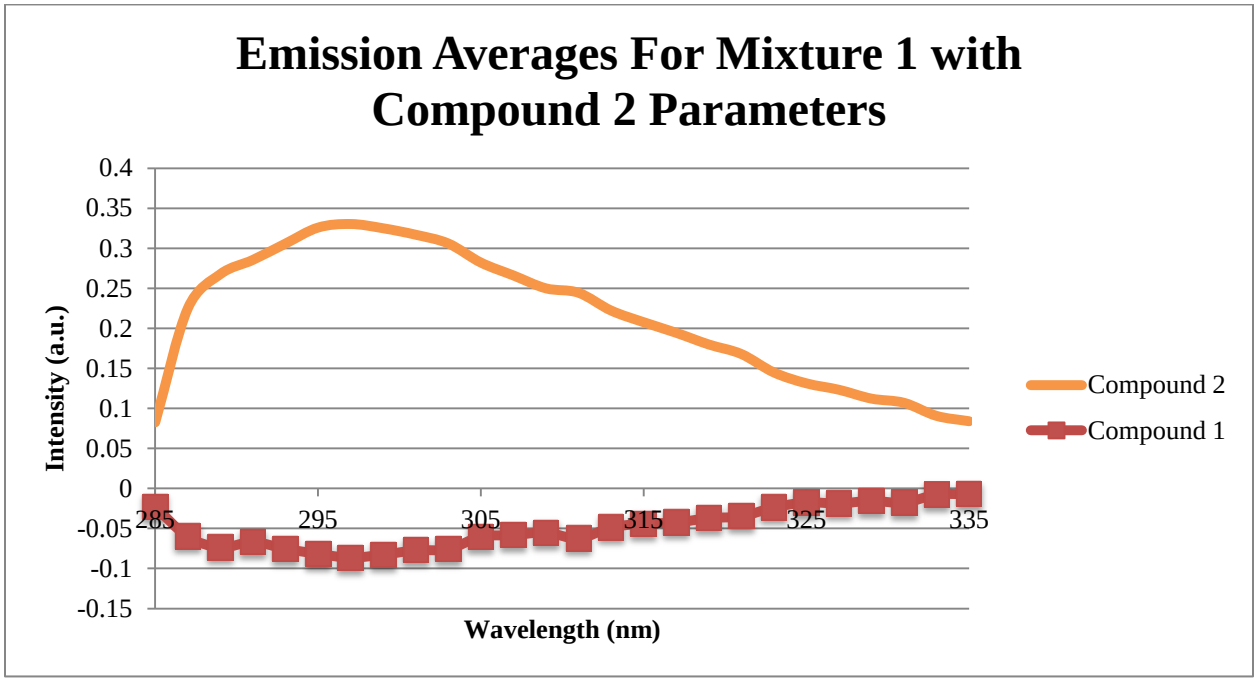

Figure 4.20:Average emission scan of Mixture 1 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 1 with compound 2 parameters for any given concentration. The maximum emission appears at 296 nm, while the other signal is background noise.

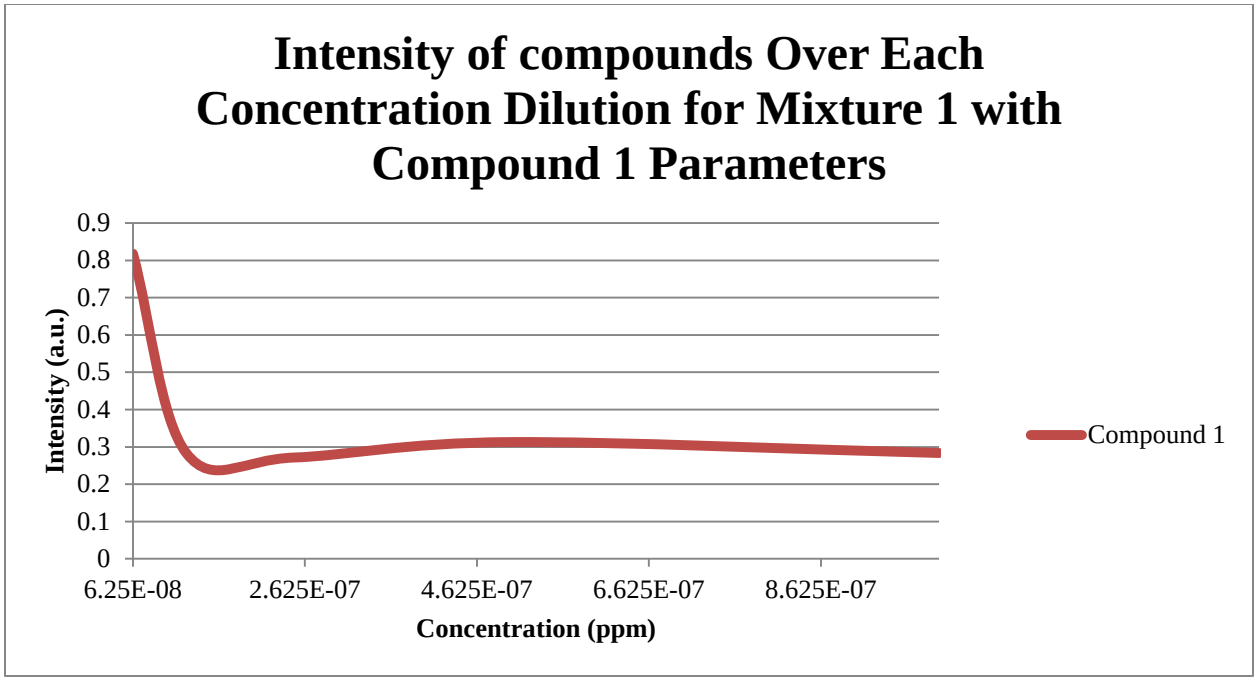

Figure 4.2: Concentration intensities for mixture 1 with compound 1 parameters after PARAFAC analysis showing a nearly linear relationship between intensity and concentration.

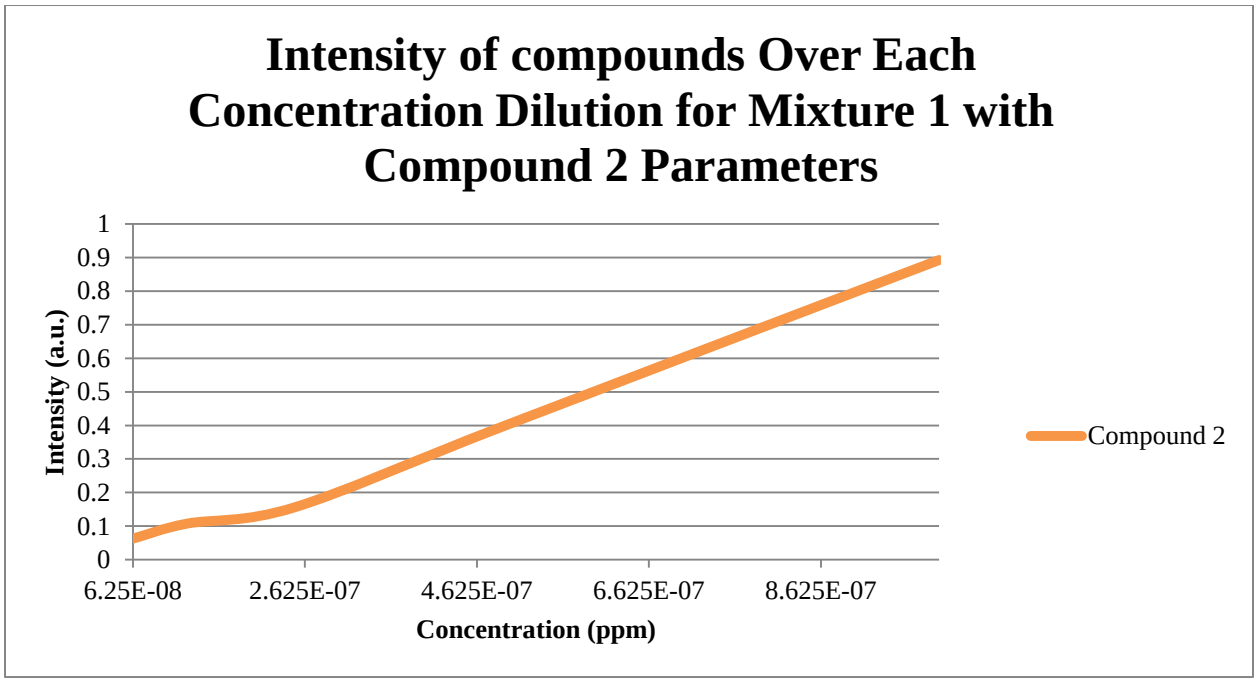

Figure 4.22: Concentration intensities for Mixture 1 with compound 2 parameters after PARAFAC analysis showing a nearly linear relationship between intensity and concentration because compound 2 was diluted in this mixture.

4.6 Mixture 2

PARAFAC analyzed excitation scans of mixture **2** with compound **1** parameters scan show a maximized peak at 313 nm for compound **1** which experienced a blue shift from the standardized peak maximum by 2 nm (Figure 4.23). The compound **2** excitation scan exhibited a maximized peak at 232 nm for compound **2** which experienced a blue shift from the standardized peak maximum by 12 nm (Figure 4.24). The compound **1** parameters emission scan exhibited maximized peak at 405 nm for compound **1** and experienced a red shift from the standardized peak maximum by 3 nm (Figure 4.25). The compound **2** emission parameters scan exhibited a maximized peak for compound **2** at 295 nm and experienced a blue shift from the standardized maximum peak by 3 nm and an exhibition of fluorescent noise for compound **1** (Figure 4.26). Some of the data had compound signals removed due to interference (Figures 4.23, 4.24, 4.25 and 4.27). Due to the nature of the PARAFAC analysis, the scan would often report information twice, this data was removed to more easily understand the output graph.

The PARAFAC analyses for compound **1** parameter scans exhibit a trend of inconsistent intensity for compound **1** and showed a sudden drop in intensity at the lowest concentration (Figure 4.27). The PARAFAC analyses for compound **2** parameter scans exhibit a fairly steady trend for compound **2** (Figure 4.28). For mixture **2** solutions the JYS limit of detection is 125ppb, PARAFAC analysis decreases this to less than 62.5ppb improving the detection by 50%.

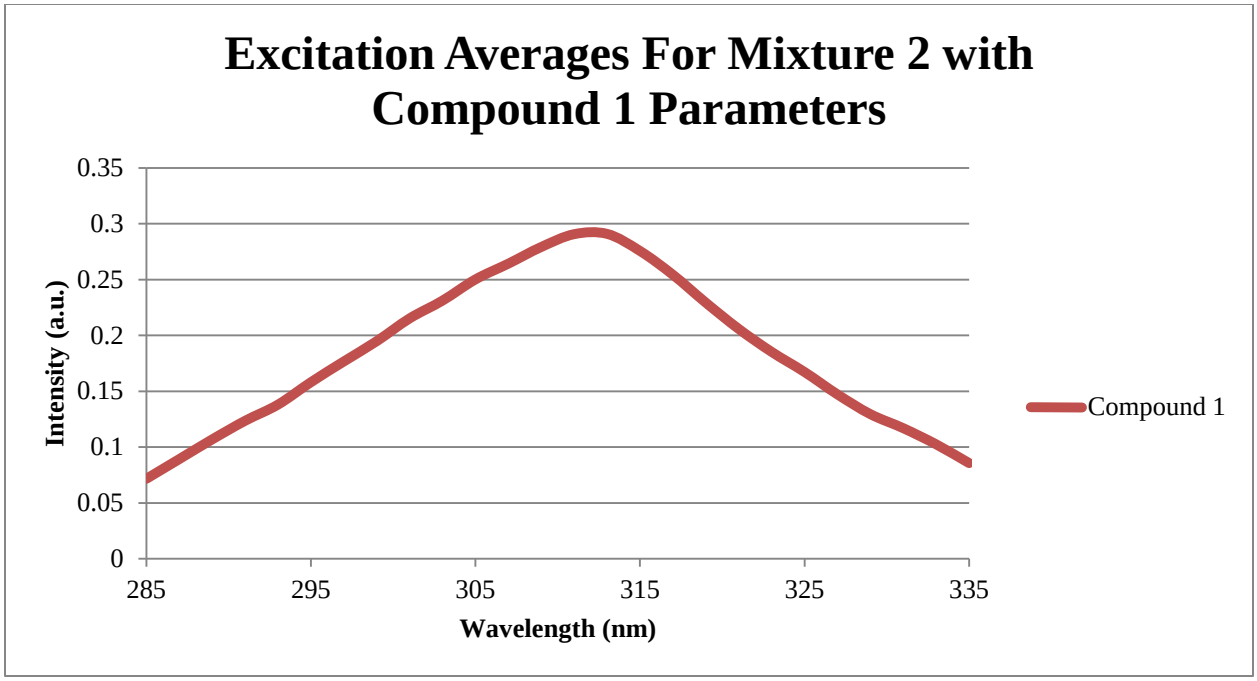

Figure 4.23: Average excitation scan with compound 1 parameters of Mixture 2 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of Mixture 2 with compound 2 parameters for any given concentration. Maximum excitation appears at 313 nm.

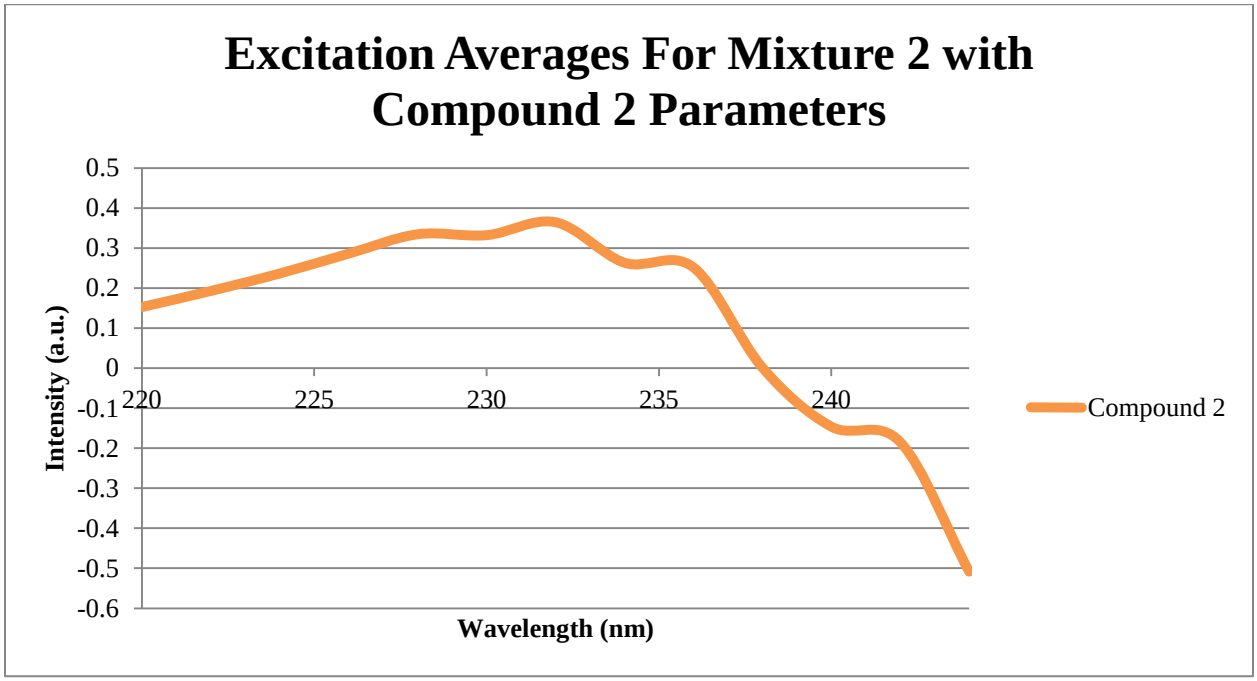

Figure 4.24: Average excitation scan with compound 2 parameters of Mixture 2 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of Mixture 2 with compound 2 parameters for any given concentration. Maximum excitation appears at 233 nm.

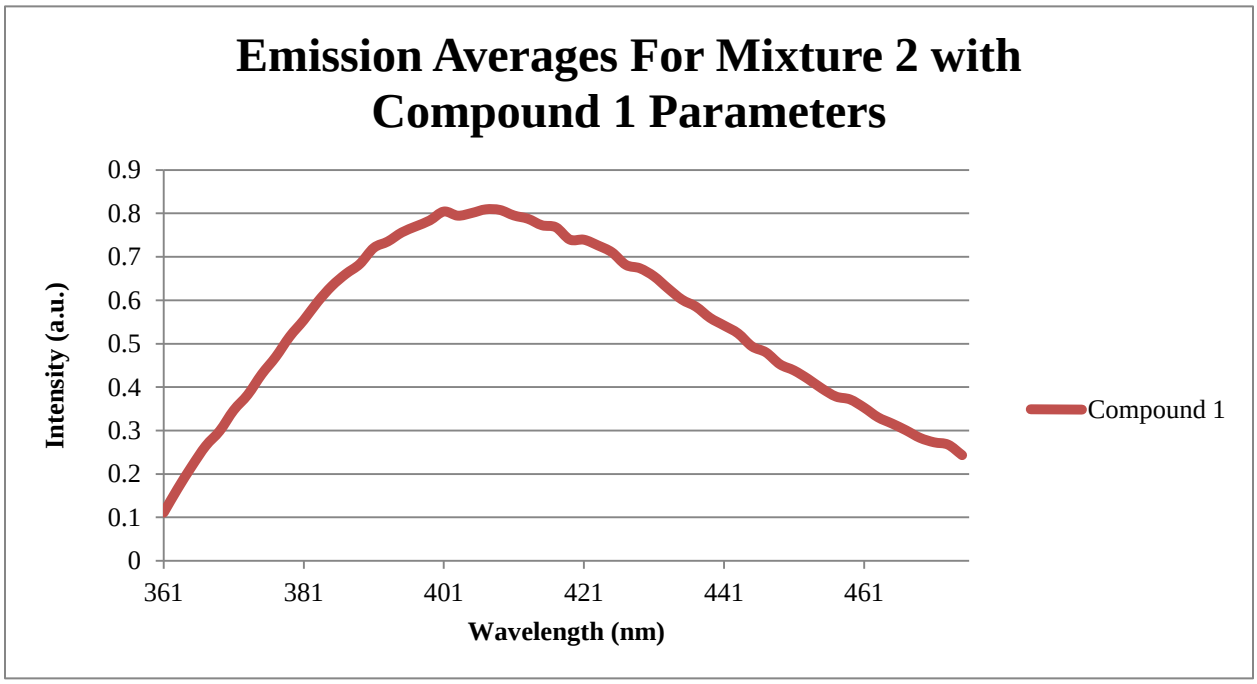

Figure 4.25: Average emission scan with compound 1 parameters of mixture 2 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 2 with compound 1 parameters for any given concentration. The maximum emission appears at 405 nm.

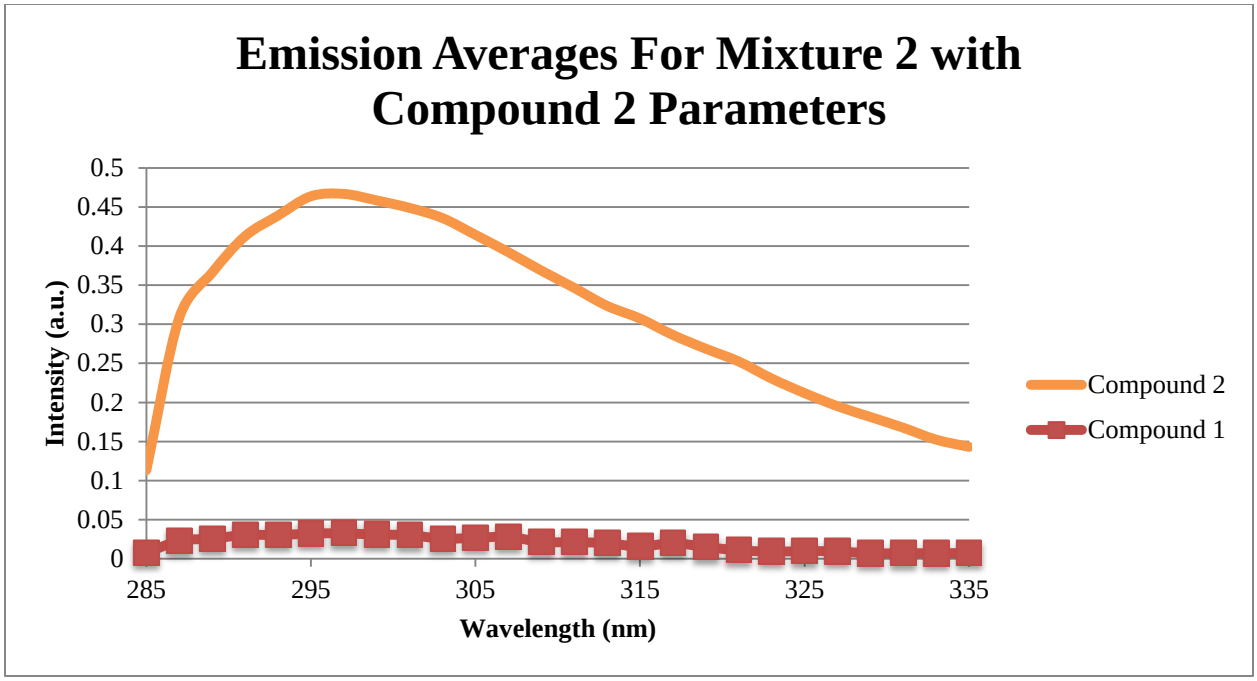

Figure 4.26: Average emission scan with compound 2 parameters of mixture 2 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 2 with compound 1 parameters for any given concentration. The maximum emission appears at 295 nm while the other signal is background noise.

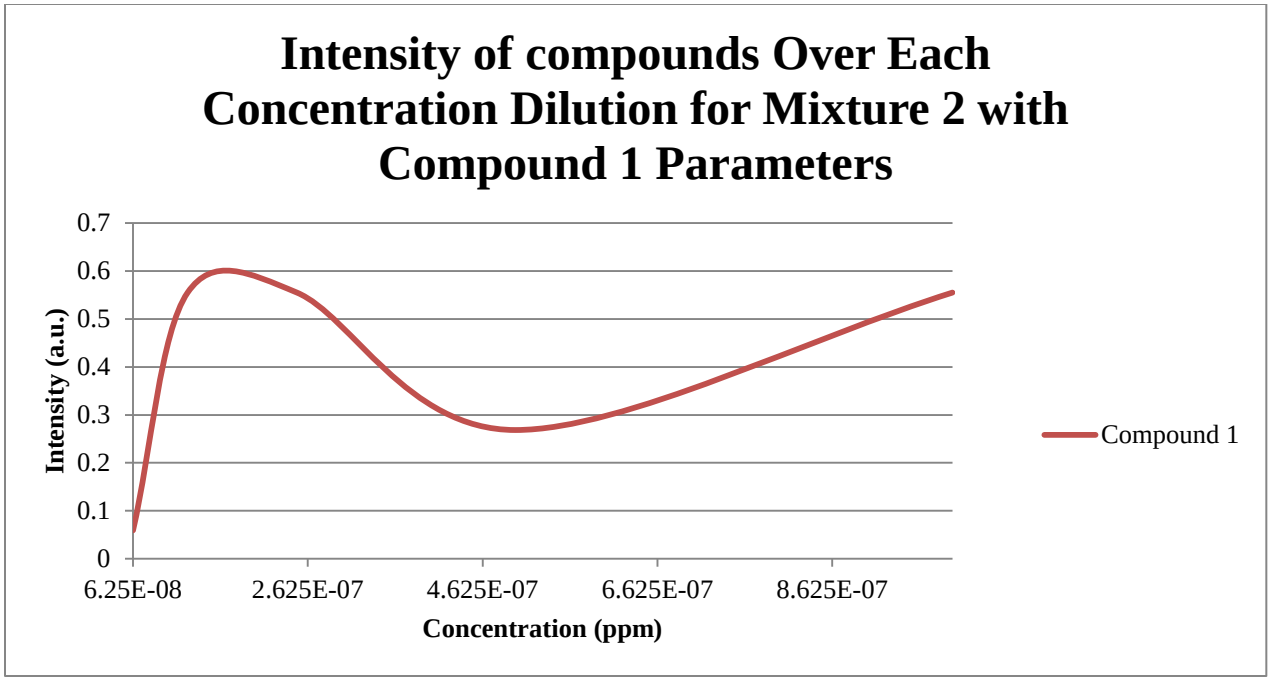

Figure 4.27: Concentration intensities for mixture 2 with compound 1 parameters after PARAFAC analysis showing a non-trending relationship between intensity and concentration.

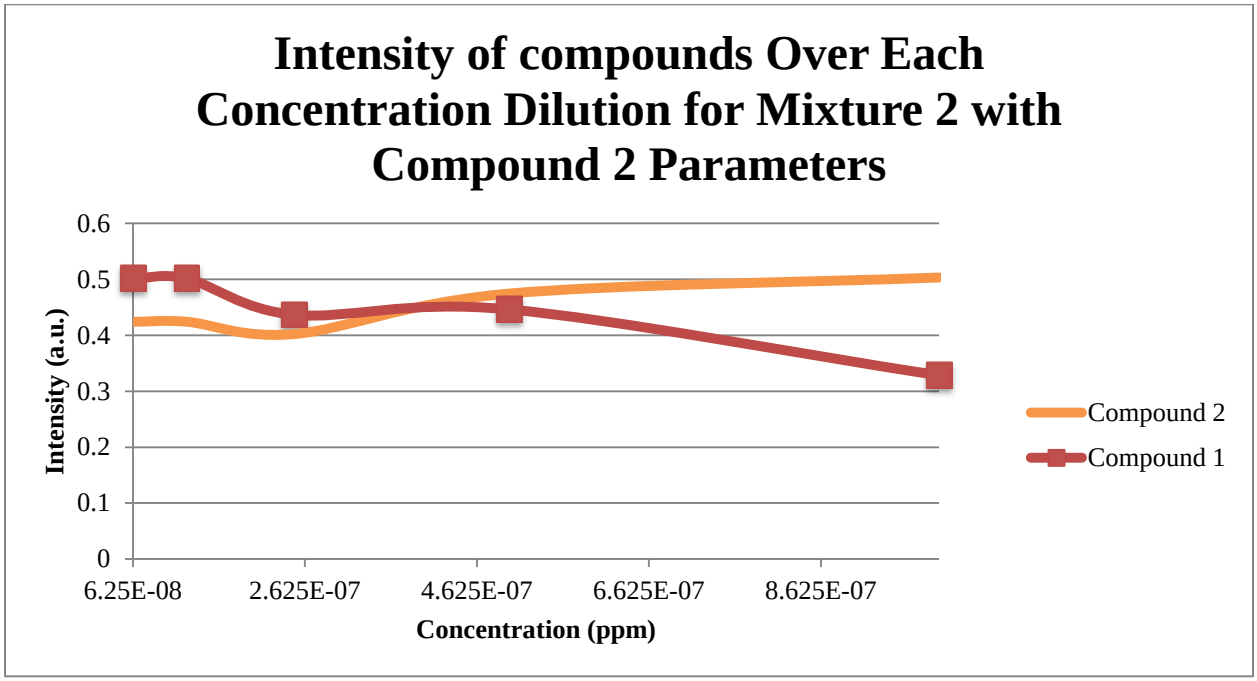

Figure 4.28: Concentration intensities for Mixture 2 with compound 2 parameters after PARAFAC analysis showing a nearly linear trend between intensity and concentration with compound 2 held at a steady concentration.

4.7 Mixture 3

PARAFAC analyzed excitation scans of mixture **3** exhibited a maximum peak at 312 nm for compound **1** which experienced a blue shift from the standardized compound peak by 3 nm and maximum peak of 328 nm for compound **4** (Figure 4.29). The emission scans exhibited a maximum peak of 402 nm for compound **1** and a maximized peak of 373nm for compound **4** (Figure 4.30). The PARAFAC analysis showed a direct relationship between concentration and intensity through compound **4**. The scan also showed a consistent trend for compound **1** with a slight increase in intensity as the concentration of compound **4** diminished indicating a mild inverse relationship between the intensities of each compound (Figure 4.31). For mixture **3** solutions the JYS limit of detection is 6.25ppb, PARAFAC analysis decreased this to less than 62.5 ppt improving the detection by 99.9%.

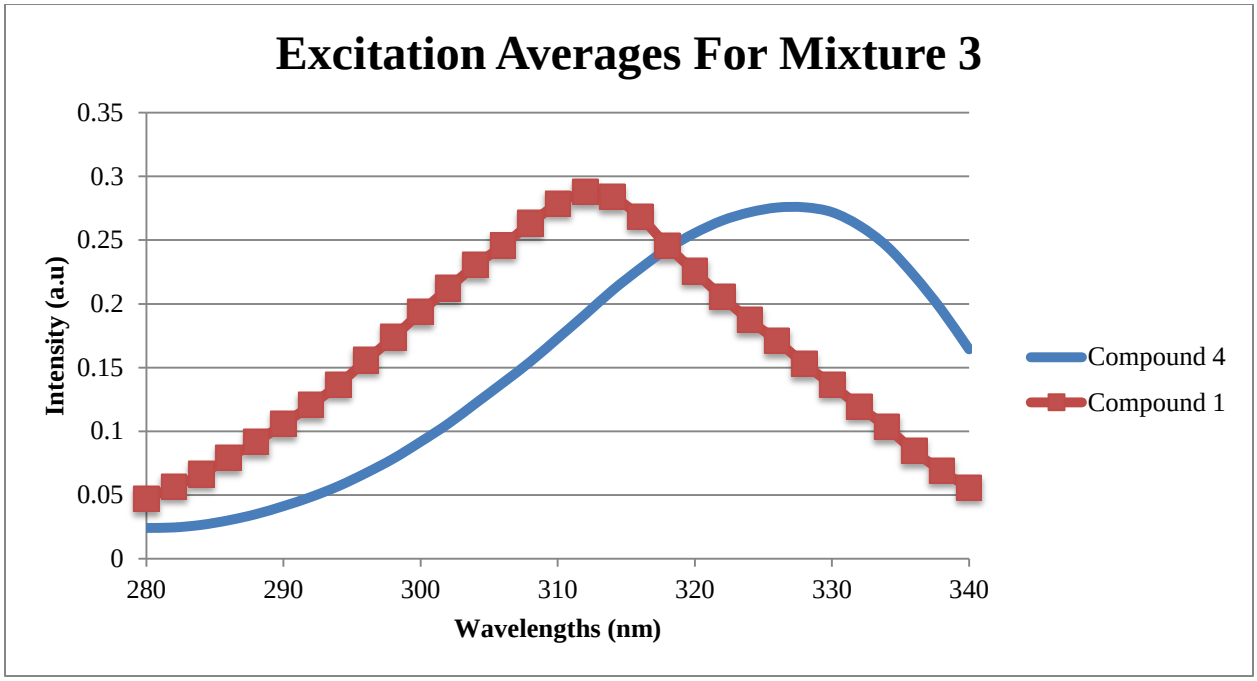

Figure 4.29: Average excitation scan of mixture 3 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 3 for any given concentration. Maximum excitations appear at 312 nm and 328 nm.

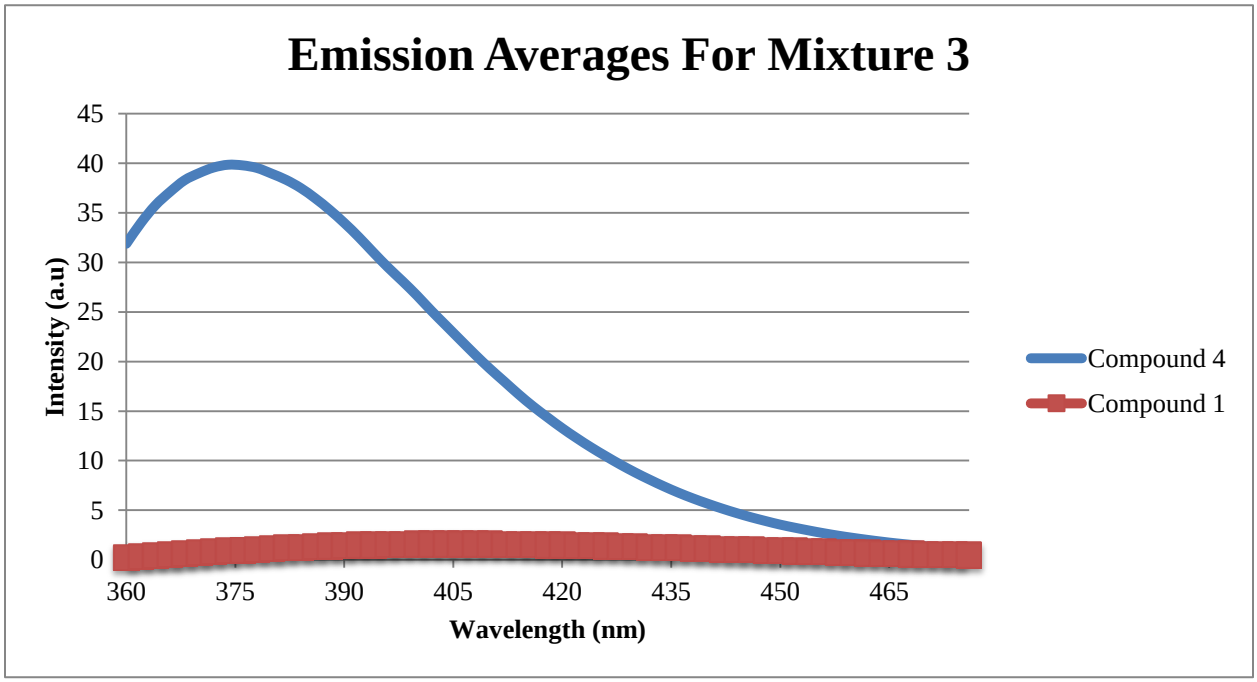

Figure 4.30: Average emission scan of mixture 3 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 3 for any given concentration. The maximum emissions appear at 374 nm and 406 nm.

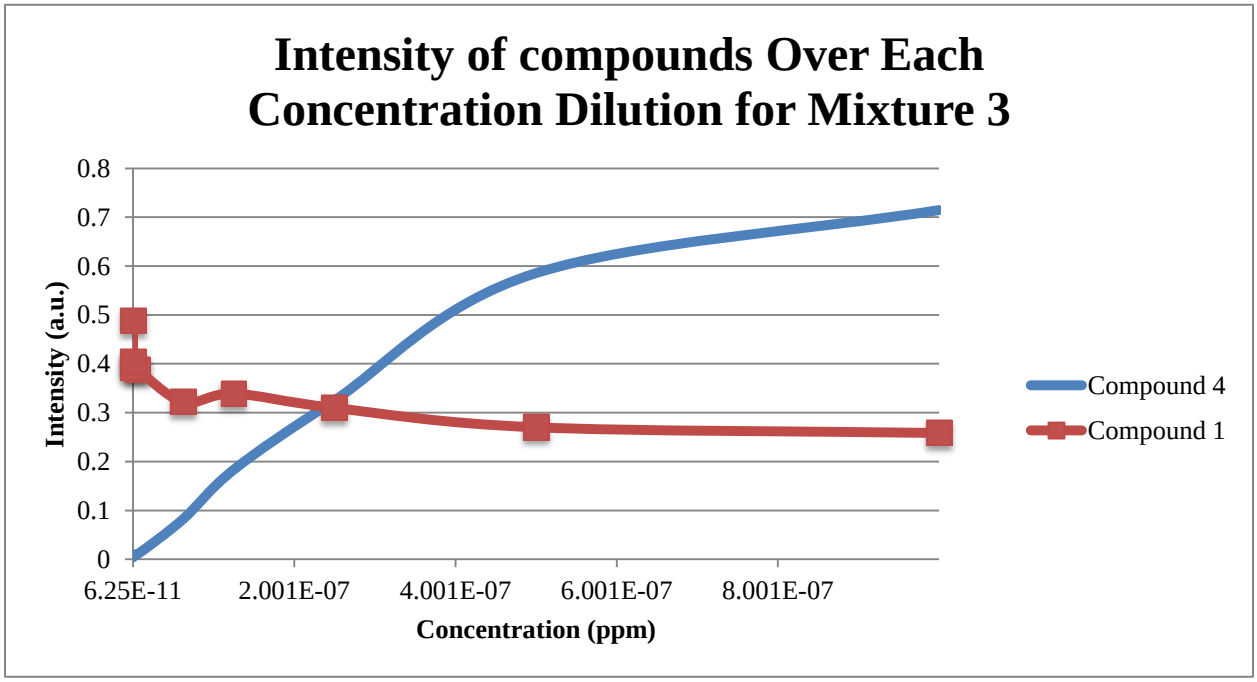

Figure 4.31: Concentration intensities for mixture 3 after PARAFAC analysis showing a nearly linear trend between intensity and concentration with compound 1 held at a steady concentration and compound 4 logarithmically diluted.

4.8 Mixture 4

The excitation scan exhibited a blue shifted maximum peak at 308 nm for compound **1** and a maximum peak of 328 nm for compound **4** and an unprecedented peak at 338 nm (Figure 4.32). The emission scan for compound **1** contained a maximum peak at 402 nm and compound **4** at 373 nm (Figure 4.33). The PARAFAC analysis showed a direct relationship between concentration and intensity through the trend line of compound **1**. The consistent compound **4** trend line showed a slight increase in intensity as the concentration of compound **1** diminished

indicating a mild inverse relationship between the intensities of the compounds (Figure 4.34). For mixture **4** solutions the JYS limit of detection is 1 ppm, PARAFAC analysis decreased this to less than 62.5ppb improving detection by 93.75%.

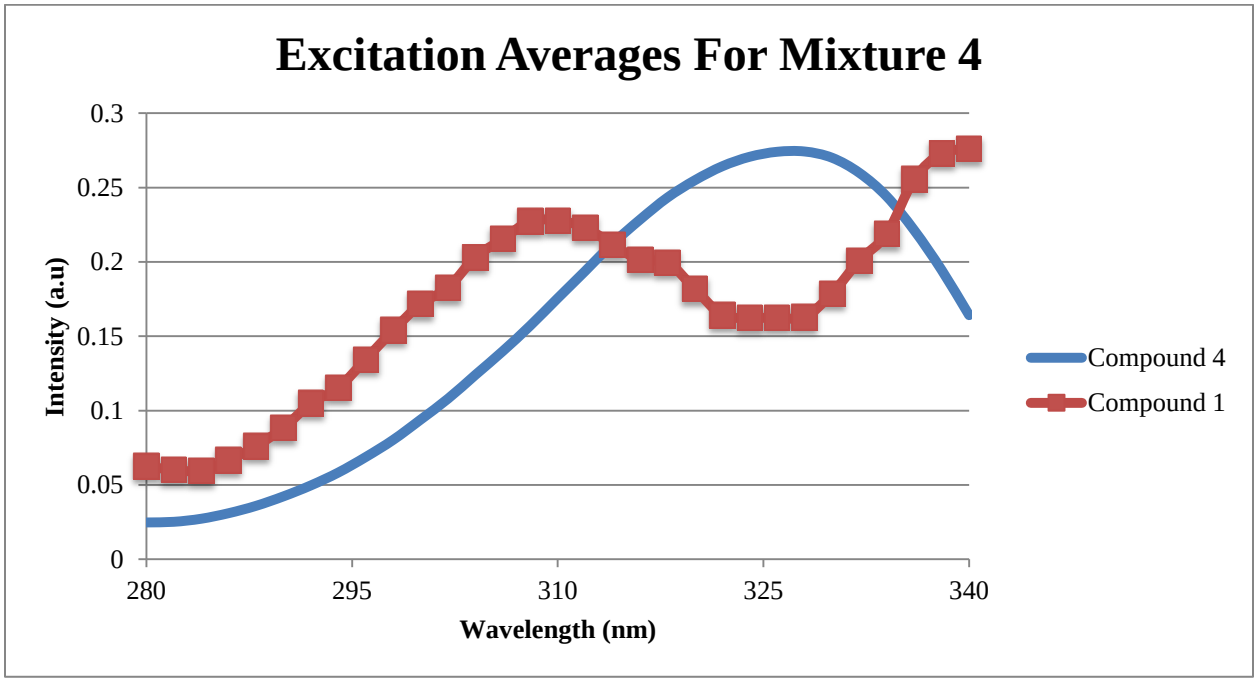

Figure 4.32: Average excitation scan of mixture 4 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of Mixture 3 for any given concentration. Maximum excitations appear at 340 nm and 328 nm.

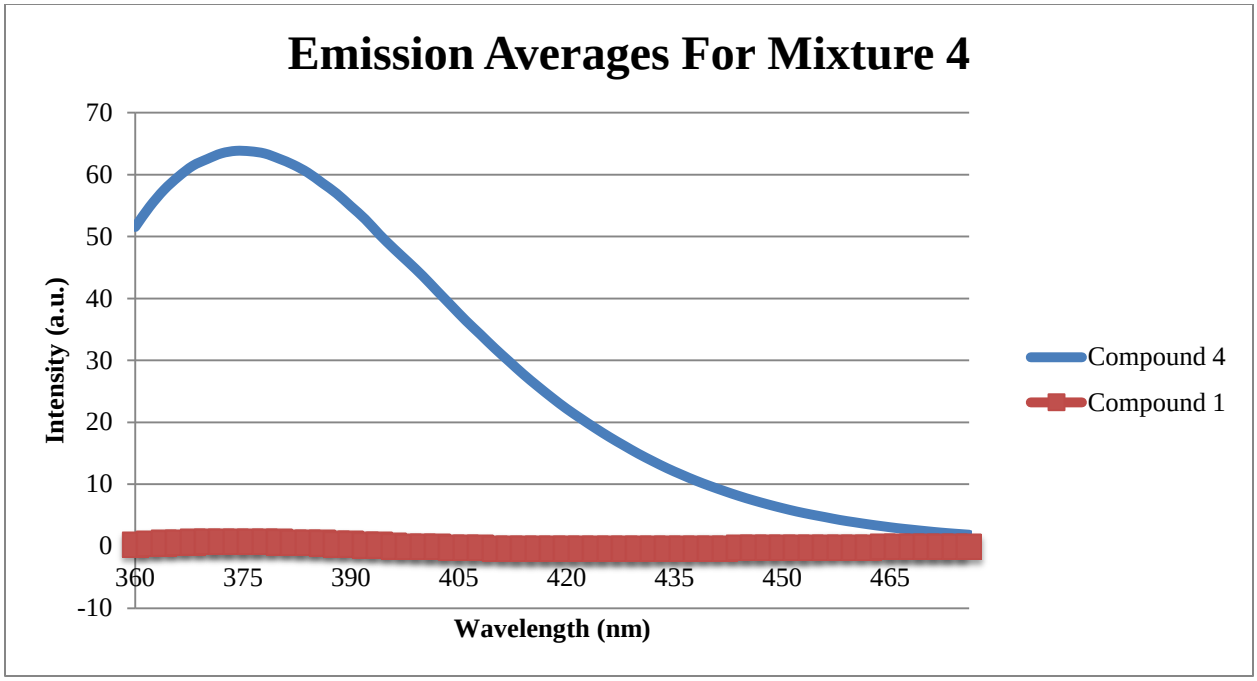

Figure 4.33: Average emission scan of Mixture 4 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 4 for any given concentration. The maximum emissions appear at 374 nm and 374 nm.

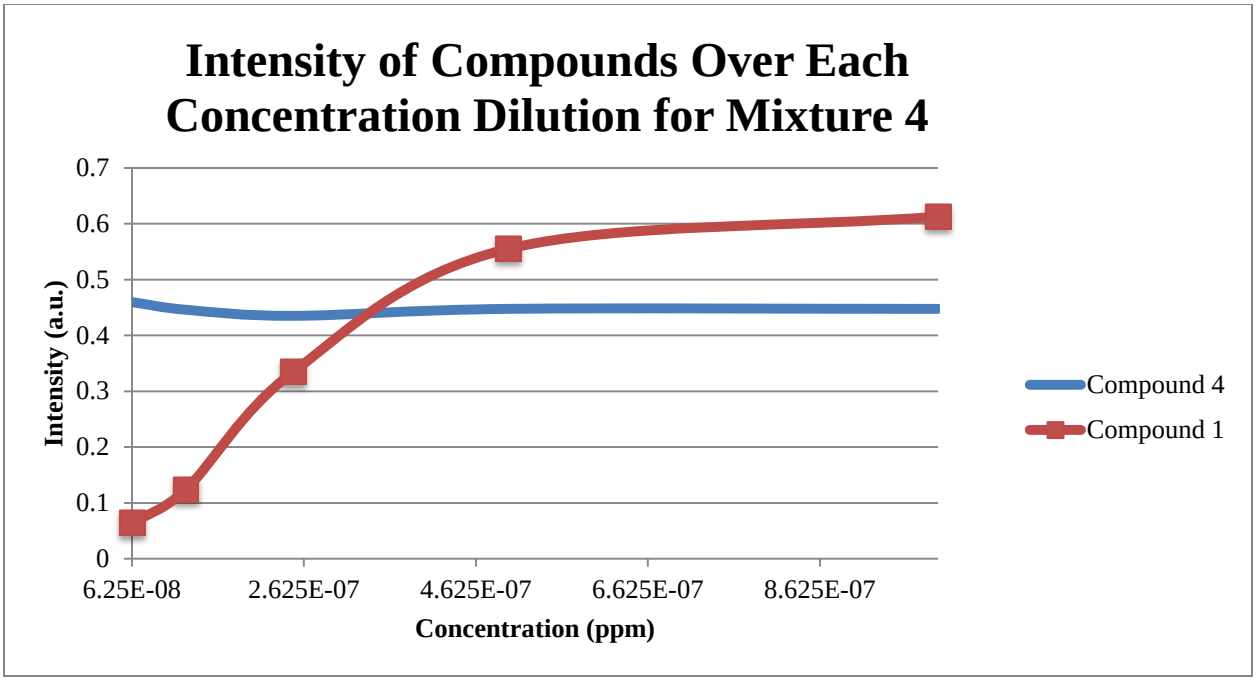

Figure 4.34: Concentration intensities for mixture 4 after PARAFAC analysis showing a linear trend between intensity and concentration with Compound 4 held at a steady concentration and Compound 1 logarithmically diluted.

4.9 Mixture 5

The PARAFAC analyzed excitation scan of mixture **5** show a maximum peak of 304 nm for compound **1** which experienced a blue shift from the standardized compound peak by 11 nm, an maximum peak of 286 nm of compound **3** and an unprecedented "interaction" peak at 235 nm (Figure 4.35). The emission scan showed a maximum peak at 407 nm for compound **1** and a maximum peak at 320 for compound **3** (Figure 4.36). The PARAFAC analysis shows a linear trend for compound **3** indicating a direct relationship between concentration and intensity. Compound **1** showed a consistent trend with a slight increase in intensity as the concentration of compound **3** diminished indicating a mild inverse relationship between the intensities of the

compounds (Figure 4.37). For mixture **5** solutions the JYS limit of detection is 250ppb, PARAFAC analysis decreased this to less than 62.5ppb improving detection by 75%.

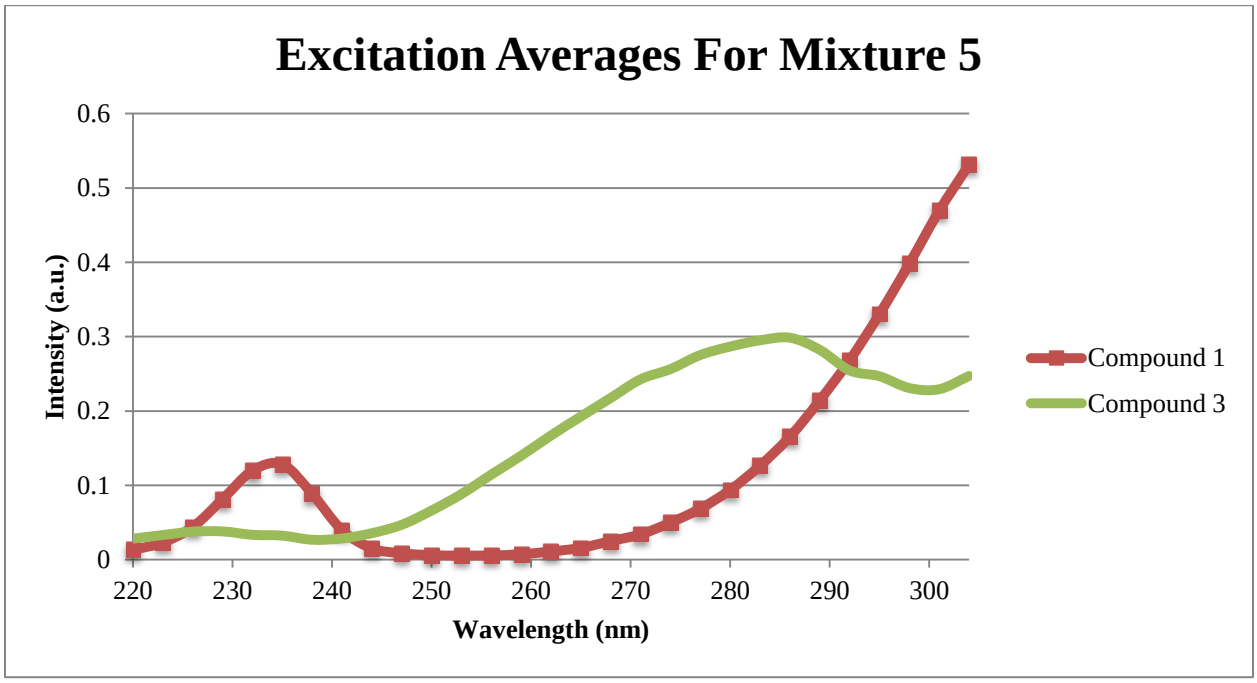

Figure 4.35: Average excitation scan of mixture 5 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 5 for any given concentration. Maximum excitations appear at 304 nm and 286 nm.

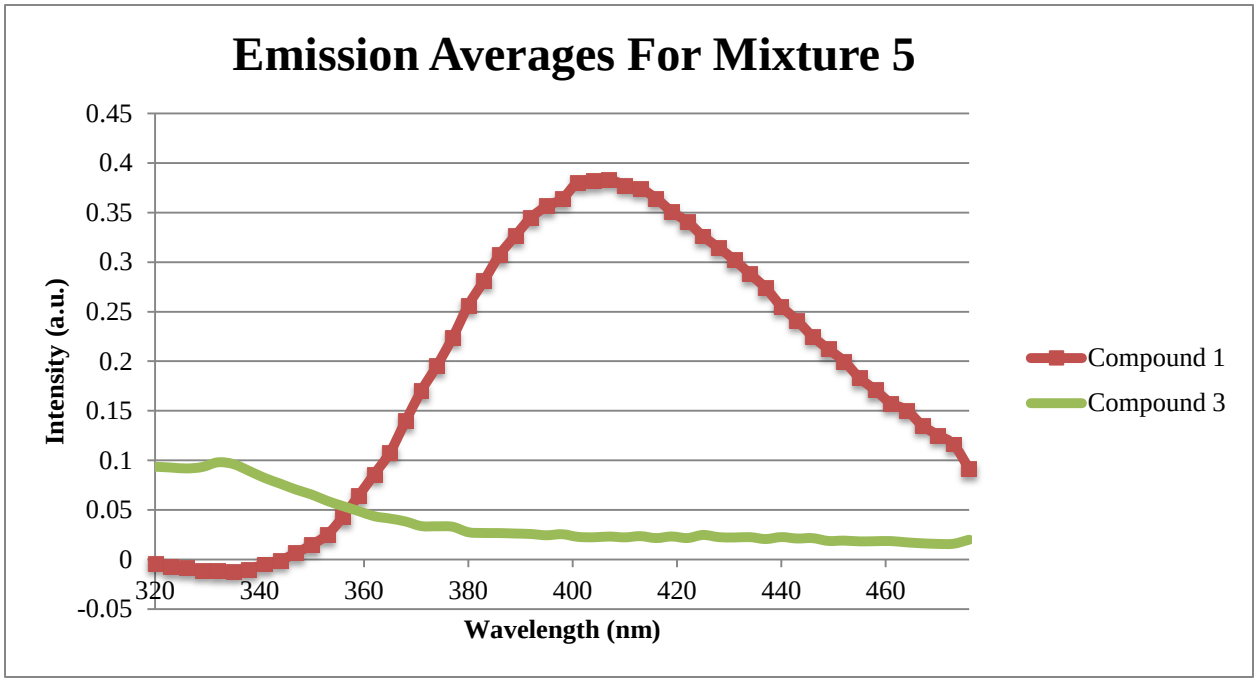

Figure 4.36: Average emission scan of mixture 5 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 5 for any given concentration. The maximum emissions appear at 402 nm and 320 nm.

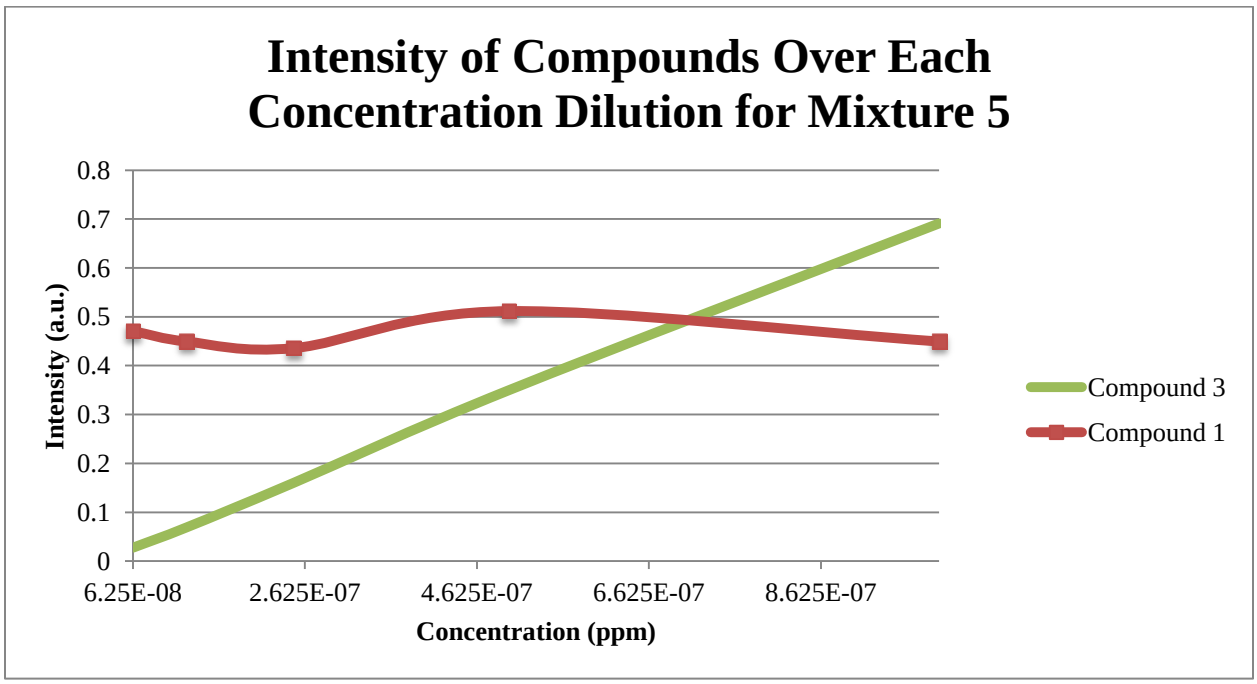

Figure 4.37: Concentration intensities for mixture 5 after PARAFAC analysis showing a linear trend between intensity and concentration with Compound 1 held at a steady concentration and Compound 3 logarithmically diluted.

4.10 Mixture 6

The PARAFAC analyzed excitation scan exhibited a maximum peak at 289 nm for compound **1** which experienced a blue shift from standardized compound peak by 26 nm, a maximum peak of 277 nm for compound **3** which experienced a red shift from the standardized compound peak by 15 nm and indication of the "interaction" peak (previously seen in mixture 5) at 240 nm (Figure 4.38). The emission scan showed a maximum peak at 401 nm for compound **1** and a maximum peak of at 326 nm for compound **3** which experienced a red shift from the standardized peak by 40 nm (Figure 4.39). The PARAFAC analysis shows a very clear linear trend for compound **1** indicating a direct relationship between concentration and intensity. Compound **3** showed a consistent trend with a slight increase in intensity as the concentration of compound **1** diminished indicating a mild inverse relationship between the intensities of the compounds (Figure 4.40). For mixture **6** solutions the JYS limit of detection is 125ppb, PARAFAC analysis decreased this to less than 62.5ppb improving detection by 50%.

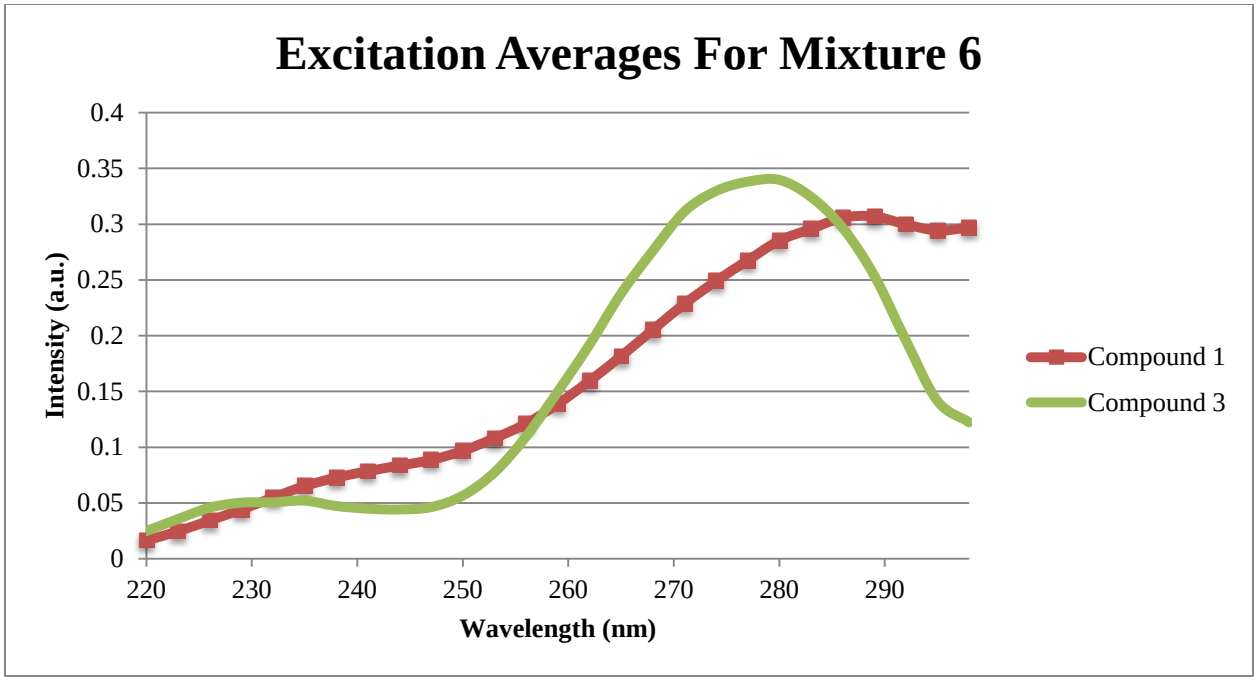

Figure 4.38: Average excitation scan of mixture 6 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 6 for any given concentration. Maximum excitations appear at 277 nm and 286 nm.

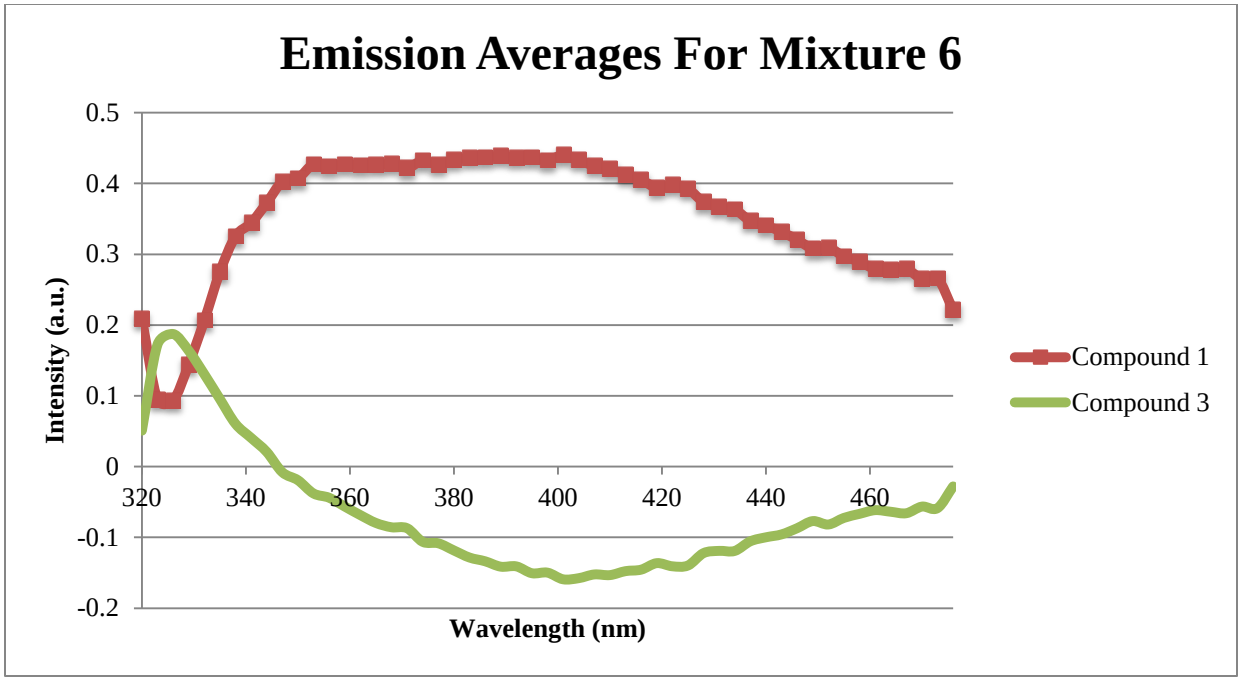

Figure 4.39: Average emission scan of mixture 6 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 6 for any given concentration. The maximum emission appears at 401 nm and 326 nm.

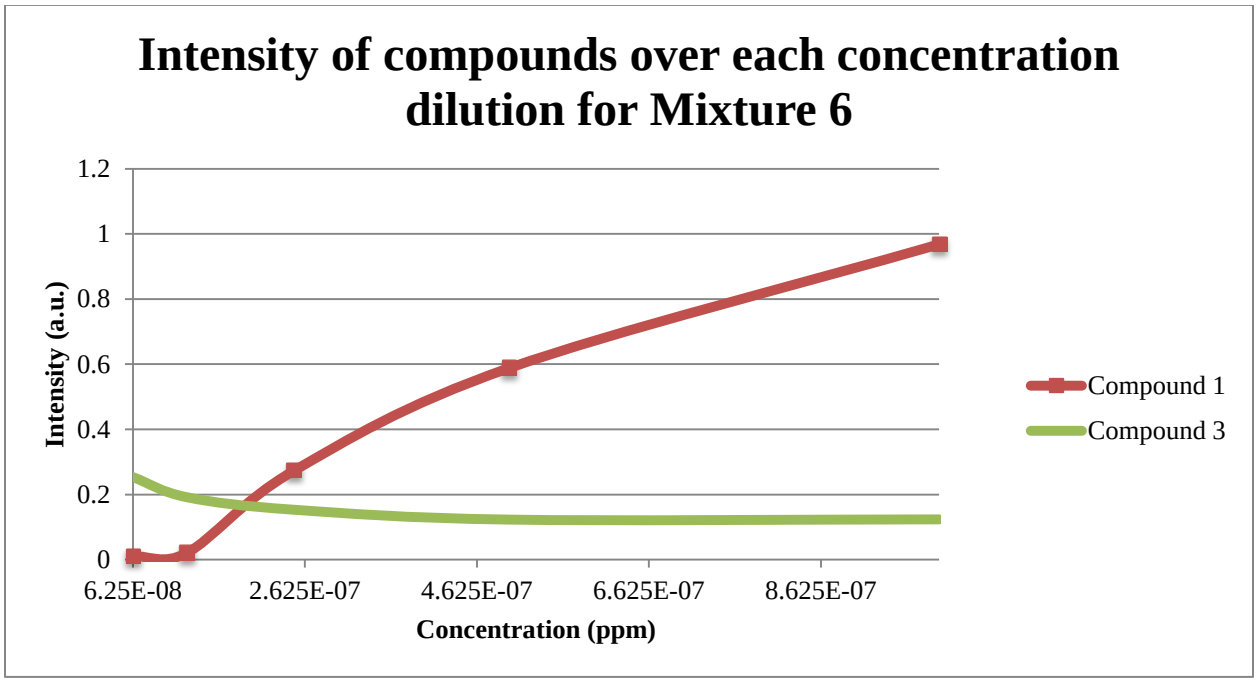

Figure 4.40: Concentration intensities for mixture 6 after PARAFAC analysis showing a linear trend between intensity and concentration with Compound 3 held at a steady concentration and Compound 1 logarithmically diluted.

4.11 Mixture 7

PARAFAC analyzed excitation scans of mixture **7** with compound **2** parameters show a maximized peak at 244 nm for compound **2.** The signal for compound **4** was removed due to interference (Figure 4.41). The excitation scan with compound **4** parameters exhibited a maximized peak at 328 nm for compound **4** and an exhibition of fluorescent noise for compound **2** (Figure 4.42). The compound **2** parameter emission scan exhibited a maximized peak at 299 nm for compound **2** which experienced a red shift from the standardized peak maximum by 1 nm and an exhibition of fluorescent noise for compound **4** (Figure 4.43). The compound **4** parameter emission scan exhibited a maximized peak for compound **4** at 373 nm and an exhibition of fluorescent noise for compound **2** (Figure 4.44).

The PARAFAC analyses for compound **2** parameter scans exhibit a nearly linear trend of intensity for compound **4** indicating a direct relationship between concentration and intensity. Compound **2** showed a consistent trend of intensity with a slight increase in intensity as the concentration of compound **4** diminished indicating a mild inverse relationship between the intensities of the two compounds (Figure 4.45). The PARAFAC analysis of compound **4** parameter scans exhibit a clear linear trend of intensity for compound **4** and a fairly steady trend of intensity for compound **2** (Figure 4.46). For mixture **7** solutions the JYS limit of detection is 6.25ppb, PARAFAC analysis decreases this to less than 62.5 ppt improving the detection by 99.9%.

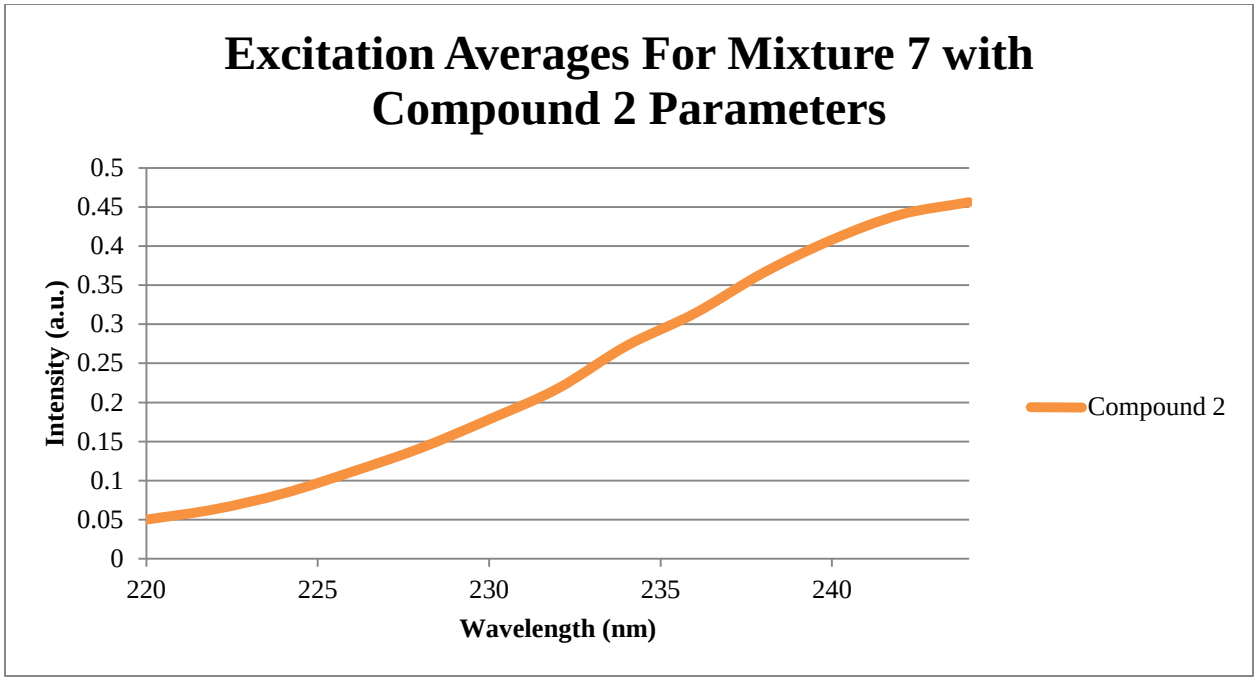

Figure 4.41: Average excitation scan with Compound 2 parameters of mixture 7 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 7 with Compound 2 parameters for any given concentration. Maximum excitation appears at 244 nm.

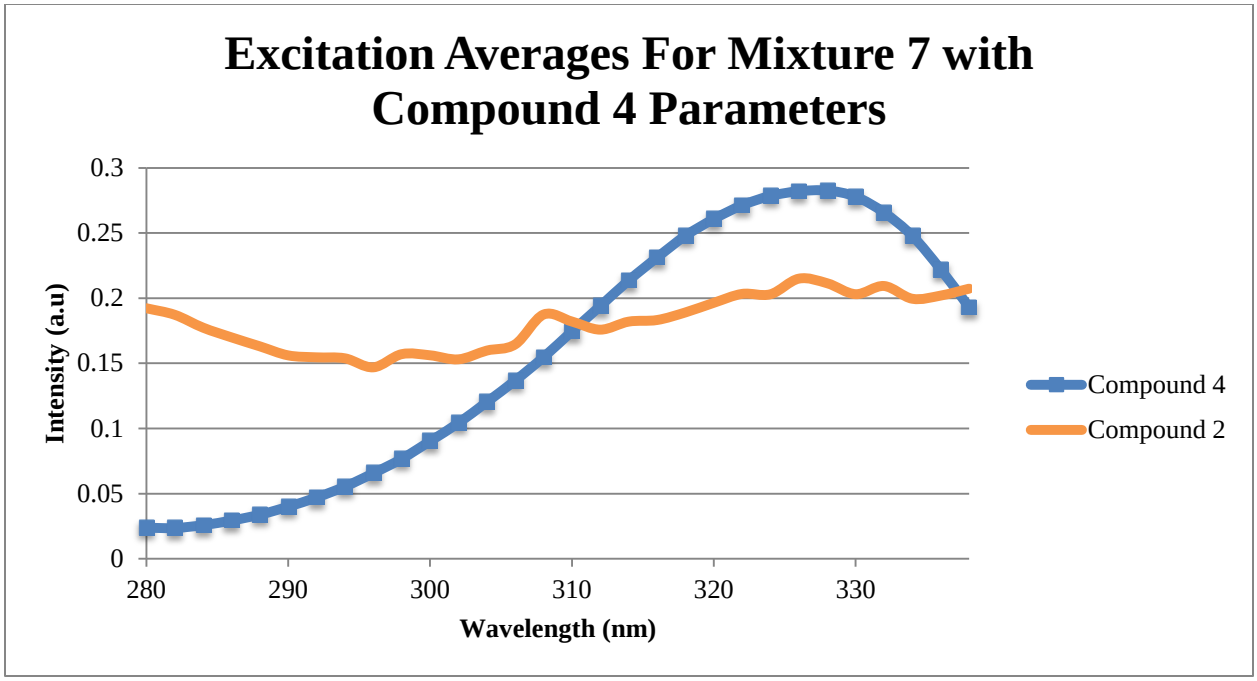

Figure 4.42: Average excitation scan with Compound 4 parameters of mixture 7 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 7 with Compound 4 parameters for any given concentration. Maximum excitation appears at 328 nm while the other signal is background noise.

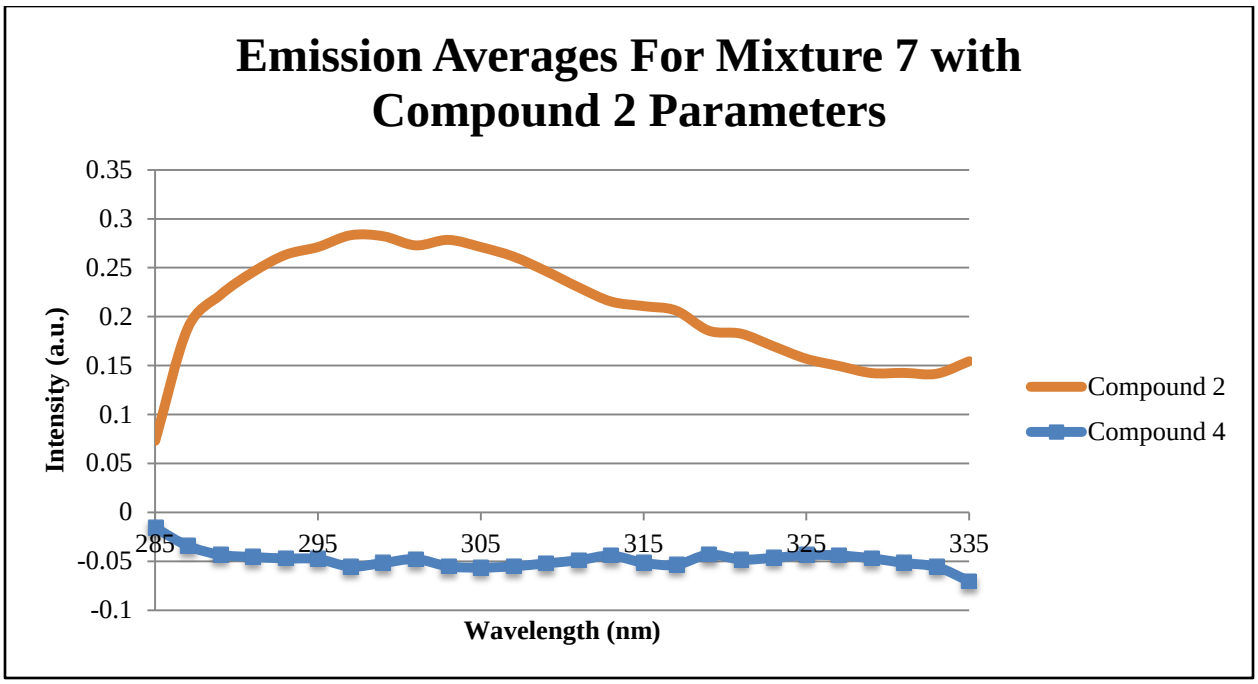

Figure 4.43: Average emission scan with compound 2 parameters of mixture 7 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 7 with compound 2 parameters for any given concentration. The maximum emission appears at 299 nm while the other signal is background noise.

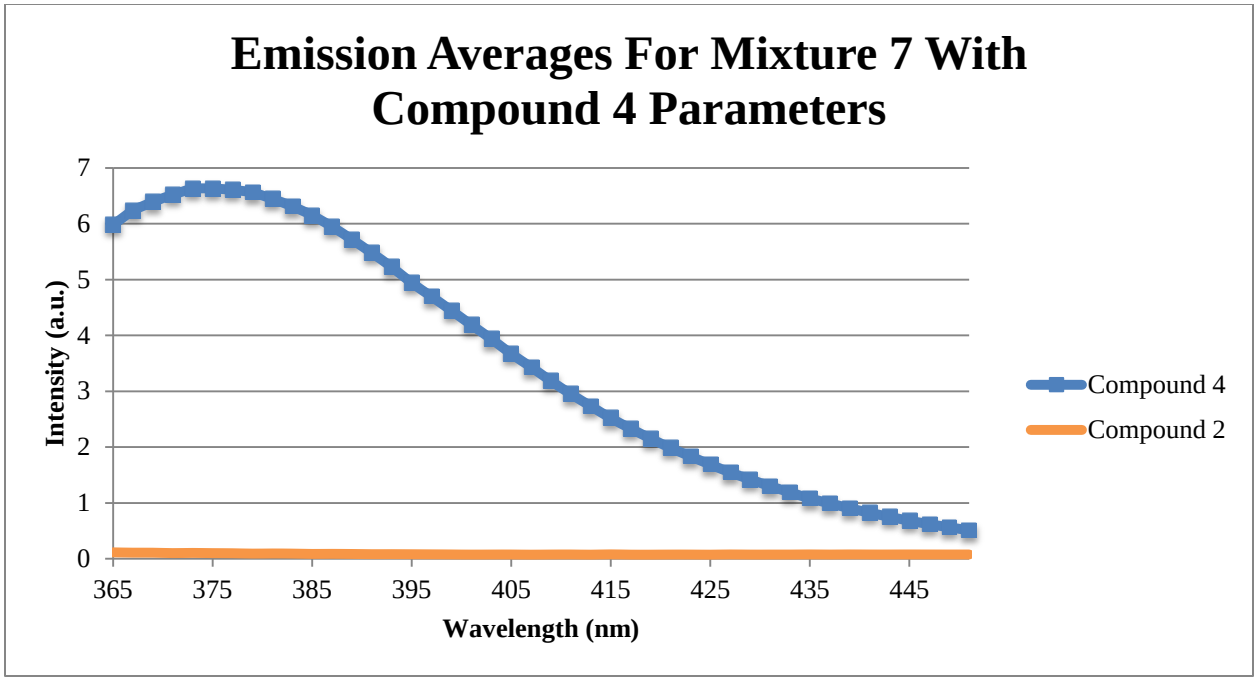

Figure 4.44: Average emission scan with compound 4 parameters of mixture 7 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 7 with compound 4 parameters for any given concentration. The maximum emission appears at 373 nm while the other signal is background noise.

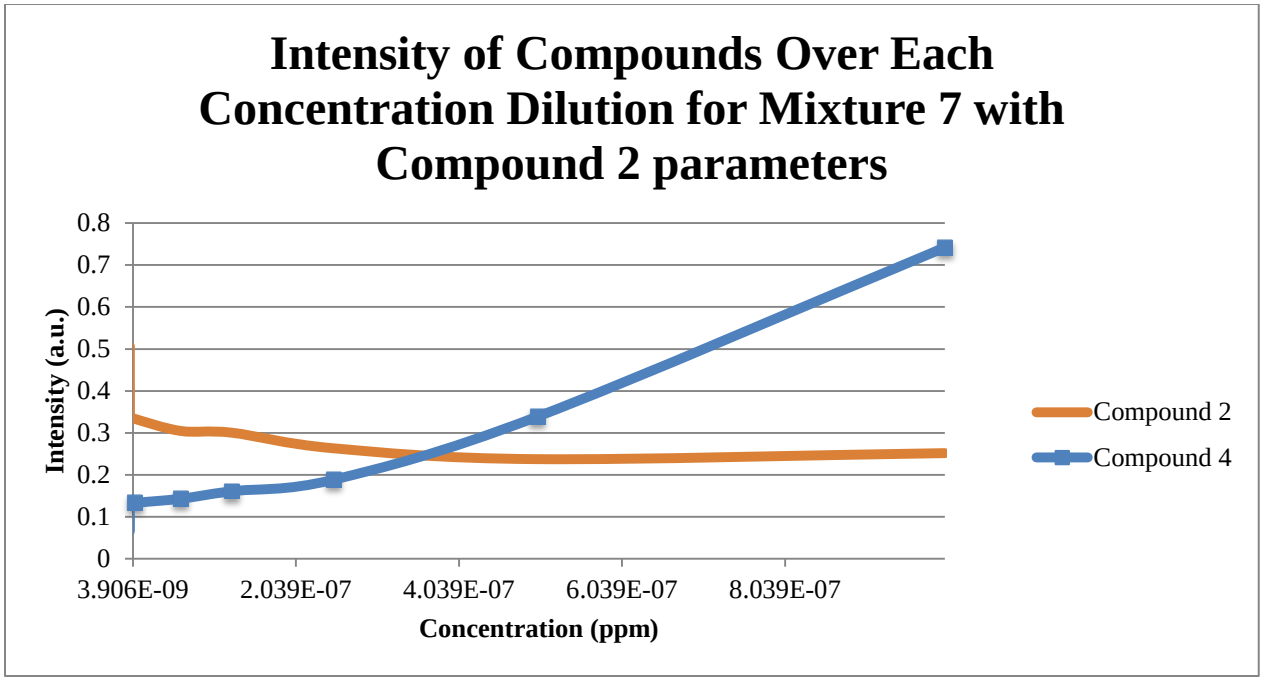

Figure 4.45: Concentration intensities for mixture 7 with compound 2 parameters after PARAFAC analysis showing a partly linear and partly steady trend between intensity and concentration with compound 2 held at a steady concentration.

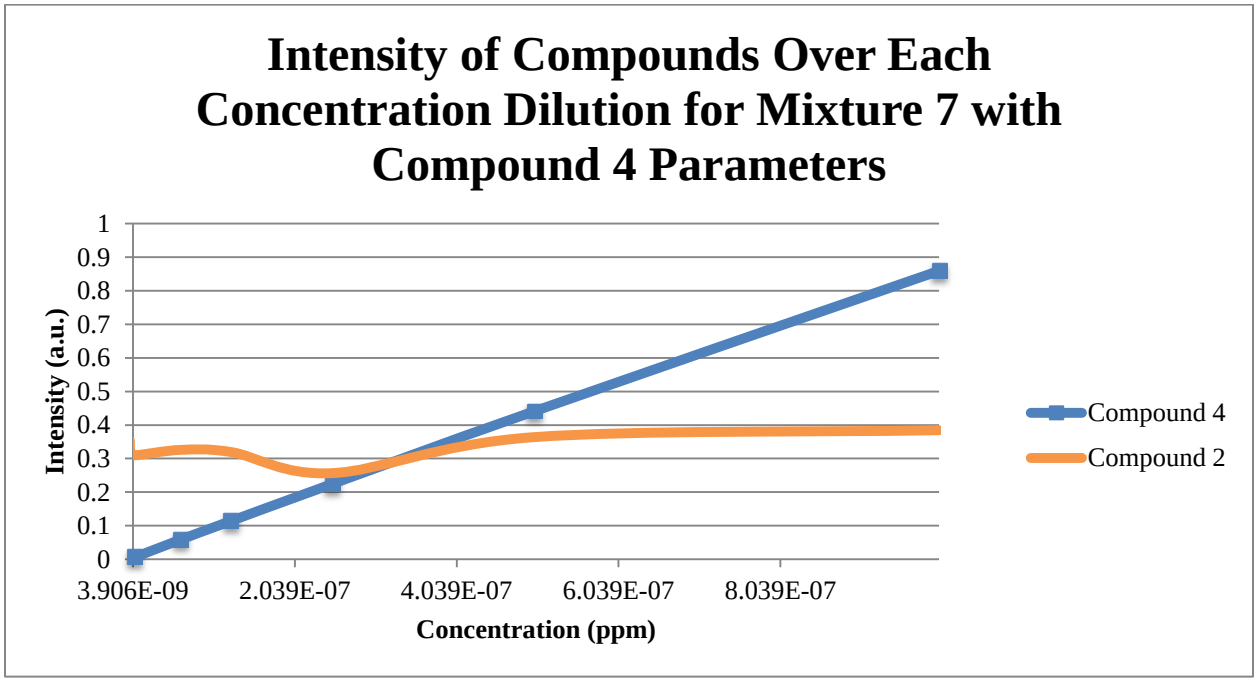

Figure 4.46: Concentration intensities for mixture 7 with compound 2 parameters after PARAFAC analysis showing a linear trend with the steady concentration of compound 2 and a direct linear trend from compound 4 between intensity and concentration.

4.12 Mixture 8

PARAFAC analyzed excitation scans of mixture **8** with compound **2** parameters exhibited a maximized peak at 244 nm for compound **2** (Figure 4.47). The excitation scan with compound **4** parameters exhibited a maximum peak at 328 nm for compound **4** (Figure 4.48). The compound **2** parameter emission scan exhibited a blue shifted maximized peak at 296 nm for compound **2** and an exhibition of fluorescent noise for compound **4** (Figure 4.49). The compound **4** scan exhibited a blue shifted maximized peak for compound **4** at 375 nm and an exhibition of fluorescent noise for compound **2** (Figure 4.50). Some of the data had compound signals removed due to interference (Figures 4.47, 4.48 and 4.52).

The PARAFAC analyses with compound **2** parameters exhibit a nearly linear trend of intensity for compound **2**. Compound **4** showed a negative and mild linear trend of intensity with an increase in intensity as the concentration of compound **2** diminishes indicating an inverse relationship between the intensities of the two compounds (Figure 4.51). The PARAFAC analyses with compound **4** parameters exhibit a clear consistent trend of intensity for compound **4** with a slight increase at the lowest concentration of compound **2** indicating a mild inverse relationship (Figure 4.52). For mixture **8** solutions the JYS limit of detection is 500ppb, PARAFAC analysis decreases this to less than 62.5ppb improving the detection by 87.5%.

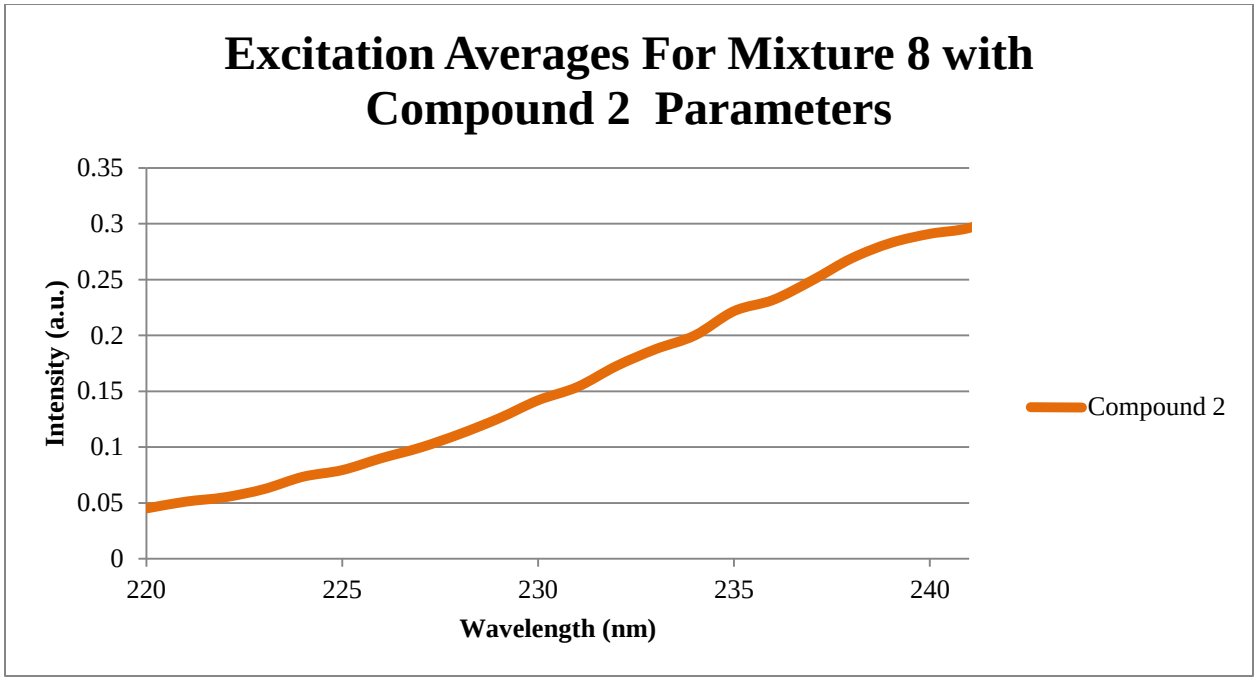

Figure 4.47: Average excitation scan with compound 2 parameters of mixture 8 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 8 with compound 2 parameters for any given concentration. Maximum excitation appears at 244 nm.

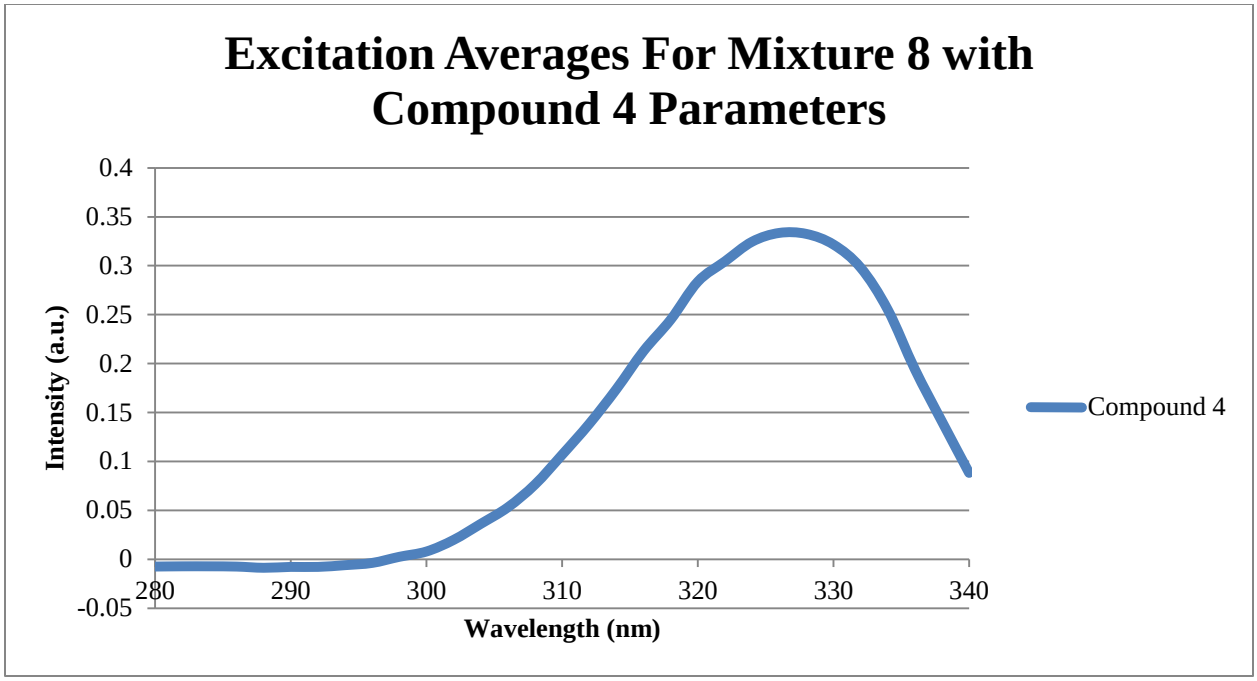

Figure 4.48: Average excitation scan with compound 4 parameters of mixture 8 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 8 with compound 4 parameters for any given concentration. Maximum excitation appears at 328 nm.

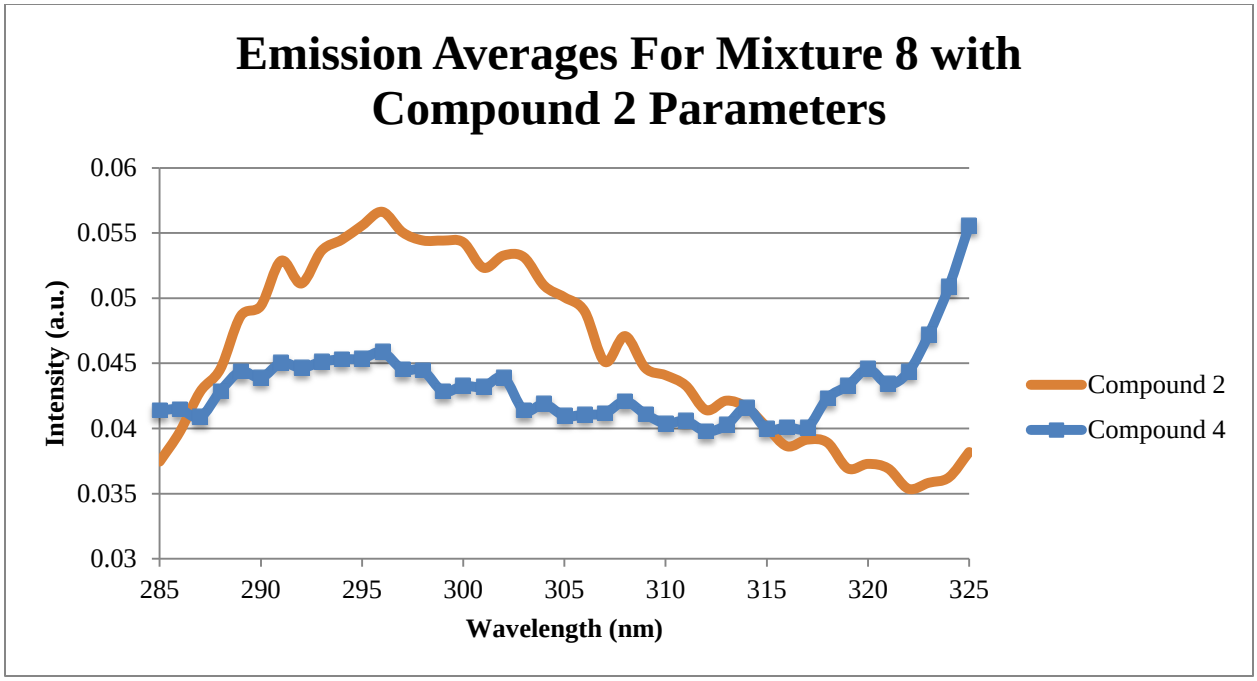

Figure 4.49: Average emission scan with compound 2 parameters of mixture 8 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 8 with compound 2 parameters for any given concentration. The maximum emission appears at 295 nm while the other signal is background noise.

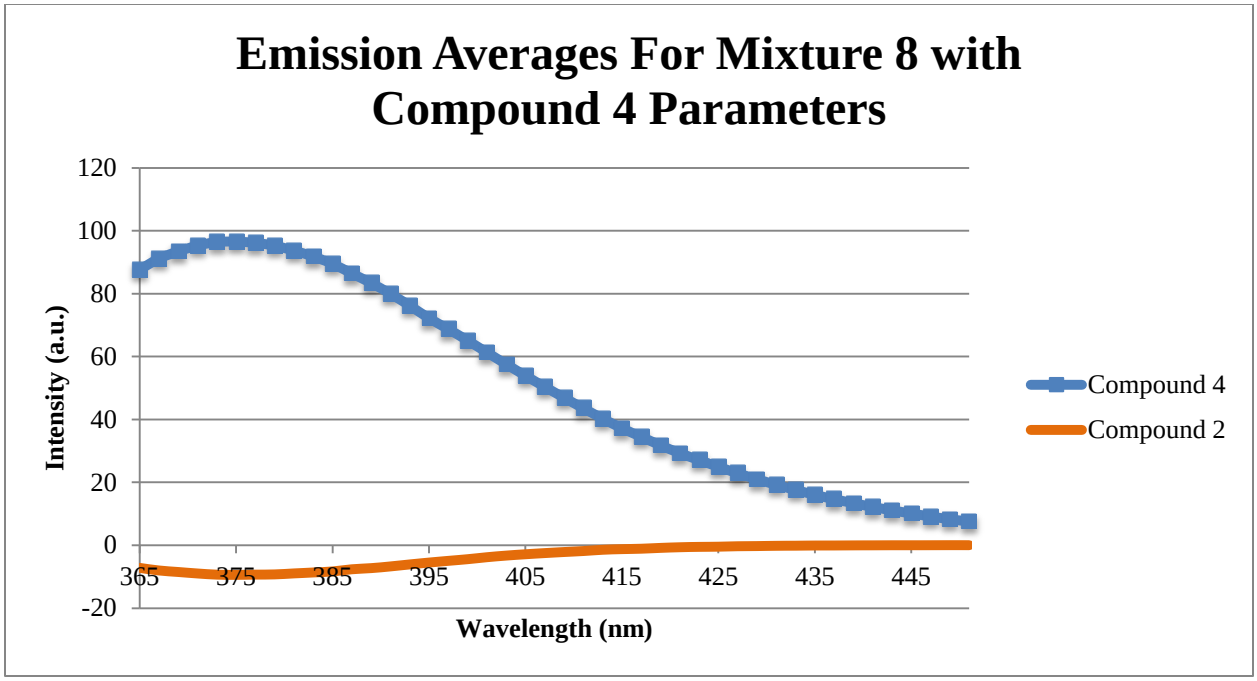

Figure 4.50: Average emission scan with compound 4 parameters of mixture 8 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 8 with compound 4 parameters for any given concentration. The maximum emission appears at 375 nm while the other signal is background noise.

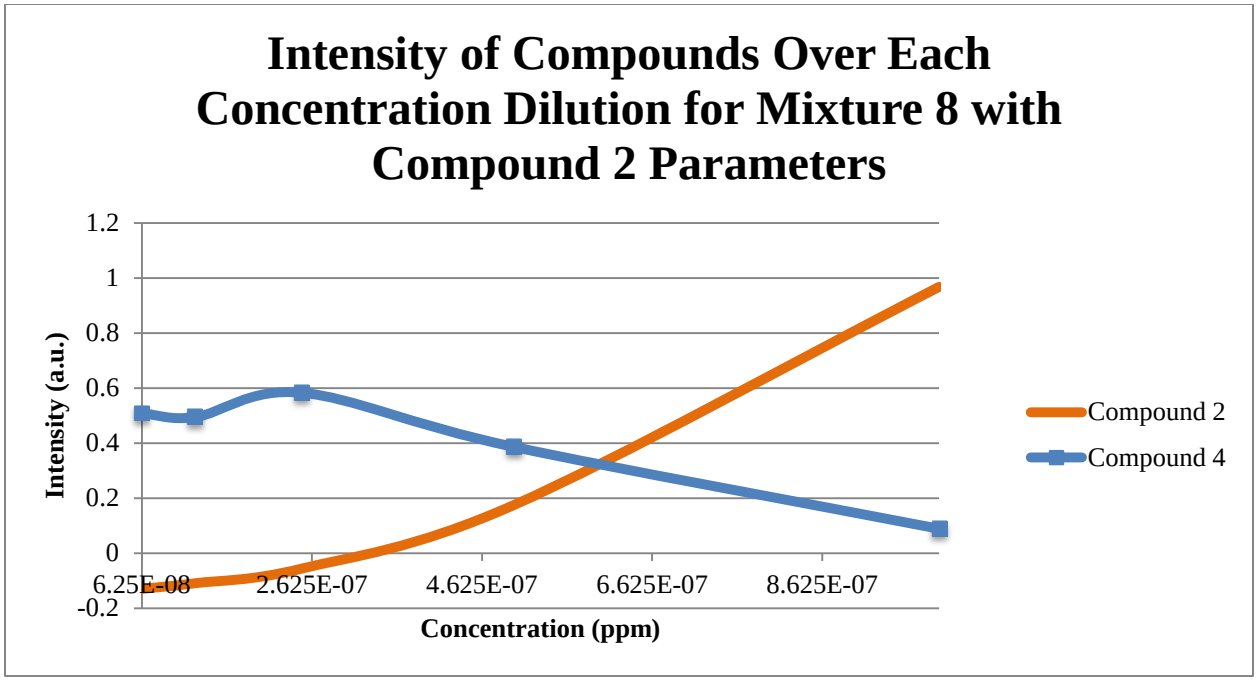

Figure 4.51: Concentration intensities for mixture 8 with compound 2 parameters after PARAFAC analysis showing a direct trend with concentration of compound 4 and an inverse relationship with the concentration of compound 2 and a direct trend from compound 2 between intensity and concentration.

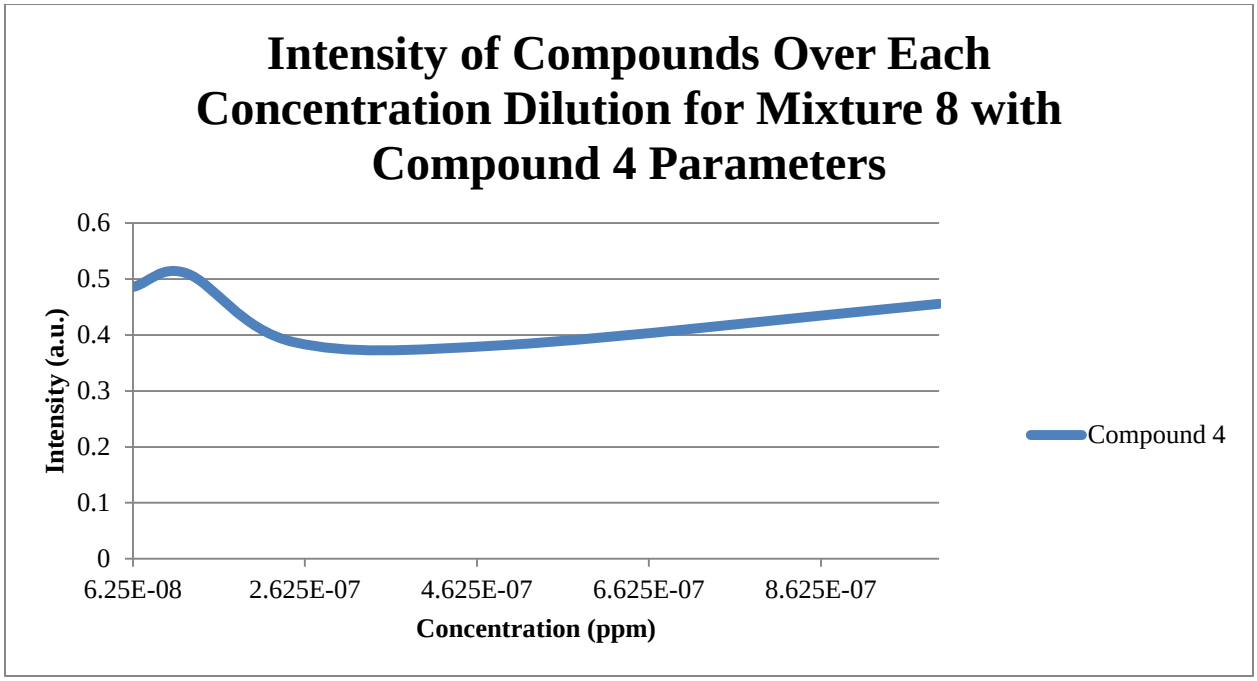

Figure 4.52: Concentration intensities for mixture 8 with compound 4 parameters after PARAFAC analysis showing a direct trend with concentration of compound 4 and an inverse relationship with the concentration of compound 2.

4.13 Mixture 9

The PARAFAC analyzed excitation scan for mixture **9** exhibited a maximum peak at 262 nm for compound **3** and a maximum peak at 270 for compound **2** which experienced a red shift from the standardized peak by 26 nm (Figure 4.53). The emission scan exhibited a maximized peak at 270 nm for compound **2** which experienced a blue shift from the standardized peak by 28 nm and a maximized peak at 270 for compound **3** which experienced a blue shift from the standardized peak by 16 nm (Figure 4.54).

The PARAFAC analysis exhibit a nearly linear trend of intensity for compound **2** indicating a direct relationship between concentration and intensity. Compound 3 showed a steady trend of intensity with a slight increase in intensity as the concentration of compound **2**

diminishes indicating a mild inverse relationship between the intensities of the two compounds(Figure 4.55). For mixture **9** solutions the JYS limit of detection is 250ppb, PARAFAC analysis decreases this to less than 62.5ppb improving the detection by 75.0%.

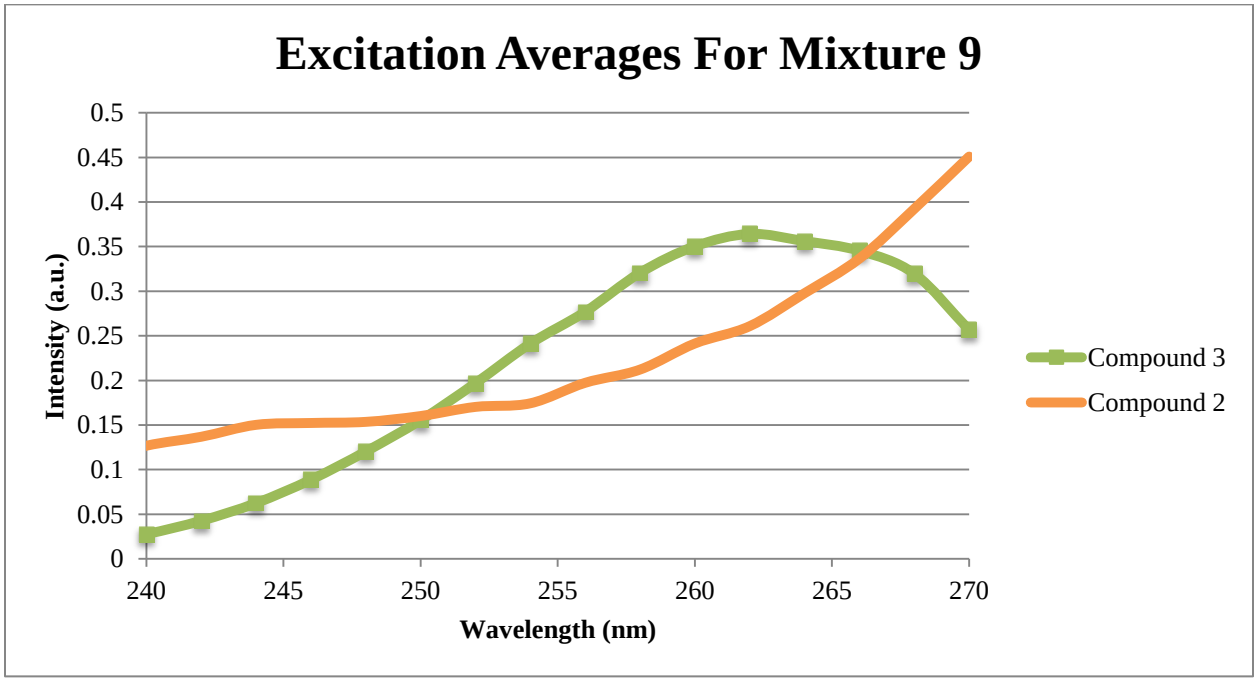

Figure 4.53: Average excitation scan of mixture 9 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 9 for any given concentration. Maximum excitations appear at 277 nm and 286 nm.

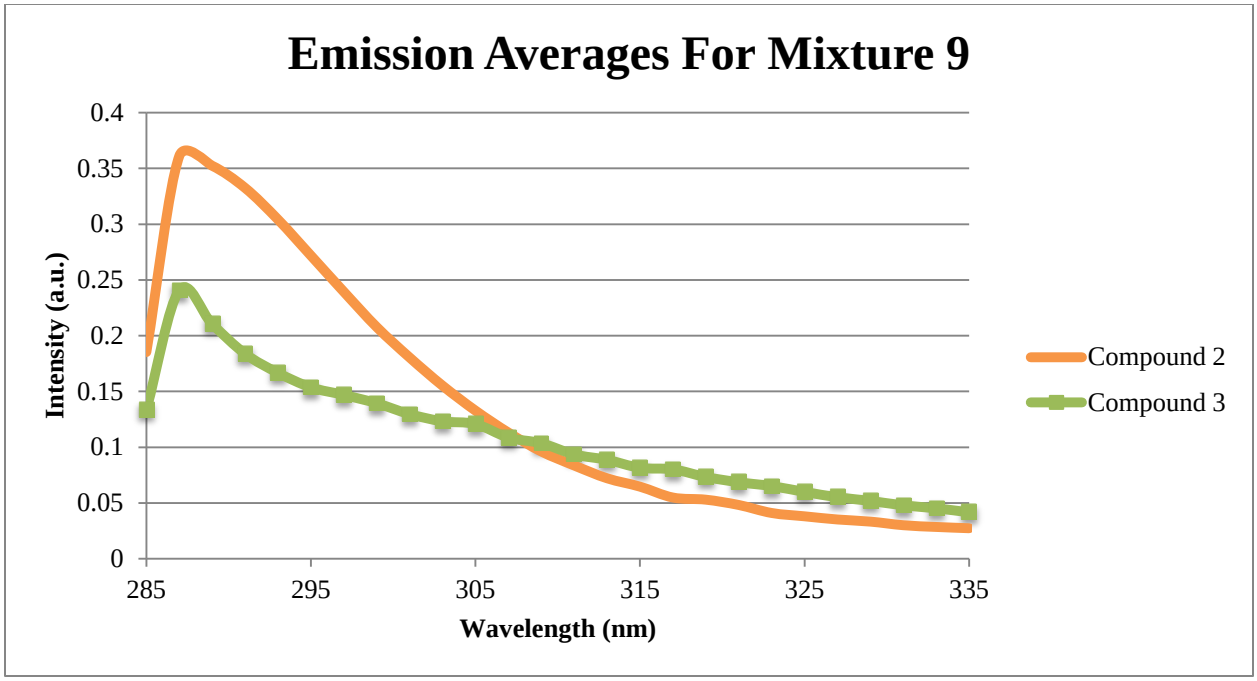

Figure 4.54: Average emission scan of mixture 9 after PARAFAC analysis of several concentrations. The above result shows the emission peaks of mixture 9 for any given concentration. The maximum emission appears at 287 nm and 287 nm.

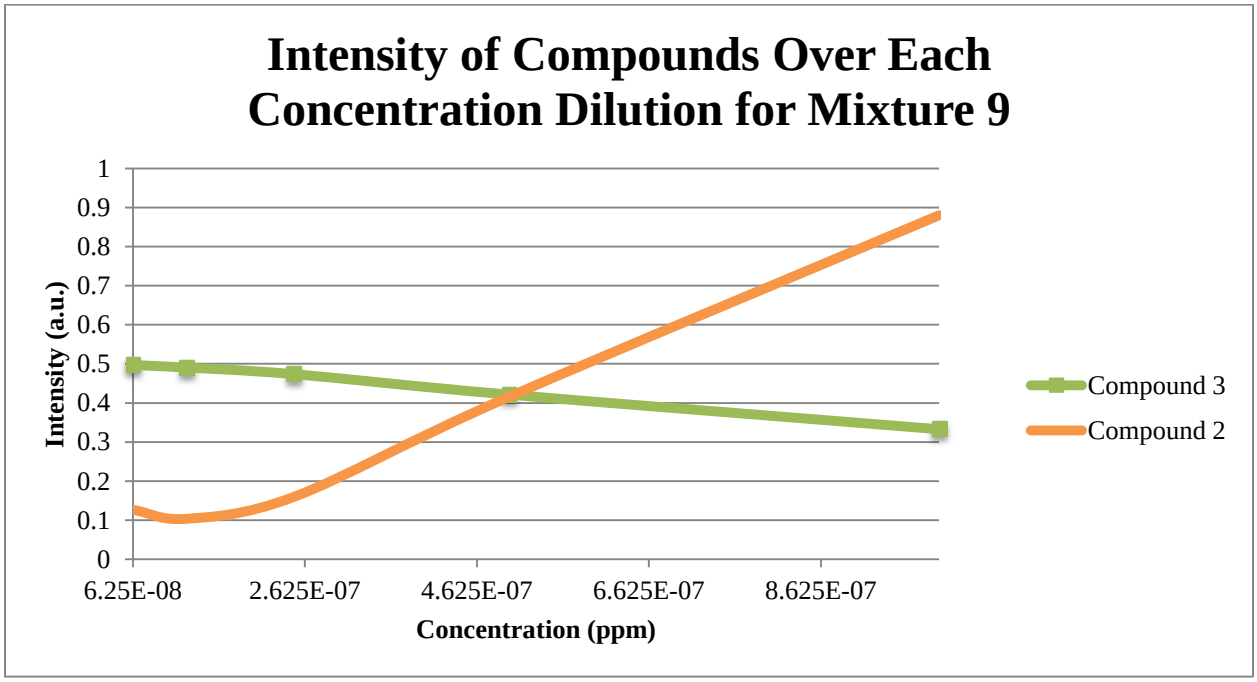

Figure 4.55: Concentration intensities for mixture 9 after PARAFAC analysis showing for compound 2 a linear trend between intensity and concentration and an inverse relationship between the two lines from the compound 3 signal.

4.14 Mixture 11

PARAFAC analyzed excitation scans of mixture **11** with compound **3** parameters show a maximized peak at 262 nm for compound **3** (Figure 4.56). The compound **4** excitation scan exhibited a maximized peak at 328 nm for compound **4** (Figure 4.57). The compound **4** parameter emission scan exhibited a maximized peak at 373 nm for compound **3** and shows fluorescent noise for compound **3** (Figure 4.58). The compound **3** emission scan exhibited a maximized peak for compound **3** at 288 nm, which experienced a red shift from the standardized compound peak by 2 nm, and shows fluorescent noise for compound **4** (Figure 4.59). Some of the data had compound signals removed due to interference (Figures 4.56, 4.57 and 4.60).

The PARAFAC analyses for compound **4** parameter scans exhibit a nearly linear trend indicating a direct relationship between concentration and intensity for compound **4** (Figure 4.60). The PARAFAC analyses of compound **3** parameter scans exhibit a consistent trend for compound **3**, but shows a direct relationship between intensity and concentration for compound **4 (**Figure 4.61). The slight increase in intensity f or compound **3** as the concentration of compound **4** diminishes indicates a mild inverse relationship between compound intensities**.** For Mixture **11** solutions the JYS limit of detection is 6.25ppb, PARAFAC analysis decreases this to less than 62.5 ppt improving the detection by 99.9%.

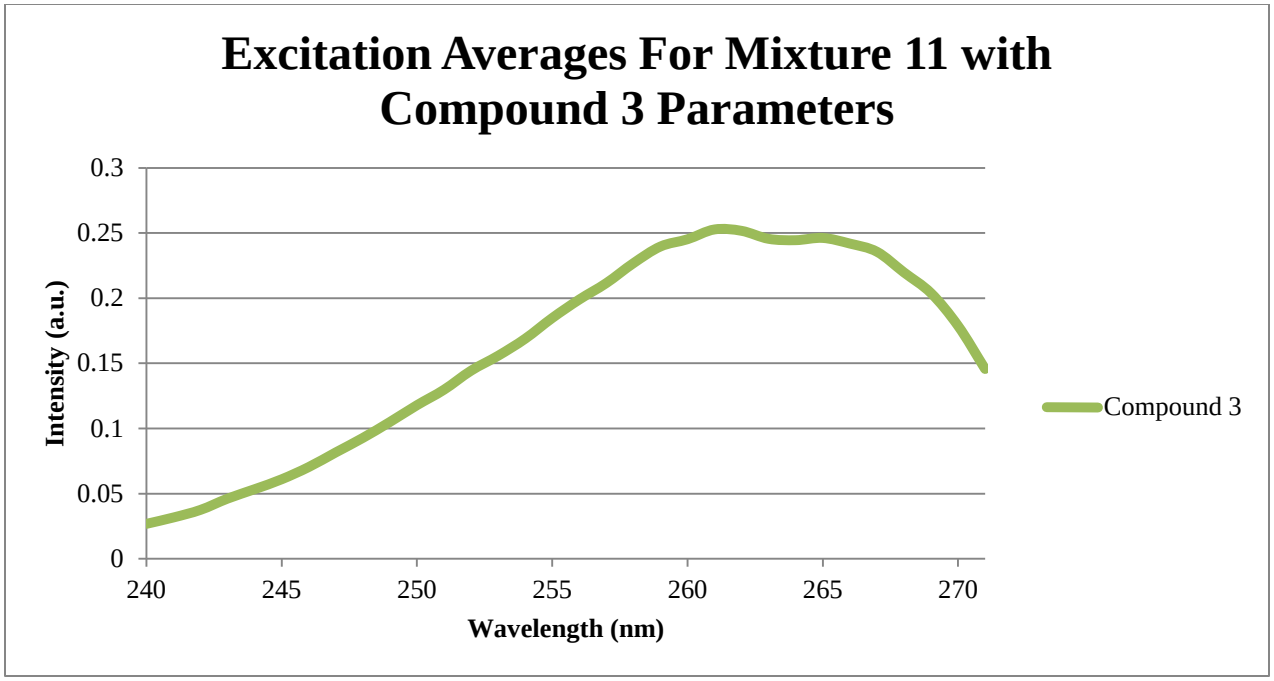

Figure 4.56: Average excitation scan with compound 3 parameters of mixture 11 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 11 with compound 3 parameters for any given concentration. Maximum excitation appears at 262 nm.

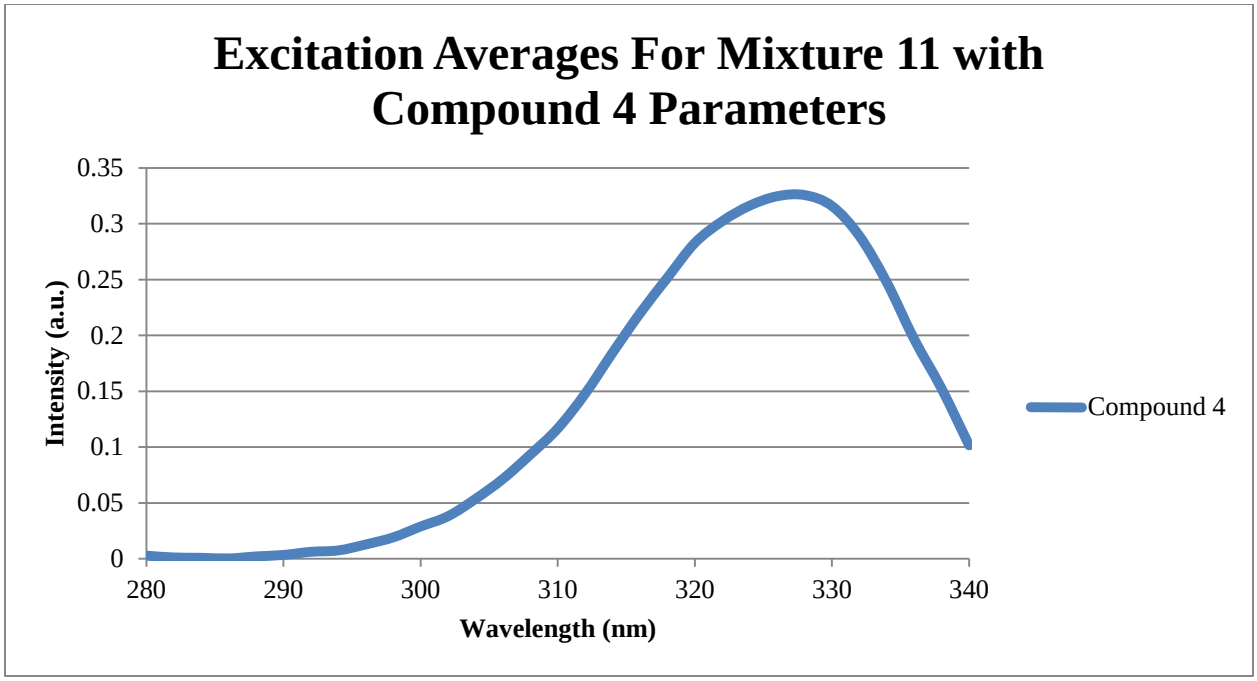

Figure 4.57**:** Average excitation scan with compound 4 parameters of mixture 11 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shape of mixture 11 with compound 4 parameters for any given concentration. Maximum excitation appears at 328 nm.

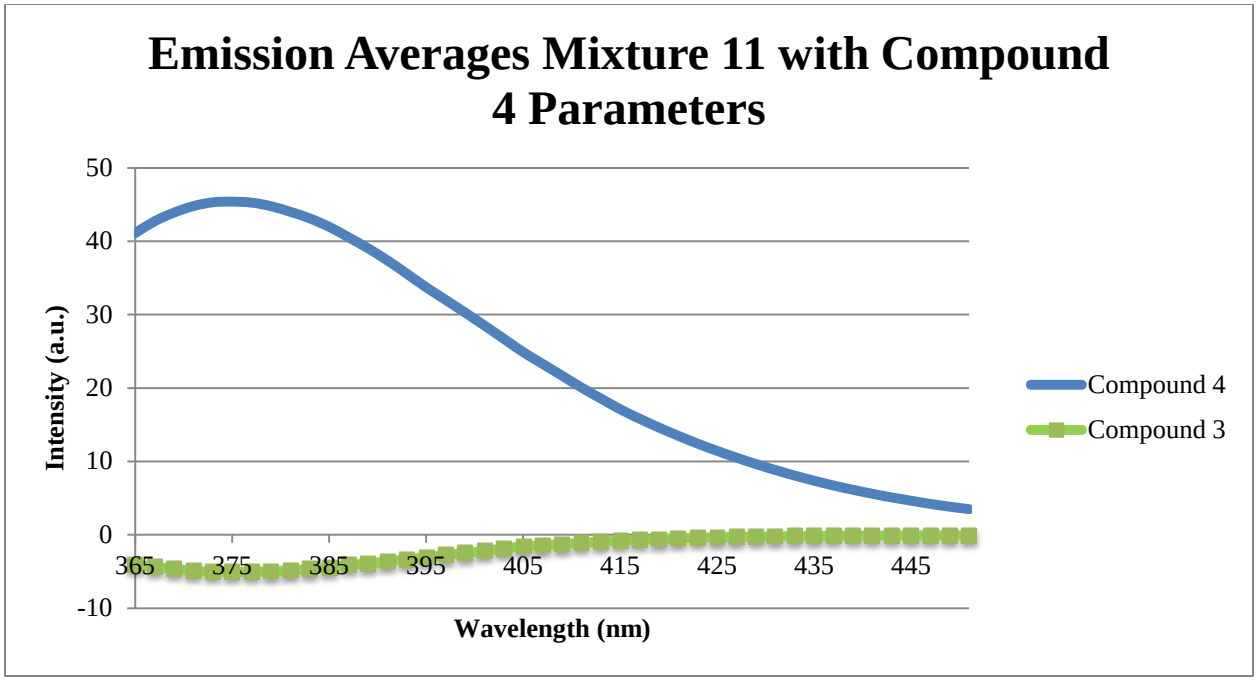

Figure 4.58**:** Average emission scan with compound 4 parameters of mixture 11 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 11 with compound 4 parameters for any given concentration. The maximum emission appears at 373 nm.

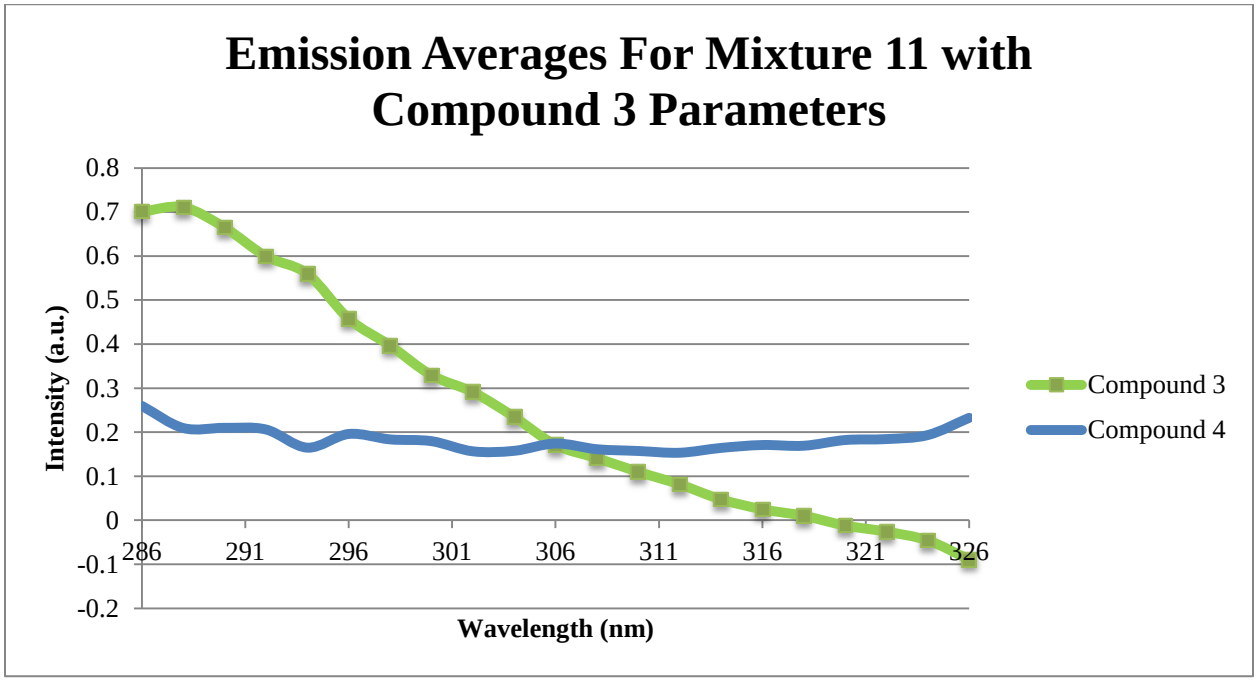

Figure 4.59**:** Average emission scan with compound 3 parameters of mixture 11 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 11 with compound 3 parameters for any given concentration. The maximum emission appears at 288 nm.

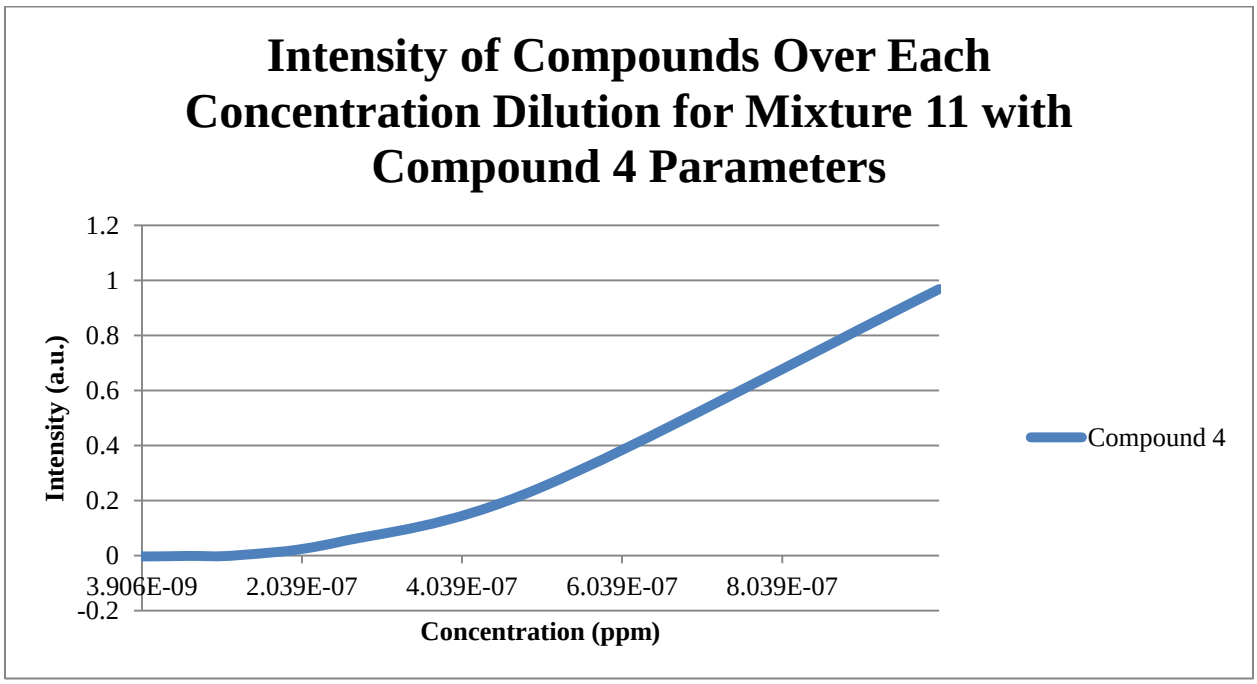

Figure 4.60**:** Concentration intensities for mixture 11 with compound 4 parameters after PARAFAC analysis showing a direct trend between intensity and concentration for compound 4.

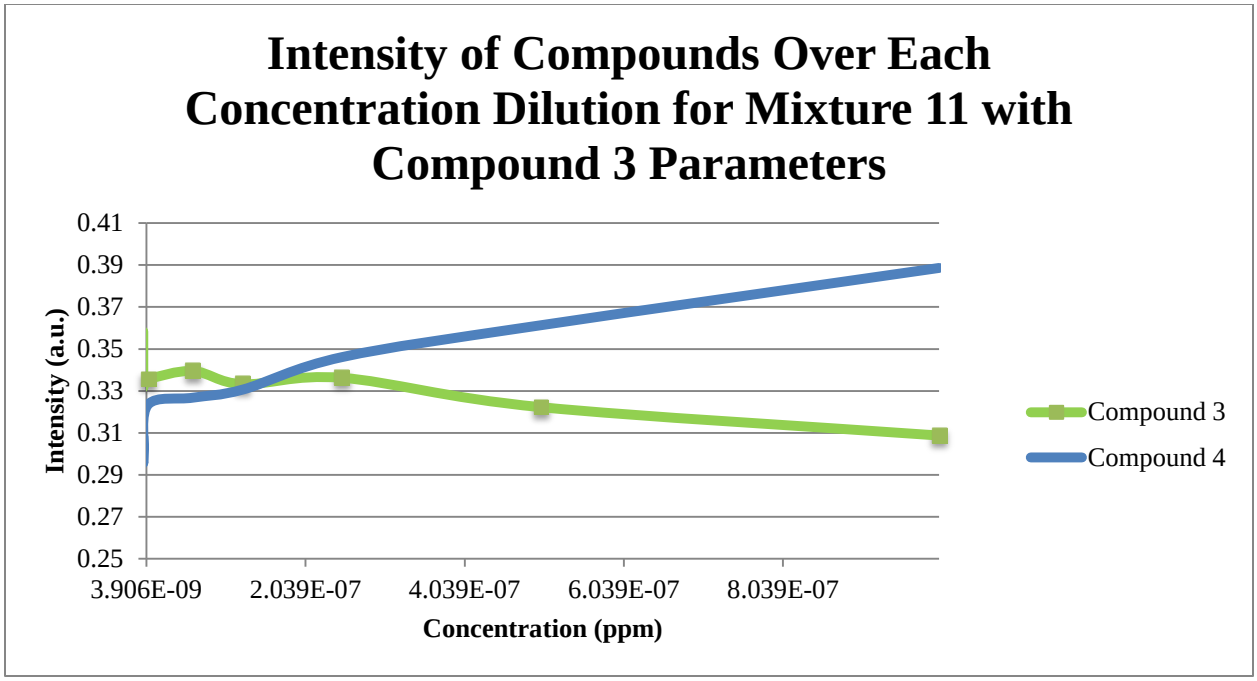

Figure 4.61**:** Concentration intensities for mixture 8 with compound 4 parameters after PARAFAC analysis showing a direct trend with the steady concentration of compound 2 and a direct linear trend from compound 4 between intensity and concentration.

4.15 Mixture 12

PARAFAC analyzed excitation scans of mixture **12** with compound **4** parameters show a maximized peak at 328 nm for compound **4** (Figure 4.62). The compound **3** excitation scan exhibited a maximized peak at 262 nm for compound **3** (Figure 4.63). The compound **4** parameters emission scan exhibited a maximum peak at 373 nm for compound **4** and fluorescent background noise for compound **3** (Figure 4.64). The compound **3** parameters emission scan exhibited a maximum peak for compound **3** at 286 nm and fluorescent background noise for compound **4** (Figure 4.65).

The PARAFAC analyses for compound **4** parameter scans exhibit a trend of consistent intensity for compound **3** and a trend of consistent intensity for compound **4**, each compound

shows a spike in intensity at the lowest concentration (Figure 4.66). The PARAFAC analyses of compound **3** parameter scans shows a consistent trend for compound **4**, but shows a direct relationship between intensity and concentration for compound **3 (**Figure 4.67). The slight increase in intensity for compound **4** as the concentration of compound **3** diminishes indicates a mild inverse relationship between compound intensities. For Mixture **12** solutions the JYS limit of detection is 250ppb, PARAFAC analysis decreases this to less than 62.5ppb improving the detection by 75.00%.

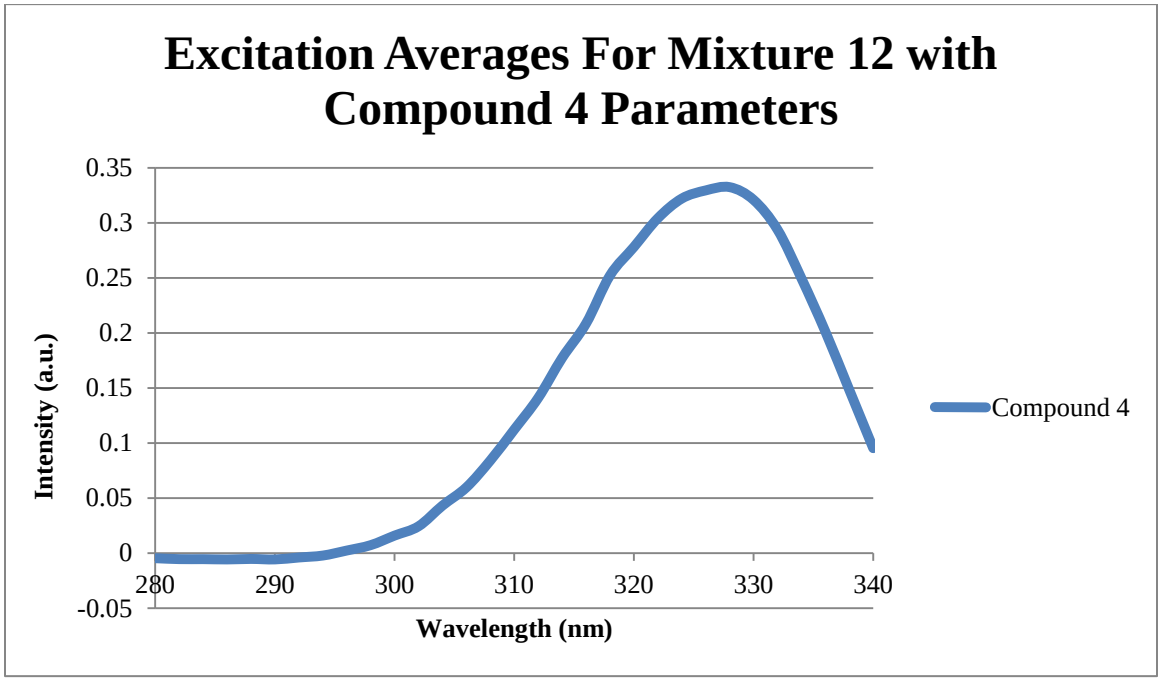

Figure 4.62**:** Average excitation scan with compound 4 parameters of mixture 12 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shape of mixture 12 with compound 4 parameters for any given concentration. Maximum excitations appear at 328 nm.

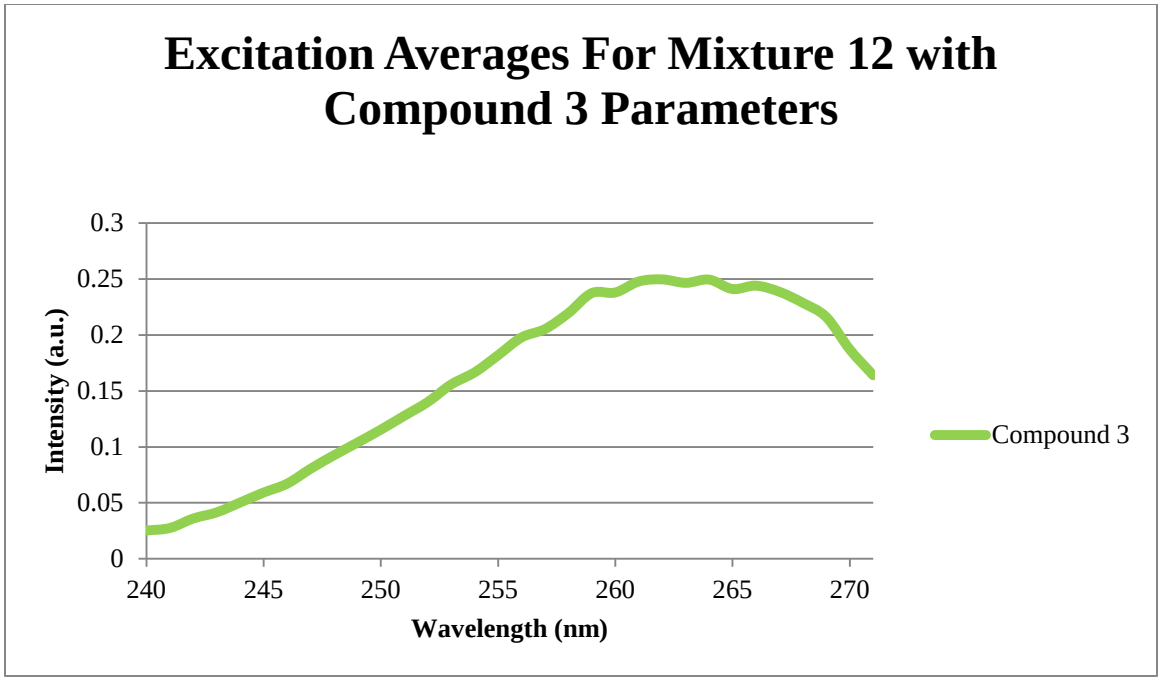

Figure 4.63**:** Average excitation scan with compound 3 parameters of mixture 12 after PARAFAC analysis of several concentrations. The above result shows the excitation peak shapes of mixture 12 with compound 3 parameters for any given concentration. Maximum excitations appear at 262 nm.

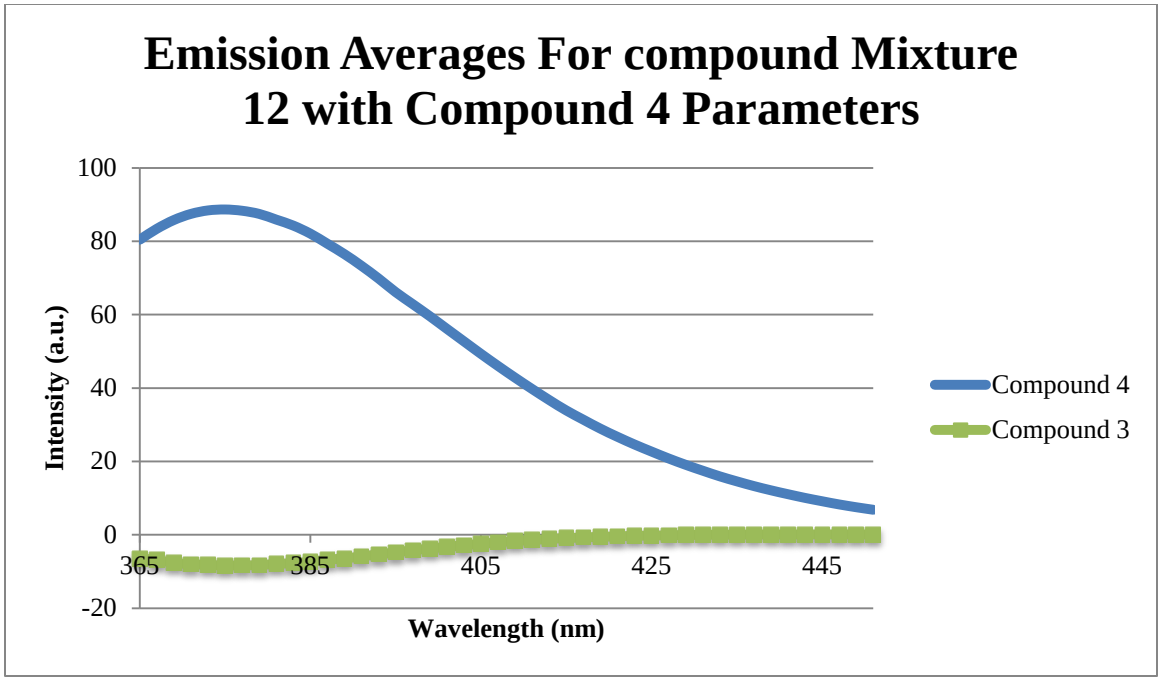

Figure 4.64: Average emission scan with compound 4 parameters of mixture 12 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 12 with compound 4 parameters for any given concentration. The maximum emission appears at 373 nm.

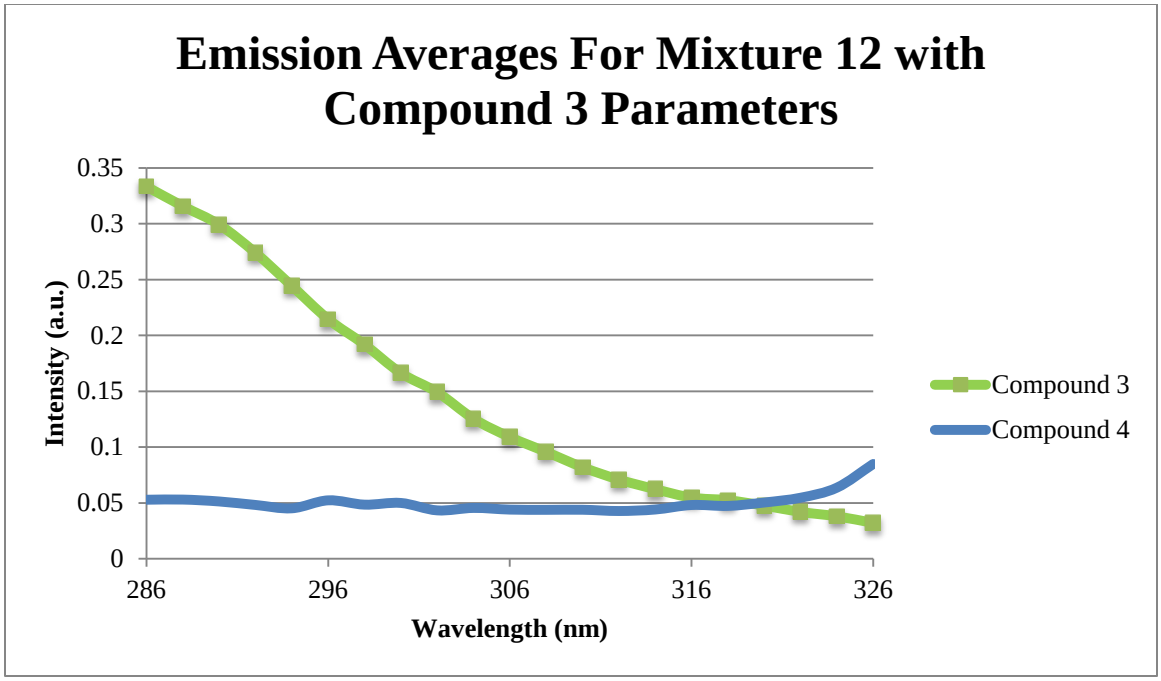

Figure 4.65**:** Average emission scan with compound 3 parameters of mixture 12 after PARAFAC analysis of several concentrations. The above result shows the emission peak shape of mixture 12 with compound 3 parameters for any given concentration. The maximum emission appears at 286 nm.

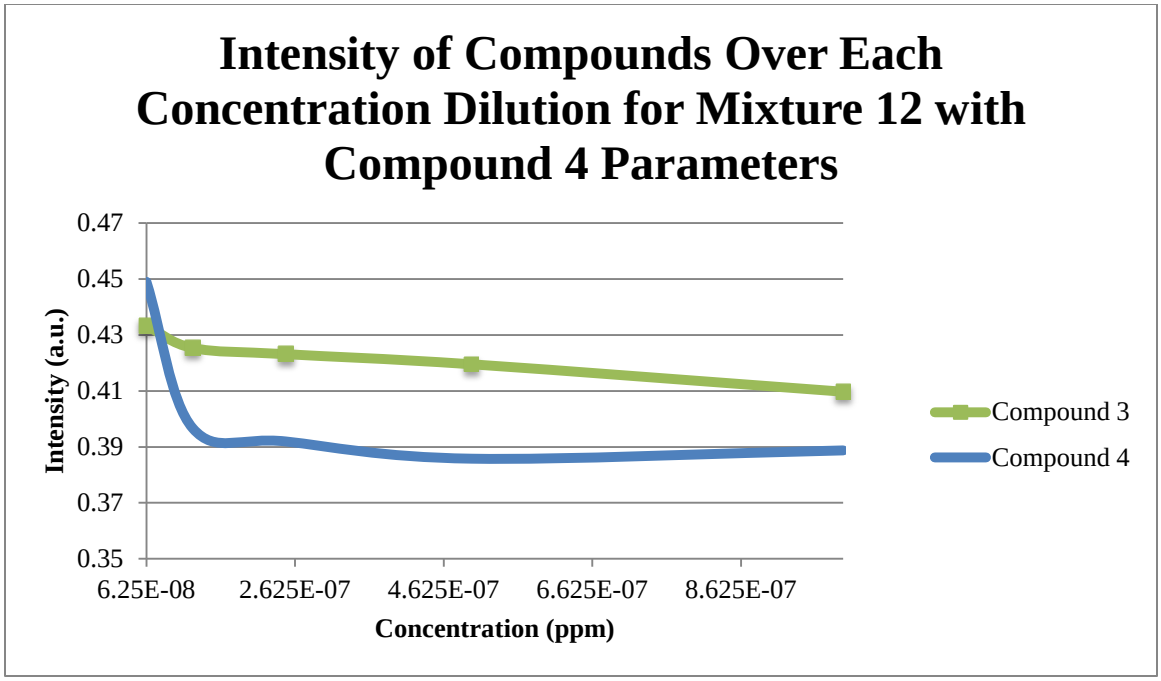

Figure 4.66**:** Concentration intensities for mixture 12 with compound 4 parameters after PARAFAC analysis showing a steady trend for compound 3 and compound 4.

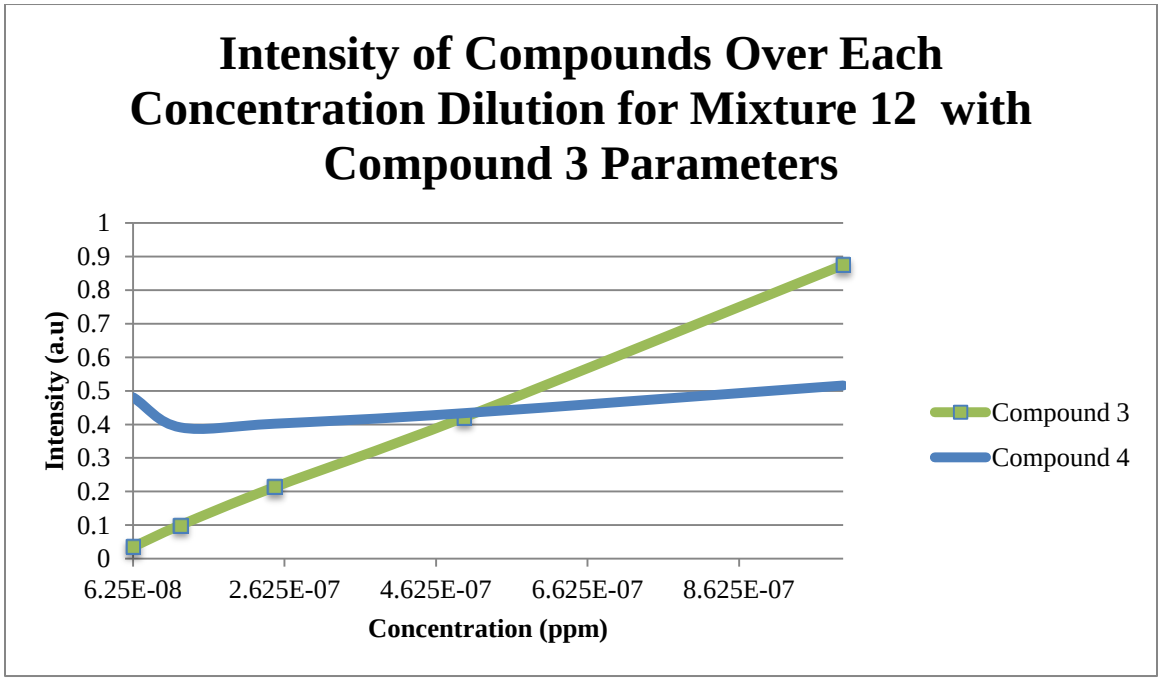

Figure 4.67**:** Concentration intensities for mixture 12 with compound 3 parameters after PARAFAC analysis showing a direct trend with the steady concentration of compound 4 and a direct linear trend from compound 3 between intensity and concentration.

Chapter 5: Discussion

5.1 Compound 1

Having determined the standardized position, shape and intensity of compound 1on an EEM with an excitation maximum of 315 nm and emission maximum of 402 nm, the shifting position, changing shape and diminishing intensity seen in the mixtures can be better observed. The JYS limit of detection was 125 ppb, paired with PARAFAC this limit of detection was improved by 50%. Although 50% is minute, particularly when compared to other rates of improvement seen in compounds **2** through **4** (75%, 75% and 90% respectively), it should be noted this improvement continues to be limited by equipment. Most JYS limits of detection occurred around 250ppb, and thus the scans were only carried on two half dilutions further. The final two concentrations of 125 ppb and 62.5 ppb resulted in an inability to differentiate using traditional methods between the peak and background noise. However, PARAFAC analysis allowed for detection at 62.5ppb. The PARAFAC analysis gave an intensity value of 0.061 a.u. where the JYS alone was unable to determine values for that concentration. Thus, PARAFAC could more than likely continue analysis into the part per trillion level.

Compound **1** was particularly luminescent and thus the limit of detection lower than the others, a similar effect seen in scans for compound **4**. The molecular structure and subsequent photochemical characteristics are important in the limit of detection. The molecular structure of compound 1 is highly conjugated and thus gives rise to a highly fluorescent molecule. This shows that the limit of detection results from a complex relationship between the inability of the JYS to measure low intensity peaks in background noise and the compound structure.

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5.2 Compound 2

Compound **2** was determined to have a standardized maximum peak at an excitation of 244 nm and an emission of 298 nm. Due to Rayleigh scattering, these maximized peak standards existed on the edges of the EEM parameters which added to the difficulty in detecting the signal. More than any other compound, compound **2** experienced the most shifting in the mixtures, thus it was easy to have the maximum easily shifted out of the EEM parameters. Having one of the weaker signals, 0.004 a.u. at a concentration of 1 ppm, the signal of compound **2** was influenced by the other compounds. The JYS limit of detection was 250 ppb which was reduced by 75.0% when paired with PARAFAC analysis. The JYS only produced background noise at the two lowest concentration, but the PARAFAC analysis gave an intensity of 0.072 a.u and could most likely continue analysis into the part per trillion level.

Although compound **2** has the weakest signal, the intensity it produced for the final concentration of 62.5 ppb after PARAFAC analysis is more intense than the stronger signal of compound **1** at this concentration at 0.071 a.u. Much like the other compounds, the limit of detection of compound **2** is influenced by compound structure and the limitations of the JYS, although compound **2** is the weaker signal, it is more distinguishable at the lowest concentration when paired with PARAFAC analysis. This is due to the size of the molecule. Being a significantly larger molecule by volume, compound **2** is more likely to be struck by the light energy than compound **1** at low concentrations and thus have a stronger signal. Conversely, at higher concentrations, the smaller in volume but more fluorescent compound **1** would have a stronger signal than compound **2**.

5.3 Compound 3

Compound **3** was determined to have a standardized peak at an excitation 262 nm and an emission of 286 nm. Compound **3**, like compound **2**, existed close to the outer range of the EEM parameters, which often lead to maximum peak to be shifted off. However, being a stronger signal than compound **2,** compound **3** was not easily overshadowed or influenced by other signals. The JYS limit of detection was 250 ppb which was decreased to 62.5 ppb when paired with PARAFAC analysis. The molecular structure of compound **3** is the least conjugated of all the compounds as well as the smallest in volume which has resulted in the weakest signal, 0.011 a.u., at the lowest concentration, but a significantly higher signal at all other concentrations. Again, the larger volume of compound **2** increased the chances it will be struck with light energy at low concentrations, the small size of compound **3** thus will have a weaker signal at low concentrations in general and when combined with larger volume molecules.

5.4 Compound 4

Compound **4** has the strongest signal of all the compounds by a factor of 50. Compound **4** was determined to have a maximum excitation at 328 nm and a maximum emission at 373 nm. The JYS limit of detection was 625 ppt, which is even lower than the PARAFAC analyzed limit of detection for compounds **1**, **2** and **3**. When paired with PARAFAC analysis, this limit of detection was decreased to 62.5 ppt and could more than likely be lower if dilutions were continued. The intensity of compound **4** can be explained thru the structure and size. The molecule itself is larger in volume than compound **1** and **3**, but also exhibits conjugation from more than just aromatic rings.

5.5 Mixture 1

Mixture **1** showed an increase in intensity for both compound **1** and **2** excitation and emission scans, this occurs because of signal mixing. The signals of the two compounds essentially stack on one another and thus the PARAFAC analysis must be done to determine the individual peaks. Mixture **1** was one of the compounds that required individual EEM scans with compound **1** and compound **2** parameters, meaning there would be individual excitation and emission scans for each compound. However, because the PARAFAC analysis is programed to distinguish two peaks, the output scans usually contained two peaks of the same compound. These discrepancies were removed to more easily understand the output data.

Looking at both excitation signals there is evidence of a slight shift caused by the mixture and fluorescence. The mixture contains two compounds absorbing the energy provided by the lamp, compound **1** absorbs at a wavelength of 313 nm, whereas its standardized peak is at 315 nm. Compound **2**, at 244 nm, takes more energy to excite. Compound **1** experiences blue shift to 313 nm thru the influence of the absorption of compound **2**.

Like all other mixtures and single compounds, mixture **1** PARAFAC concentration analysis showed a direct relationship between concentration and intensity. The sudden intensity at the lowest concentration of the compound **1** PARAFAC concentration analysis output can be explained by the inner filter effect. When there are a great number of molecules in a solution (high concentration) light beams can become trapped within it bouncing off the surrounding molecules and trapping emitted photons. The monochrometer is unable to detect the intensity within the solution and thus it reports a low intensity. Upon lowering the concentration and number of molecules, the intensity spikes as light beams escape solution.

The JYS limit of detection was 125 ppb and was lowered to less than 62.5 ppb. As previously discussed, due to the limitations of the JYS, no dilutions were carried further than 62.5 ppb. However, PARAFAC establishes a value at this low concentration indicating it could go further. This improvement is less than that of compound **2** but equal to the improvement of compound **1.**

5.6 Mixture 2

Much like the counterpart mixture **1**, mixture **2** experienced shifting of excitation and emission peak maximums and increased intensity thru combine fluorescent signals. The blue shift occurred due to the influence of compound **2** on compound **1**. The PARAFAC analysis of the compound **1** concentration scans show a sudden increase and then decrease in the intensity. The inner filter effect could explain this non-trending relationship. The JYS determined the limit of detection to be 500 ppb and was lowered to 62.5 ppb with a 87.50% improvement, this is better than the improvement seen in compound **1** and compound **2**. This is due to the signal mixing, making it difficult for the JYS to distinguish between peaks, especially when the weaker compound, compound **2**, is the mixture component undergoing dilution. However, PARAFAC analysis is able to recognize even the smallest shift in trends.

5.7 Mixture 3

Mixture **3** utilized combined EEM ranges and thus allowed for observation of both peaks in one excitation and emission scan. The intensities in the excitation and emission scans of compound **1** experienced a slight increase in intensity compared to single compound intensities due to combined fluorescent signals. Compound **4** experienced a dramatic loss compared to the single compound **4** intensities, from 12 a.u. to 0.275 a.u. in excitation and from 55 a.u. to 40 a.u. This is likely due to the inner filter effect. Although compound **4** is a large and highly conjugated

molecule, compound **1** is entirely conjugated, thus in a combined EEM range, compound **1** will absorb more of the light energy. This effect is intensified as seen in the PARAFAC output of concentration intensities as the concentration of compound **4** decreases and the intensity of compound **1** increases with a steady concentration. The JYS limit of detection for mixture **3** was 6.25 ppb but this was decreased by 99.9% as the PARAFAC analysis was able to distinguish one one-hundredth of that concentration at 62.5 ppt. This substantial improvement is in part due to the dramatic intensity of compound **4**.

5.8 Mixture 4

Mixture **4** utilized combined EEM ranges and thus allowed for observation of both peaks in one excitation and emission scan. The intensities in the excitation and emission scans of compound **1** were both lower than that of the single compound intensities; this is due to the decreasing concentration of compound **1** in mixture **4.** Compound **1** experienced a dramatic blue shift due to the influences of compound **4.** Compound **4** experienced a dramatic loss in excitation compared to its single compound intensities, from 12 a.u. to 0.275 a.u. It also experienced a great increase in emission intensity. The loss is likely due to the inner filter effect. Although compound **4** is a large and highly conjugated molecule, compound **1** is entirely conjugated, thus in a combined EEM range, compound **1** will absorb more of the light energy. However, the emission intensity increase is most likely due to the decrease in compound **1** concentration. This effect is intensified as seen in the PARAFAC output of concentration intensities as the concentration of compound **1** decreases and the intensity of compound **4** increases while maintaining a steady concentration. The JYS limit of detection for mixture **4** was 1 ppm, much higher than the others due to the steady concentration of compound **4** completely blocking out the presence of compound **1** which was not even visible at 1 ppm. The PARAFAC analysis

improved the detection by 93.75%, down to 62.5 ppb. More than likely the limit of detection is even lower.

5.9 Mixture 5

Mixture **5** utilized combined excitation and emission ranges and this allowed for observation of both peaks in a single excitation and emission scan. Compound **1** showed increased intensity for both excitation and emission, this is due to its steady concentration. Compound **3** exhibited an increase in excitation intensity due to combined fluorescence absorption intensities, but a decrease in emission energy due to lessening concentration. However, the more interesting component of this mixture is the interaction peak seen from 225 to 240 nm. If particular mixtures create new peaks in the EEM matrix, these can be used to better identify the components of an unknown mixture.

The JYS limit of detection was 250 ppb was decreased to less than 62.5 ppb improving detection by 75.0%, this is equivalent to the JYS and PARAFAC limit of detection for compound **3.** This mirrored improvement shows PARAFAC can distinguish peaks in and out of mixtures.

5.10 Mixture 6

Mixture **6** utilized combined excitation and emission ranges and this allowed for observation of both peaks in a single excitation and emission scan. Compound **1** showed increased intensity for both excitation and emission, this is due to the combined fluorescent absorption. Compound **3** exhibited an increase in excitation intensity due to combined fluorescence absorption intensities, but negative emission energy. There is a hint of the interaction peak seen in mixture **5** from 225 to 240 nm. It is more faint due to the steady

concentration of compound **3**, the decreasing compound **3** in mixture **5** allowed the peak to be seen rather than hidden under the intensity.

The JYS limit of detection was 125 ppb was decreased to less than 62.5 ppb improving detection by 50.00%, this is equivalent to the JYS and PARAFAC limit of detection for compound **1.** This mirrored improvement shows PARAFAC can distinguish peaks with equivalent precision in and out of mixtures.

5.11 Mixture 7

Mixture **7** was characterized utilizing the standardized peaks of compound **2** and compound **4**. Mixture **7** showed an increase in intensity for compound **2** and a loss in intensity for compound **4** in excitation and emission scans. The repeated loss of intensity for compound **4** is due to the extremity of intensity. The other three compounds, alike in fluorescent intensity, appear greater in intensity when mixed because it is difficult to distinguish between similar peak signals, thus they are combined. The compound **4** signal is so intense it is entirely distinct from the other signals, thus in mixture combination does not enhance the signal of compound **4**, but hinders it. Compound **2** also experienced a red shift in the emission spectrum due to the intensity of compound **4**. Despite these difficulties, the excitation and emission scans show distinct peaks for compounds **2** and **4**.

Like all other mixtures and single compounds, mixture **7** PARAFAC analysis showed a direct relationship between concentration and intensity. Both PARAFAC analyses of the concentrations show compound **4** decreasing in intensity with shrinking concentration, while compound **2** demonstrates an inverse relationship between the intensities of the compounds. This is explained by the inner filter effect and signal hindrance. With a larger amount of molecules in solution, light energy is more easily trapped,

so as the concentration of one mixture component goes down, the intensity of the other increases despite being held at a steady concentration.

The JYS limit of detection for mixture **7** was 6.25 ppb and was improved by 99.90% down to one-thousandth of the original limit of detection, 62.5 ppt. However, PARAFAC established an intensity value at this low of a concentration indicating that it could go even further.

5.12 Mixture 8

The counterpart to mixture **7**, mixture **8** experienced red shift in the emission peak of compound **2** from the influence of compound **4**. There was also greater intensities seen in compound **2** and lesser intensities seen in compound **4**. The PARAFAC concentration analysis once again showed the direct relationship between intensity and concentration and the inverse relationship between compound intensities, especially exhibited in the compound **2** parameter scan. The compound **4** parameter scan had to have compound **2** removed, but demonstrates the relationship between intensity and concentration with a steady trend line from a compound of steady concentration. Thus far the data from mixtures with combined EEM parameters and those that utilized the parameters of single compound EEMs have reported comparable data with similar results, thus indicating compounds with incompatible excitation and emission ranges can be analyzed using this method. The JYS limit of detection for mixture **8** was 500 ppb and was improved by 87.50% down to one eighth of the original limit of detection, 62.5 ppb, although this limit is likely lower.

5.13 Mixture 9

Mixture **9** utilized combined EEM ranges and thus allowed for observation of both peaks in one excitation and emission scan. The intensities in the excitation scan, compound **2** experienced a decrease in intensity compared to single compound intensities. Compound **3** also experienced a decrease in intensities, although less so than compound **2**. Compound **2** is a large molecule with low conjugation likely loosing light energy to compound **3** which is highly conjugated. Thus in a combined EEM range, compound **3** will absorb more of the light energy. The emission scan also showed a decrease in intensity. There was also significant shifting in mixture **9**, it is uncertain why this particular mixture resulted in shifts of such magnitude also seen in greater magnitudes in mixture **10**. However, despite these shifting peak maximums and intensity decreases, the PARAFAC concentration analysis showed a strong direct relationship between intensity and concentration similar to other mixtures and single compounds. This would indicate that no matter how mixing changes the peak shape, wavelength placement, the intensities remain in a ratio that allows PARAFAC to interpret concentration correctly.

The JYS limit of detection for mixture **9** was 250 ppb and was improved by 75.00% down to one fourth of the original limit of detection, 62.5 ppb, but more than likely could be lower.

5.14 Mixture 11

Mixture **11** was characterized utilizing the standardized peaks of compound **3** and compound **4**. Mixture **11** showed an increase in intensity for compound **3** and a loss in intensity for compound **4** in excitation and emission scans. Other than the gains and losses in intensity, the excitation scans are unremarkable with compounds peaks in the standardized position. The emission scan of compound **3** experienced a red shift due to the emission of compound **4.**

Like all other mixtures and single compounds, mixture **11** PARAFAC analysis showed a direct relationship between concentration and intensity. Both PARAFAC analyses of the concentrations show compound **4** decreasing in intensity with shrinking concentration, while compound **3** demonstrates an inverse relationship between the intensities of the compounds. This is explained by the inner filter effect and signal hindrance. With a larger amount of molecules in solution, light energy is more easily trapped, so as the concentration of one mixture component goes down, the intensity of the other increases despite being held at a steady concentration. The JYS limit of detection for mixture **11** was 1 ppm and was improved by 93.75% down to one sixteenth of the original limit of detection, 62.5 ppb, but is likely lower than this.

5.15 Mixture 12

The counterpart to mixture **11**, mixture **12** showed greater intensities seen in compound **3** and lesser intensities seen in compound **4**. The PARAFAC concentration analysis once again showed the direct relationship between intensity and concentration and the inverse relationship between compound intensities, especially exhibited in the compound **3** parameter scan. The compound **4** demonstrates the relationship between intensity and concentration with a steady trend line from a compound of steady concentration (compound **4**) and a compound that should have no change in intensity due to the parameters used (compound **3**). The JYS limit of detection for mixture **12** was 250 ppb and was improved by 75.0% down to one fourth of the original limit of detection, 62.5 ppb, although this limit is likely lower.

Chapter 6: Conclusion

Overall the many mixture and single compound scans showed several patterns. The excitation and emission intensities of the compounds combined in mixtures and create more intense peaks, except for compound **4** which was so intense it is too distinct to combine. This combined intensity increases and decreases along with the concentration. In combined EEM parameters the intensities would be lowered in both compounds compared to the individual compound intensity. In mixtures that were scanned with individual compound EEM parameters, the intensity was less for the compound that was being diluted while the other remained similar to the individual compound intensity. The compounds often caused red shift and blue shift in mixtures, but were usually miniscule with one exception (mixture **9**). The inner filter effect also offered difficulties, sometimes skewing the intensity trend. Despite these difficulties, PARAFAC analysis is able to distinguish between peaks and determine the trend of their concentration dilutions. PARAFAC analysis can decrease limits of detection to environmentally relevant concentrations (part per billion) and beyond (part per trillion). Although the limit of detection is dependent upon the equipment and the structure and volume of the molecule, this research has shown PARAFAC can improve the LOD whether or not (1) the molecule is particularly fluorescent, or (2) if the spectrometer has a low capacity for detection. The most fluorescent molecules and those that are easiest to work with will be those that are large and highly conjugated, but a small molecule with limitedly conjugation can still be detected.

Pairing PARAFAC analysis with fluorescence spectroscopy is a plausible and useful tool for water contaminant detection down to the single part per billion and trillion dilution. It has been particularly evident that PARAFAC allows for the elimination of background noise interference. This was best represented by the successful determination of contaminant presence

beyond the limit of detection of the fluorescence spectrometer despite utilizing EEM scans that contained interference. It has been shown that when search for mixtures, although a hybridized EEM range yields the best and most cohesive results, scanning mixtures at their individual component's EEM parameters is a useful technique to avoid limiting the JYS's ability to scan any mixture.

The most obvious way fluorescence can rival GC/LC MS is by comparing the speed at which the analysis can be done. GCLC /MS scans take a long time, a few hours for one sample, but the greatest hindrance of quick analysis is availability of GC/LC MS. Most GC/LC MS analysis will take place in limited professional laboratories. Most samples needing to be analyzed will have to be mailed. The time it takes to get the samples to the facility, wait in the queue of priority for your samples to be analyzed, run through the GS/LCMS by a professional, analyzed by another professional, and finally written up and mailed back will take weeks at a time. The analysis performed for this experiment only took a few hours. Fluorescence may only be able to give an estimate to the concentration range, but the speed at which it can determine contaminant presence is the key factor in its usefulness. Detection of contaminant pesticides, additives, waste and run off can be determined quickly and thus allows for real time decisions concerning water health and its availability to the public.

Chapter 7: Future Directions

Continuations of this project would best be seen in expansion of the number of components in a mixture, compound type and field sampling. This project only explored mixtures of two compounds that experienced shifting, interaction peaks and fluorescent intensity variations. Increasing the amount of mixture components is likely to exaggerate these effects and offer a more challenging, but more extensive and realistic, dataset to analyze. To even further this experiment's validity compounds other than organic synthetics should be tested such as inorganic phosphor contaminants, contaminants in the presence of dissolved organic carbon species such as fulvic and humic acid and perhaps even non-fluorescent contaminants that could be marked with a fluorescent tag. Ultimately, the project should aim to include field sampling in its next installment in order to determine if the EPA standardized filtration methods interfere with the fluorescent monitoring process.

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