UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias e Ingenierías

Characterization of contaminants by different treatments for their removal in water: Use of Caffeine as a model compound

Artículo académico

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Química

Trabajo de titulación presentado como requisito
para la obtención del título de
Licenciada en Química

Quito, 21 diciembre de 2016

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

COLEGIO POLITÉCNICO

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Characterization of contaminants in water by different treatments: Use of Caffeine as a model compound

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Abstract

Samples of three Advanced Oxidation Processes AOPs such as Fenton, Photo-Fenton, and photocatalysis, and samples of an adsorption process: *moringa oleifera* were characterized at different times to monitor their caffeine removal process. An analytical method was developed through Reverse Phase High Performance Liquid Chromatography (RP-HPLC) for caffeine determination. The obtained calibration curve was found to be linear (R^2=0.99948) for a [5:100] ug/mL range. Caffeine's retention time varied between 10.5-10.7 minutes. Precision and reproducibility were achieved for the characterization method. The different methods have been tested as water treatments procedures for removal of organic compounds, being caffeine an organic compound model. The coagulation and flocculation process of *moringa oleifera* was found to be working up to 65% caffeine removal, and the AOPs were found to achieve up to 90% removal. Degradation of caffeine was monitored by UV for the Advanced Oxidation Processes. The different methods for caffeine removal were found to be statistically different ($p < 0.05$).

Keywords. Caffeine, AOPs, Moringa oleifera, Characterization, Removal, Fenton, Photo-Fenton, photocatalysis , HPLC

Resumen

Muestras de tres procesos de oxidación avanzada, AOPs por sus siglas en inglés, como Fenton, Foto-Fenton, Fotocatálisis, y muestras del proceso de abdsorción: *moringa oleifera,* fueron caracterizados para el monitoreo de su proceso de remoción de cafeína. Un método analítico fue desarrollado en Cromatografía de Alto rendimiento en modo fase reversa RP-HPLC para la determinación de cafeína. La curva de calibración obtenida presentó linealidad (R^2=0.99948) en un rango de [5:100] ug/mL. El tiempo de retención de la cafeína varió entre 10.5-10.7 minutos. Se alcanzó precision y repetitividad para el método. Los diferentes métodos fueron analizados como procesos alternativos en el tratamiento de aguas residuales para la remoción de contaminantes orgánicos, siendo la cafeína un modelo de contaminante. El proceso de floculación-coagulación de la *moringa oleifera* conllevó a una eliminación del 65%, mientras que los procesos de oxidación avanzada alcanzaron una eliminación del 90%. La degradación de la cafeína fue monitoreada mediante UV para los procesos de oxidación. Los diferentes métodos fueron hallados estadísticamente diferentes ($p< 0.05$).

Keywords. Cafeína, AOPs, Moringa Oleifera, Caracterización, Remoción, Fenton, Foto-Fenton, Fotocatálisis , HPLC

1. Introduction

Modern Instrumental Laboratory experiments

Kagel and Farwell [1], in 1983, described the quantification of the separation of the components of analgesics: acetaminophen (4-acetamidophenol) and aspirin (2-acetoxybenzoic acid), and caffeine (1,3,7-trimethylxanthine). Their experiment entailed mobile phase optimization for the quantification of aspirin and caffeine in Vanquish tablets. They found that with a C8 (25 cm, 46 mm i.d.) column, a methanol/acetic acid/ water mobile phase led to an optimal separation of the analgesics. However, in Kagel's experimentation replicate injections were not performed to minimize the error. Nowadays, Modern Instrumental Laboratory techniques involve the mean of a series of replicates for more accurate analysis, due to the feasibility of the determinations owed to the efficient columns and short running times.

This paper describes a modern characterization of caffeine removal in artificial water by four different methods: Fenton, Photo-Fenton, photocatalysis, and adsorption with *moringa oleifera* seeds. The chromatographic conditions described in this work yielded high efficiency and resolved peaks, and represent the application of modern instrumental laboratory experiments. In addition, even though normal phase LC is still used, this is a good example of how efficient a modern reverse phase liquid chromatography is, specially when it comes to the separation of polar organic compounds (analgesics).

Organic Contaminants: A Caffeine Model

Organic contaminants such as caffeine, hormones, antiseptics, etc. are not being highly removed in wastewaters in Ecuador [9,21]. Therefore, one of the main concerns in wastewater treatment should be the removal of different emergent contaminants, included the variety of organic compounds. The wastewater effluents from different types of industries contain a large pollutant quantity of organic compounds [3-6]. Even though, these compounds could be, in some extent, deemed as innocuous to the human being, they can certainly affect aquatic animal life and get into the food chain [21]. It is also known that wastewater contaminants could get into the soil

and increase the danger of poisoning when forage crops are grown [3]. Even though, caffeine might seem to be inoffensive, it is getting into our food chain. For example, according to Amiel [4] the use of wastewater to irrigate agricultural land is getting to be a common practice in countries where fresh water is limited.

Despite the fact that a lot of methods for organic compounds' removal have been developed for physical and chemical degradation [8], there is a growing need, especially in developing countries like Ecuador, for affordable and sustainable wastewater treatment procedures. Due to the need of a model of organic compounds' removal in wastewater, caffeine (Fig.1) was used as an example of a polar organic compound. In this sense, the present project could be used as a model for all kinds of polar organic compounds considered to be secondary contaminants [10,11].

Moreover, it is expected that the presented caffeine model could be used as a model for dye, or other pharmaceutical products removal in wastewaters. According to Beltrán-Heredia [13], over 50 000 tons of dye are discharged annually into effluents. This clearly states a problem with organic contamination. In the case of Ecuador, the lack of emergent contaminants' removal such as metals and organic compounds is a problem with unknown consequences, therefore is imperative to study alternative ways for water treatment in national conditions.

Caffeine, with the chemical name 1,3,7-trimethyl-xanthine and classified as belonging to the purines, was chosen as a model for all kinds of organic compounds due to its water solubility of 2.17%, which makes it a compound likely to persist in water [19]. Also, caffeine is part of a myriad of preparations such as analgesics and remedies, and it is also a popular additive of carbonated drinks [18,19]. Caffeine was also chosen since it is a chemical compound that can be naturally found in plant sources, and constitutes the most abundant contaminant in wastewaters in Quito, Ecuador (5597 μg/L) [21]. Caffeine is an emerging contaminant EC, which are compounds whose presence in the environment has not been noticed since little is known about them and their impact [11].

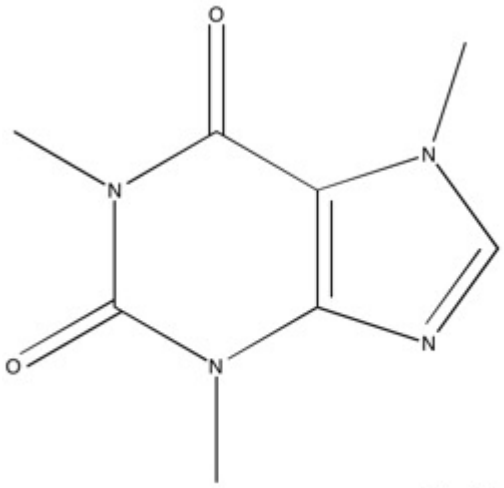

Figure 1. Caffeine (1,3,7-trimethyl-xantine) Structure

Advanced Oxidation Processes

AOPs are physical-chemical processes that generate and use transitory species such as hydroxyls (OH*), which take part in the oxidation of organic compounds [28-29]. The reduction potential of the hydroxyl radical OH* is high, which means it is a good electron acceptor [24]. Therefore, hydroxyl would attack caffeine and help to degrade it.

Fenton-like Processes

Caffeine can effectively scavenge hydroxyl radicals via Fenton reactions [14], suggesting a caffeine-derived radical formed in the reaction of caffeine with OH*, which could provide a biochemical basis to understand the anti-carcinogenic properties of caffeine [18].

Fenton's (Eq.1), and Photo-fenton's (Eq.2) oxidation have been found in a comprehensive review, Neyens and Baeyens [30], to be a very effective and promising method for the removal of hazardous organic pollutants from wastewaters.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^- \qquad (1)$$

$$Fe^{3+} + H_2O + h\upsilon \rightarrow Fe^{2+} + OH \cdot + H^+ \qquad (2)$$

Photocatalysis: TiO_2

Photocatalysis with TiO_2 involves a very similar process to Fenton, with the difference that here the TiO_2 constitutes the catalyst, a semiconductor that will absorb light (UV in this case), and enhance the destruction of the pollutant without the catalyzer being affected [28]. The excited electrons of the TiO_2 would then take part in the oxidation-reduction process, with the absorbed species OH-, water, and oxygen [25]. In this photocatalytic process, the radical OH* will be formed, and would also be the responsible of the degradation of caffeine due to its reduction potential (+2.8V @25ºC) [23].

Moringa Oleifera MO

Moringa Oleifera, depicted in Figure 2, is native to South Asia, but it is a crop widely cultivated across the tropics [20]. Hence, moringa plantations do exist in Ecuador (Pedernales, Manabí), so this crop could be used as an alternative option for wastewater treatment based on its adsorption potential [20-21]. Moreover, previous studies [13,21] have shown the heavy metal removal potential of this crop in wastewaters.

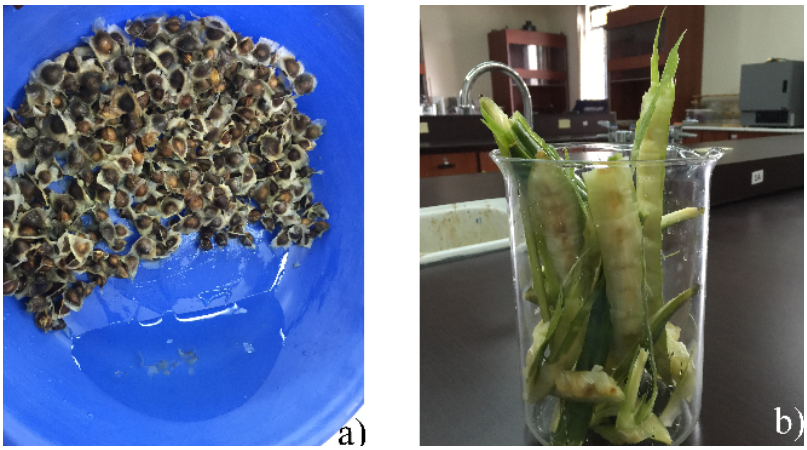

Figure 2. Moringa Oleifera a) seeds, b) plant. Photography taken by Jaime Cahuasquí.

The use of Moringa oleifera has been previously reported [13,20-22], and the coagulant/flocculant ability of the seed extract has been found promising, due to its lack of external dependency on reagents such as $Al_2(SO_4)_3$. The fact that moringa oleifera adsorption is not technologically difficult in operation, has made this process an efficient way of wastewater treatment, especially in developing countries [22].

Overview

The mobile phase for the analyses should be prepared at least the day before the day of the analyses. It is better if the mobile phase is also used as the solvent in the solutions preparation, however, E-pure-grade water can be used as well. Approximately 6-7 hours provides sufficient time to run the chromatographic analyses with the samples previously provided the day before.

The HPLC system requires at least 45 min before the first injection to equilibrate. Triplicate injections of all four caffeine standard solutions and samples should be performed for statistically relevant data and accuracy. In the analysis of the data, a relative peak area versus caffeine concentration graph should be generated for concentration determination.

2. Experimental Procedure

Reagents

Caffeine 97% m/m (purchased from Quifatex), Caffeine 99% m/m (purchased from Aldrich), TiO_2 (Merck), H_2O_2 33% m/m (Merck), ferrous sulfate heptahydrate 99% (Aldrich), sodium thiosulfate 99.5% (Aldrich), HPLC grade Methanol (Fisher Scientific), and glacial acetic acid (Fisher Scientific) were used as received without any further purification. E-Pure-grade water was produced in the laboratory, and used to prepare all the solutions in all experiments.

Apparatus and Operating Conditions: HPLC

High-performance liquid chromatography HPLC analyses were conducted using a Buck Scientific BLC-10/11 system equipped with a manual injection valve with a 20 μL sample loop, and a fixed-wavelength UV detector set at 254 nm, as depicted in Figure 3. An isocratic pump was used to deliver the eluent at a constant rate of 1 mL/min. The detector was set for 10-mV output, and resolution was set to 0.5.

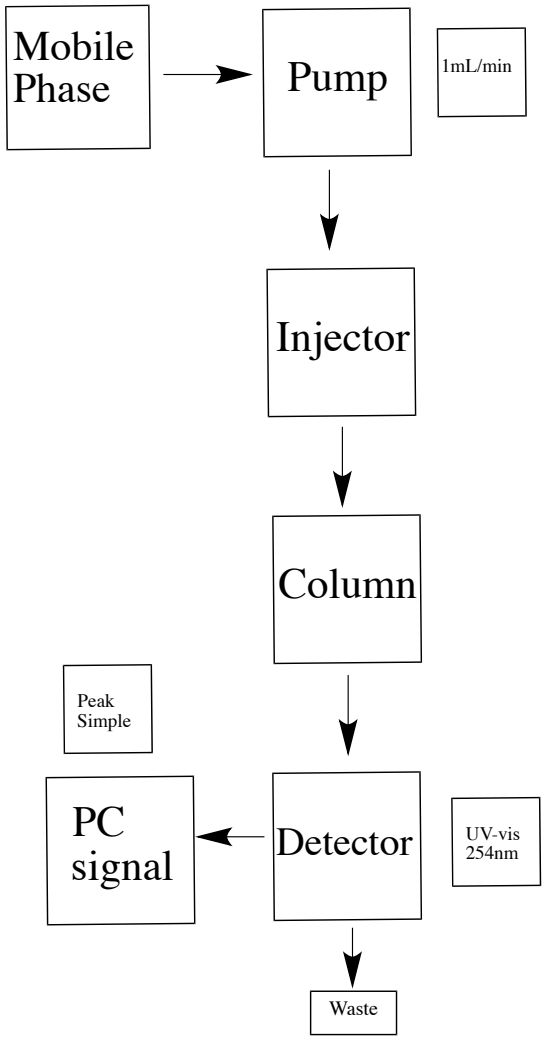

Figure 3. HPLC setup.

A Pinnacle II C18 (5-μm particle size, 150 mm x 4.6 mm i.d.) reversed-phase (RP) analytical column with no guard column was used at room temperature. A PeakSimple (4.26 version, 6 channels) integrator was operated to record chromatograms and peak areas. The computer software used was PeakSimple (4.17 version).

Mobile Phase

The mobile phase used for all experiments was a mixture of 34:15:1 (v/v/v) water: methanol: acetic acid. It was prepared by placing 300 mL of methanol and 20 mL of acetic acid water into a 1-L volumetric flask and diluting to volume with E-pure water. The mobile phase was mixed and filtered with PVDF 0.45 μm (Millipore, Millex-HV) nylon filter membrane, and degassed for 30 minutes with ultrasonic cleaner (model 5510R-DTH) prior use. An isocratic elution was performed with a flow rate of 1 mL/min.

Reverse phase-ion suppression mode was used for the separation. Hence, acetic acid was added to the mobile phase as a buffer to suppress ionization of future analgesics to be analyzed in future work (i.e. salicylic acid). This way a buffered mobile phase would promote the retention time of the acids relative to the bases (caffeine) [1]. The radio of methanol and the buffered solution used was the one reported by Kagel [1], so that proper isocratic conditions would be obtained.

Apparatus and Operating Conditions: UV-Vis

Ultraviolet (UV) absorbance measurements were performed using a UV-VIS Cecil (model CE2041) equipped with quartz cell (1 cm path length). Absorbance was measured with a water baseline correction. The scan rate used was 300 nm/min with a data point interval of 0.5 nm, in a 200-400 nm wavelength range.

Preparation of Caffeine Standard Solutions

Dilutions were made to give rise to a 100 μg/mL caffeine stock solution. Serial dilutions were performed in 100 mL flasks to prepare 5, 10, 50, 100 μg/mL solutions used later to obtain the calibration curve. Standards were degassed for 30 minutes with ultrasonic cleaner (model 5510R-DTH) prior injection. Approximately 3 mL of each solution were filtered with nylon syringe filters (Millipore, Millex-HV) and placed in PCR vials.

Preparation of Fenton and Photo-fenton Samples

The pH of a caffeine aqueous solution (100 μg/mL) was adjusted to 2.9±0.2 with sulfuric acid 0.1 M. Under stirring, ferrous sulfate (1-10 mg) and hydrogen peroxide (0.5mL) were added. For Photo-fenton process, the solution was irradiated by a monochromatic lamp (254 nm). 1.5 mL aliquots were withdrawn from the aqueous solution at different times for a one-hour period. Reaction was stopped with an excess of sodium thiosulfate. Aliquots were filtered with nylon filters and protected from light for approximately 12 hours in a 4 ºC refrigerator prior HPLC analysis.

Preparation of Photocatalysis (TiO_2) Samples

The pH of a caffeine aqueous solution (100 μg/mL) was not adjusted. 0.1 g of TiO_2 was added to the aqueous solution. The solution was irradiated by a monochromatic lamp (254 nm). 1.5 mL aliquots were withdrawn from the aqueous solution at different times for a one-hour period and submitted to centrifugation at 3000 rpm. Samples were filtered with nylon filters and protected from light for approximately 12 hours in a 4 ºC refrigerator prior HPLC analysis.

Preparation of Moringa Oleifera Samples

A caffeine aqueous solution (100 μg/mL) was used without any pH adjustment. Different procedures were characterized in order to find the most successful method of adsorption: *moringa oleifera* seed extract. Samples provided for HPLC analysis, were treated with NaCl 1M in order to extract the *moringa oleifera* protein. Aliquots were withdrawn from the aqueous

caffeine-seed extract solution at different times, filtered, and protected from light in a 4ºC refrigerator prior HPLC analysis.

Calibration Curve: Collection and Treatment of Data

Triplicate injections were performed with each caffeine standard solution. Peak areas were recorded with PeekSimple (4.26 version, 6 channels) integrator. However, manual integration was carried out for the caffeine peak (10.7 min). Results were exported to Microsoft Excel 2011, and the calculated average areas at each concentration were used. Relative peak areas were calculated by dividing each average area by the smallest recorded area. A calibration curve of relative peak area against concentration (µg/mL) was plotted using Microsoft Excel 2011. A concentration equation was depicted from the calibration curve.

Quantitative Analysis of Samples

All samples were analyzed by triple injections. Areas were recorded the same way as with the standard caffeine solutions. Each caffeine peak area value was divided by the smallest area of the standards, and the calibration curve equation was used in order to determine the caffeine concentration at each sample time. Software Minitab 17 was used in order to find differences between all the methods that were characterized.

3. Results and Discussion

Determination of Caffeine's retention time and Limit of Detection LOD

Detection Limit LOD is a fundamental part of method validation, and several estimation methods for this limit have been implemented. Herein a method based on the dispersion characteristic of the regression line was used (Eq.3).

$$LOD = 3.29\sigma \quad (3)$$

Where σ represents the dispersion found between the values in the calibration curve.

Official guidelines for HPLC analyses present different techniques for LOD calculation, but they are all based in the fact that LOD is the smallest quantity of analyte of which can be said that is present in the sample [32]. Figure 4 shows how LOD depends on two risk values α,β set to 5%, which means that a confidence interval of 95% would be used for the analyte concentration. The critical value Lc equals to zero, so that it is fixed and the LOD solely depends on β. While α represents the risk of detecting the analyte when it is not present, β represents the risk of not detecting the analyte when it is present in the sample [31]. As shown in Fig.4, a Gaussian distribution is assumed since the dispersion σ is constant in the blank.

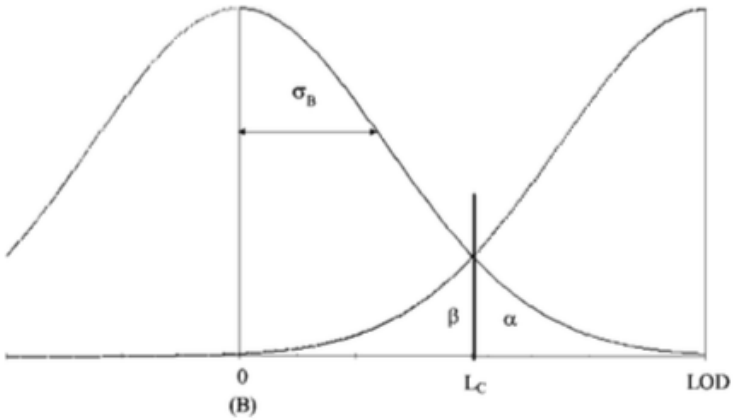

Figure 4. Representation of LOD [retrieved from ref 31]. LOD depends on the risk values α,β set to 5%, σ_b was estimated by the standard deviation of the intercept of the regression line.

In the present paper, a LOD of 5.336 μg/mL was found for caffeine. However, visual LOD was found to be 5 μg/mL. LOD was calculated with equation 3, using ordinary least Squares Regression Data at four levels of significance (5-100 μg/mL). A regression line of the chromatographic peak area against caffeine concentration was used, assuming independence of the area dispersion in relation to caffeine quantity [31,32]. The standard deviation of the blank used in equation 3 was estimated by the standard deviation of the intercept, so that the calculated

LOD corresponds to the caffeine concentration for which the peak area is equal to 3.29 times the chosen standard deviation, as shown in eq.3.

Caffeine's retention time *rt* was found to be in the range of 10.5-10.7 minutes as shown in the displayed Chromatogram of Fig.5. Retention time for caffeine was found to be constant in all the samples through visual check.

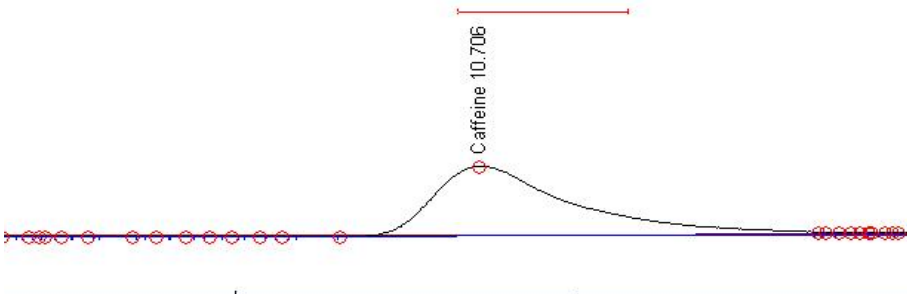

Figure 5. Caffeine retention's time rt. Displayed Chromatogram.

Resolution

The Buck Scientific BLC-10/11 HPLC system used for the experiments was equipped with a resolution channel, so a visual resolution analysis was carried out. Standards of caffeine were injected, and retention times were recorded. Figure 6 shows some of the resolutions tested; it was found that the lower the resolution used, the higher the signal recorded by the HPLC. No significant differences were found in terms of accuracy when using different resolutions. A resolution of 0.5 was set for all the experiments reported in the present paper, due to its clean chromatograms and stability of the calibration curve.

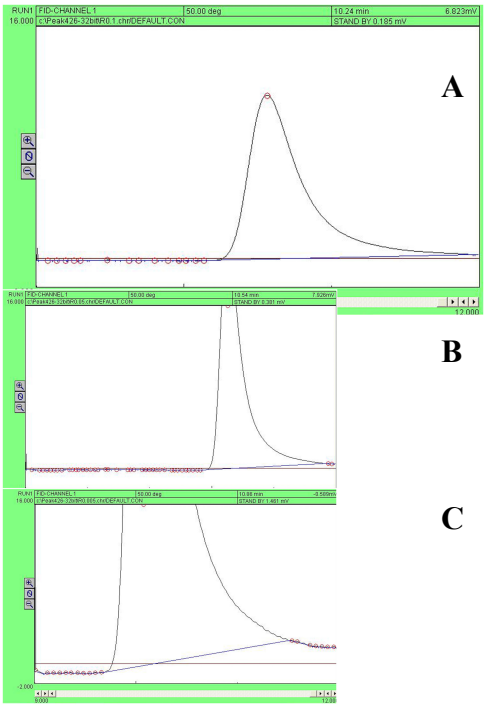

Figure 6. Caffeine peak at different resolutions. **A:** 0.5, **B:** 0.05, **C:** 0.005.The lower the resolution, the higher the signal recorded by the HPLC.

Calibration Curve

During each Standard solution run, area of the caffeine peak was recorded from the integrator in order to obtain a calibration curve as shown in Table 1. The average area at each concentration was calculated and divided by the smallest are to give rise to relative peak areas. A calibration curve was obtained, by plotting relative peak area against caffeine concentration (Figure 7). The average areas were reported as intra-day areas for the repeatability analysis.

Caffeine Calibration curve was found to be linear in the [5-100 µg/mL] range. The calibration plot was obtained with Microsoft Excel 2011.

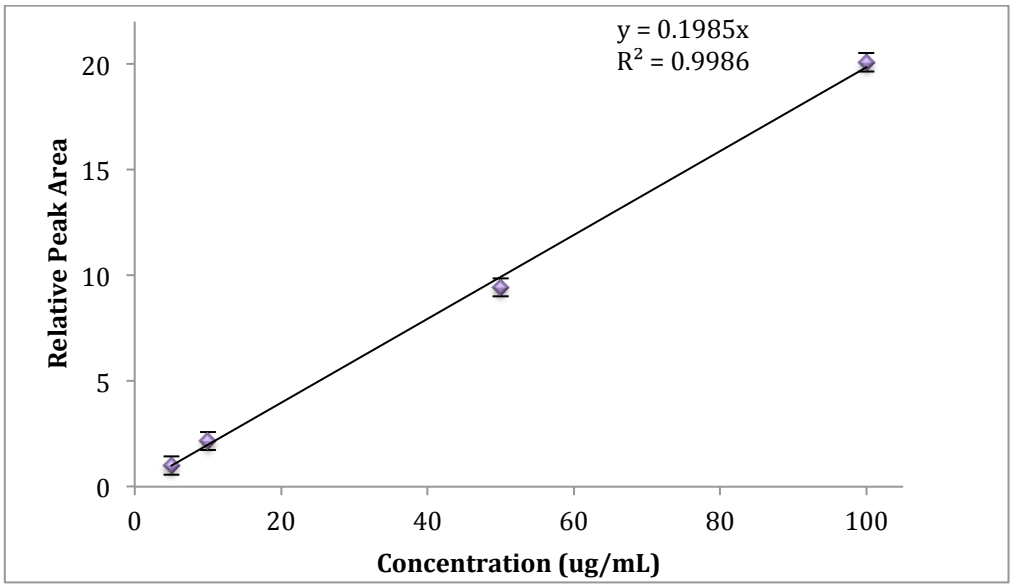

Figure 7. Caffeine Calibration curve.

Table 1. Recorded Caffeine-Peak Areas.

Concentration (µg/mL)	Peak Area			
	5	10	50	100
	Level 1 Response	Level 2 Response	Level 3 Response	Level 4 Response
1	1.533	4.727	19.50	40.96
2	2.173	4.308	19.58	42.69
3	2.532	4.432	19.75	41.55
Intra Day (Average)	2.0791	4.489	19.61	41.73
SD	0.4133	0.1759	0.1044	0.7186
RSD	0.1988	0.03918	0.005325	0.01722
Relative Peak Area	1.000	2.159	9.433	20.072
Average SD	0.3530			
Slope	0.1985			
LOD	5.336			

Repeatability and Reproducibility

The accuracy of the characterization method used in this paper was tested through repeatability and Reproducibility, two fundamental elements of method validation. For this purpose, Intra-day data was recorded by triplicate at different periods of time for a four months period, and an Inter-day average was calculated as depicted in Table 2.

Table 2. Recorded Caffeine-Peak Areas for Method Validation. Stability of Peak Areas.

Concentration (µg/mL)	Peak Area			
	5	10	50	100
	Level 1 Response	Level 2 Response	Level 3 Response	Level 4 Response
Intra Day 1	2.446	4.283	20.073	43.29
Intra Day 2	2.329	4.180	20.14	43.74
Intra Day 3	2.282	4.752	20.16	41.98
Intra 1 Week	1.875	4.658	19.88	40.35
Intra 1 Month	2.0791	4.489	19.61	41.73
Intra 4 Months	1.844	3.704	18.21	39.66
Inter Days (Average)	2.143	4.344	19.68	41.79
SD	0.228	0.3477	0.6813	1.455
RSD	0.1062	0.08005	0.03462	0.03481
Average SD	0.6779			

As found in Table 2, the method presented in this paper is robust, which means it can be done at different times and stability in the calibration curve would remain, as shown in Figure 8.

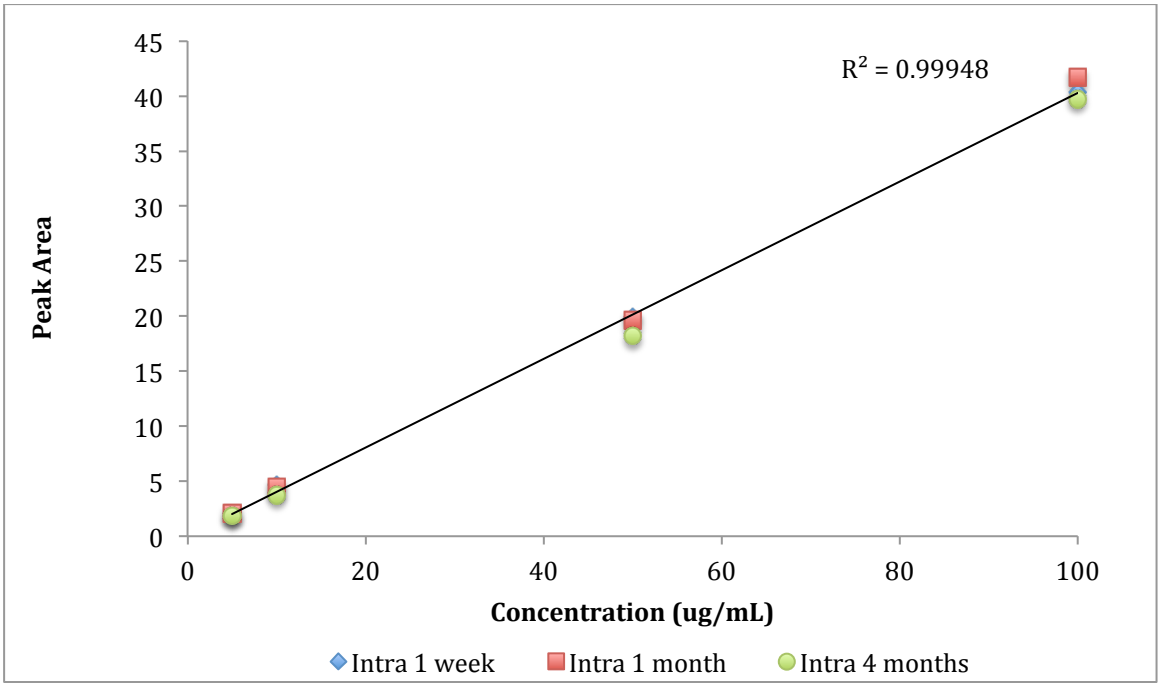

Figure 8. Caffeine Calibration curve stability.

The stability of the calibration curve can be explained by the HPLC instrument stability, which was achieved by using the same instrumentation for mobile phase preparation, and the same caffeine for all the analysis. The stability found in the calibration curve does not only show that the method used in this paper is robust, but it also suggests that a calibration curve does not have to be obtained every time a sample is to be analyzed. When using a different brand caffeine (Aldrich) with higher purity a stable calibration curve (Figure 9) was also obtained, but higher peak areas were recorded due to its higher purity (99%). However, the calibration curve reported in Figure 8 was used for the analysis due to its linear fit (R^2=0.9995).

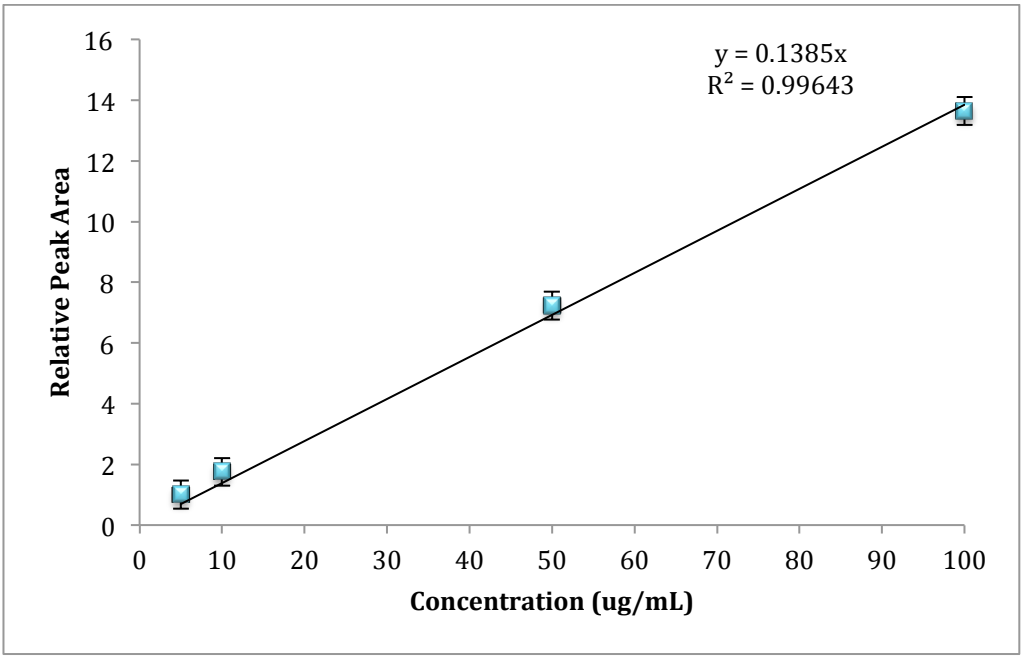

Figure 9. Caffeine (Aldrich) calibration curve.

AOPs Samples Characterization

For Fenton, preliminary experimentation was carried out using commercial H_2O_2 in a 10 µg/mL caffeine aqueous solution (Fig.10). It was found that purity of the Hydrogen peroxide is important in the caffeine degradation, and that higher caffeine removal is observed with a purer peroxide solution.

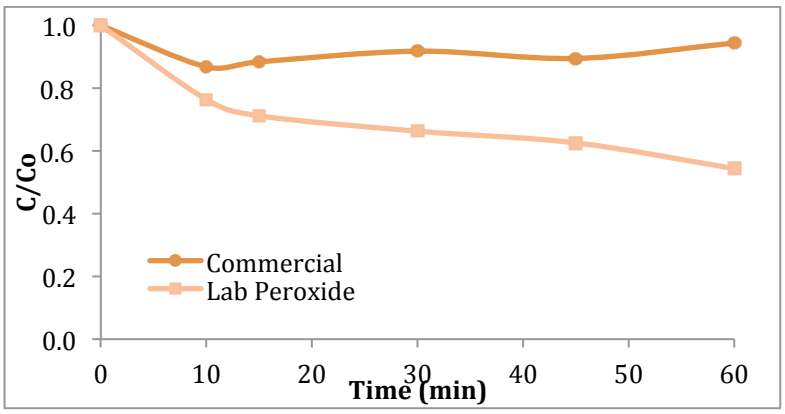

Figure 10. Caffeine removal with Commercial Hydrogen Peroxide vs. Laboratory 30%m/m Peroxide.

Due to its higher caffeine removal, pure Hydrogen Peroxide was chosen. Different concentrations of FeII were used for the Fenton degradation (Fig.11). It was found that higher Fe (II) concentrations led to a higher caffeine removal. When 30 mg of Fe were used a 100% caffeine removal was recorded, this might be because of a removal out of the LOD or because of a faster degradation that could not be monitored through HPLC.

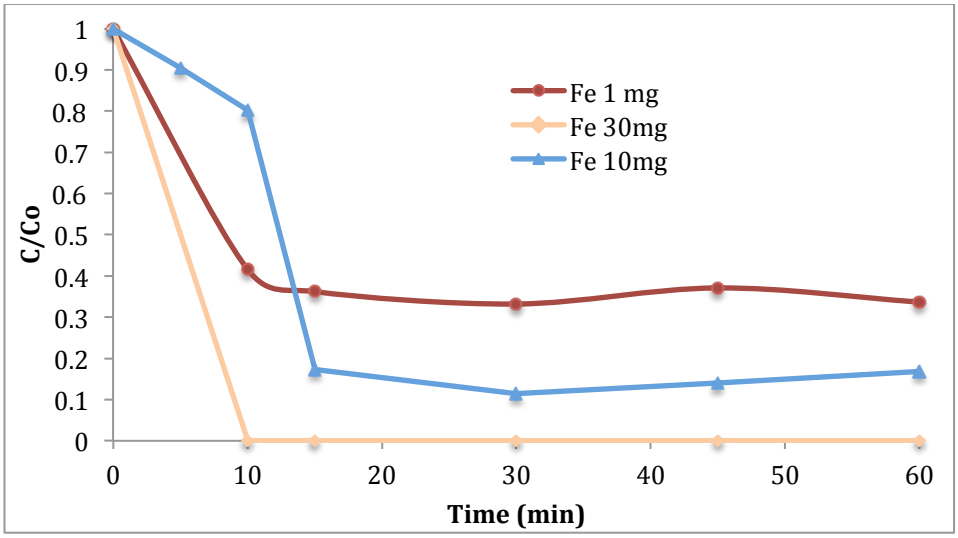

Figure 11. Fenton Process. Higher caffeine removal was achieved with higher concentrations of Fe.

For Photo-fenton a similar kinetics to that observed in Fenton process was obtained (Fig.12). Moreover, higher caffeine removal was accomplished with photo-fenton due to the light irradiation that speeded up the caffeine degradation [26].

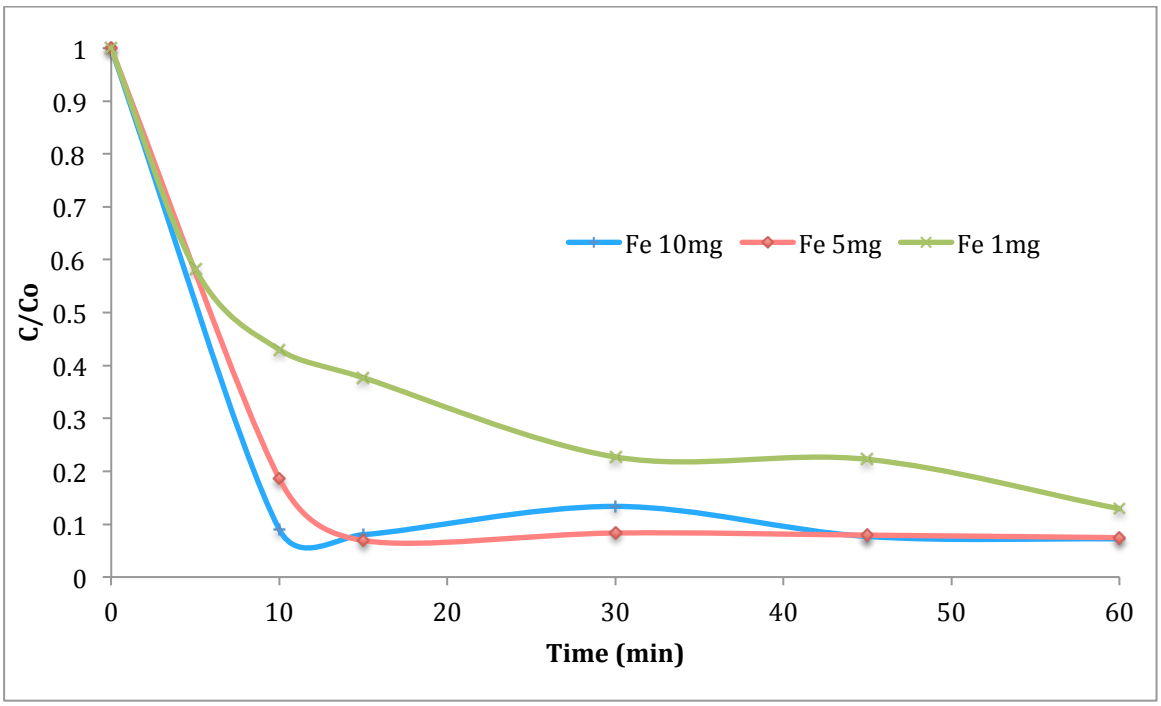

Figure 12. Photo-fenton Process. Higher caffeine removal (up to 95%) was achieved with higher concentrations of Fe.

When compared to the Fenton-like processes, photocatalysis with TiO_2 resulted in the lowest caffeine removal (53%) (Fig.13). This can be explained by the fact that no pH control was carried out in the photocatalytic process. All processes were compared with the UW/Caff/H2O, showing that Fe II has an important role in caffeine degradation when compared to Hydrogen Peroxide, and that is the reason why Fe II concentration was chosen to be varied throughout the experimentation protocols. In addition, Toscano [28] reported similar dye removal percentages with a similar photocatalytic method, so we believe that further experimentation with TiO_2 would lead to slightly different caffeine-percentage removals.

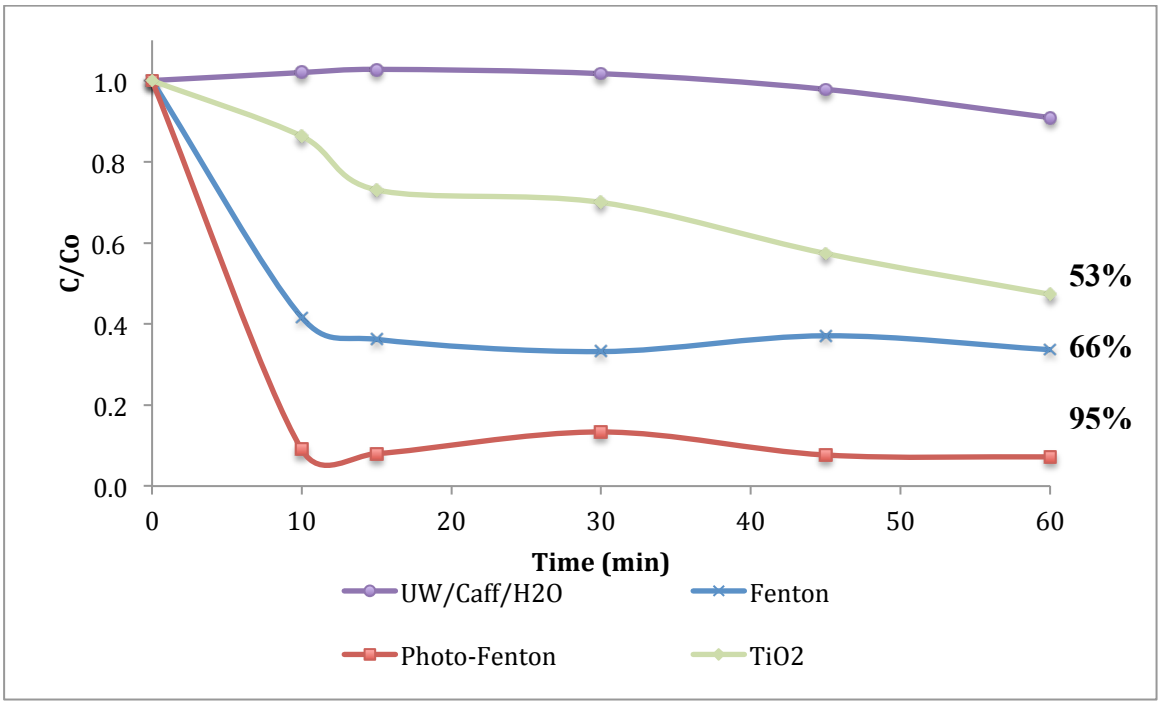

Figure 13. AOPs Comparison. TiO_2 yielded a 53% caffeine removal, Fenton 66%, and Photo-fenton 95%.

Moringa Oleifera Samples Characterization

For *moringa oleifera* different treatments were applied for the seeds. However, as depicted in Figure 14, a higher removal was found with the use of NaCl protein-seed extracts as described in the experimental section (Fig.15).

It was found that *moringa oleifera* yielded a higher caffeine removal when compared to TiO_2, but a lower removal when compared to the other AOPs. However, due to the simplicity of the *moringa oleifera* procedure, it can be easily used as a method for wastewater treatments.

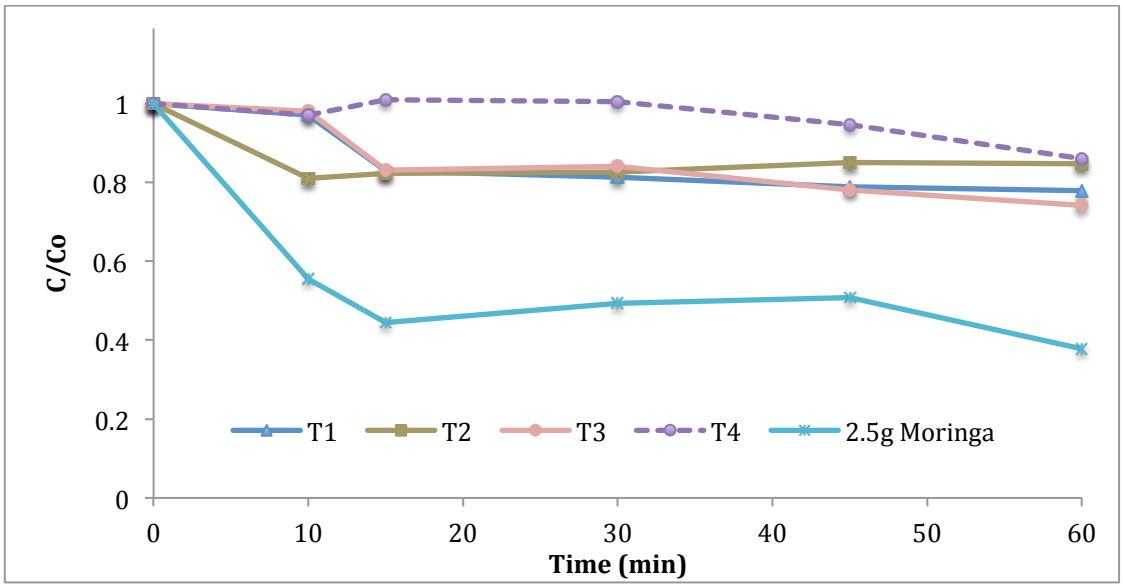

Figure 14. Moringa Oleifera Adsorption. Different treatments (T1-T4) were applied, however higher caffeine removal (65%) was achieved with NaCl- seed extract (2.5g Moringa).

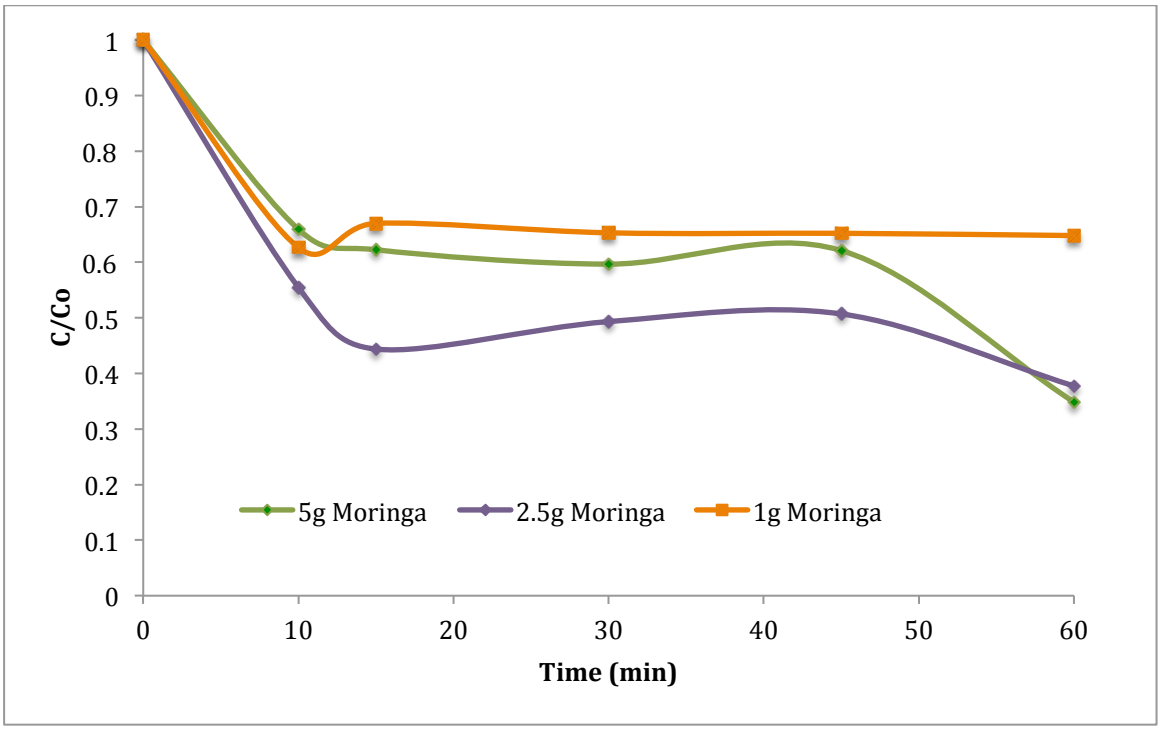

Figure 15. Seed extract Caffeine Removal. 62-65% caffeine removal was achieved with different quantities of *moringa oleifera* NaCl- seed extract. Higher concentrations of extract yielded higher caffeine removal.

Moringa Oleifera seed extract versus AOPs

Through UV-Vis spectra, differences between AOPs and MO methods were observed.

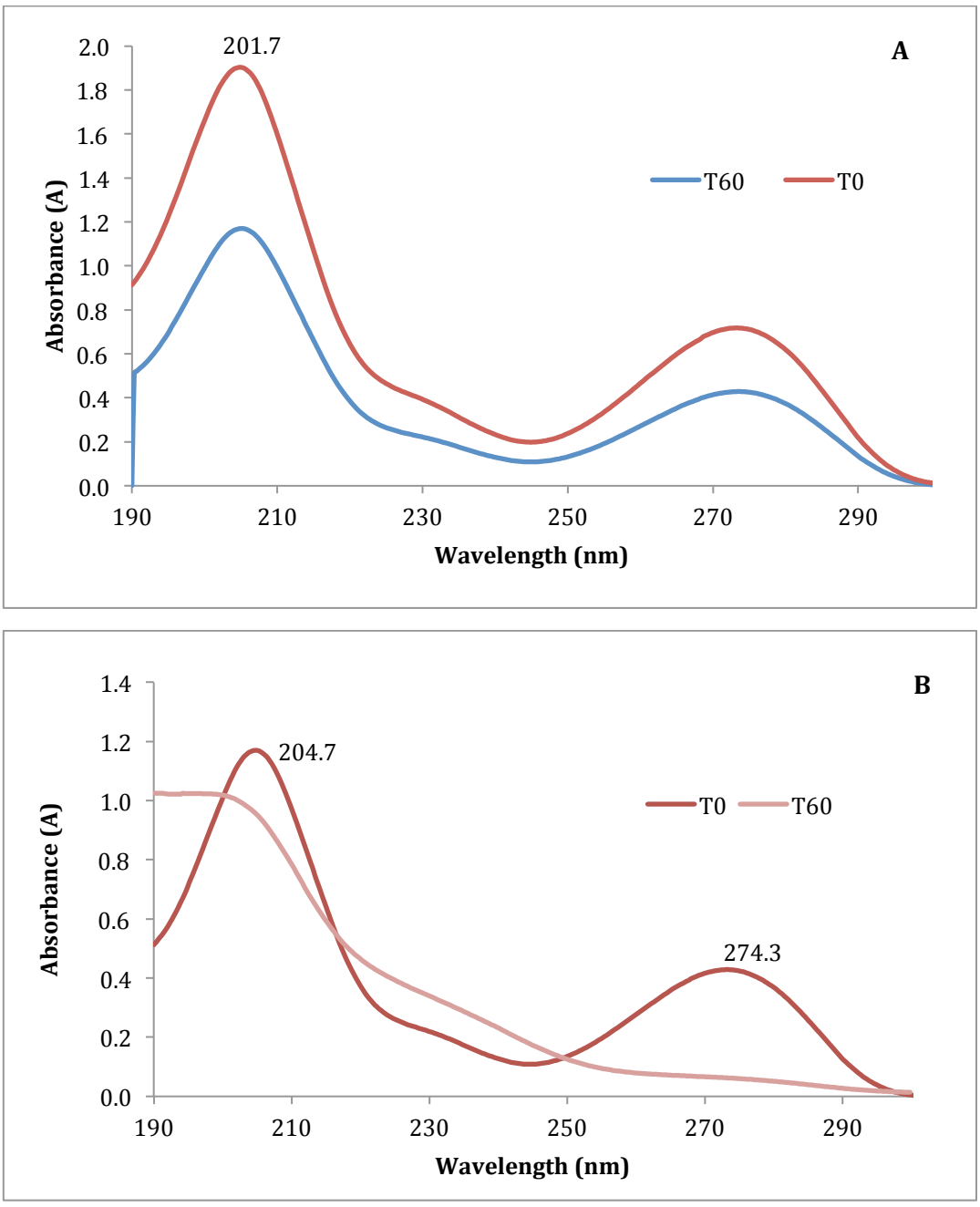

Figure 16. UV spectra of Moringa and Photo-fenton samples. A: caffeine removal with *moringa oleifera* was achieved through an adsorption process. **B:** caffeine removal in Photo-fenton was achieved through a degradation process.

UV spectra showed that as proposed, Moringa is an adsorption process while Fenton-like processes are based in the degradation of the organic compound as observed in Fig.16

The different methods were found to be statistically different ($p<0.05$) (Fig.17), and a Fisher test was carried out (Fig.18) for the means, which were found to be different. No significant differences were found between TiO_2 and Moringa (1g of seed extract), which is logical since they both led to similar caffeine removal percentages.

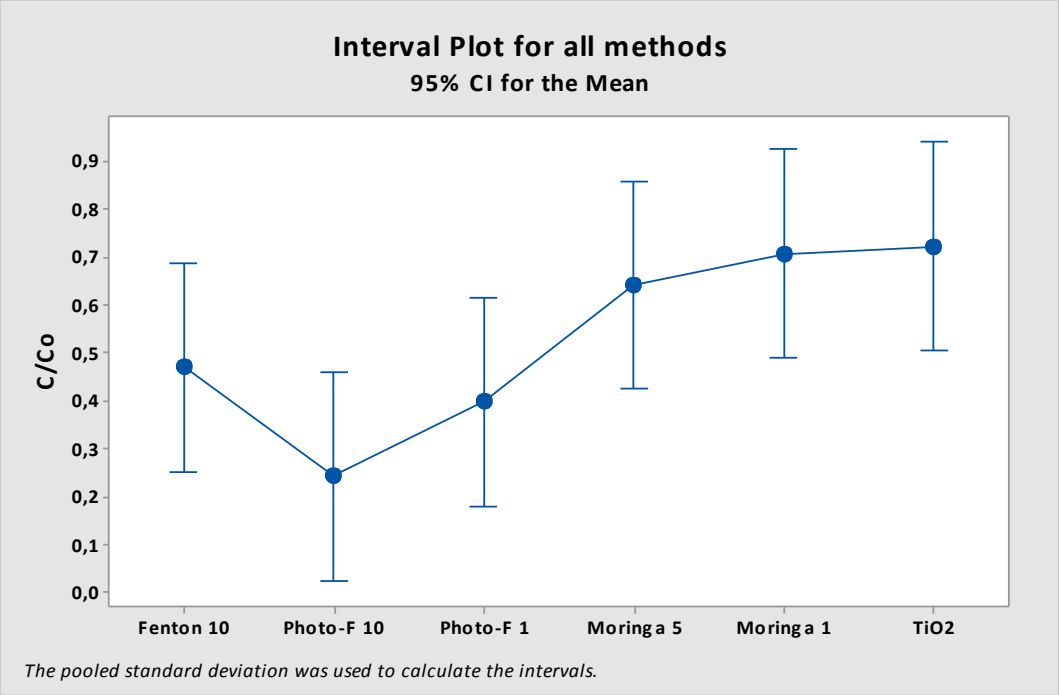

Figure 17. ANOVA. 95% CI for the mean of different methods. The most representative methods were found to be significantly different

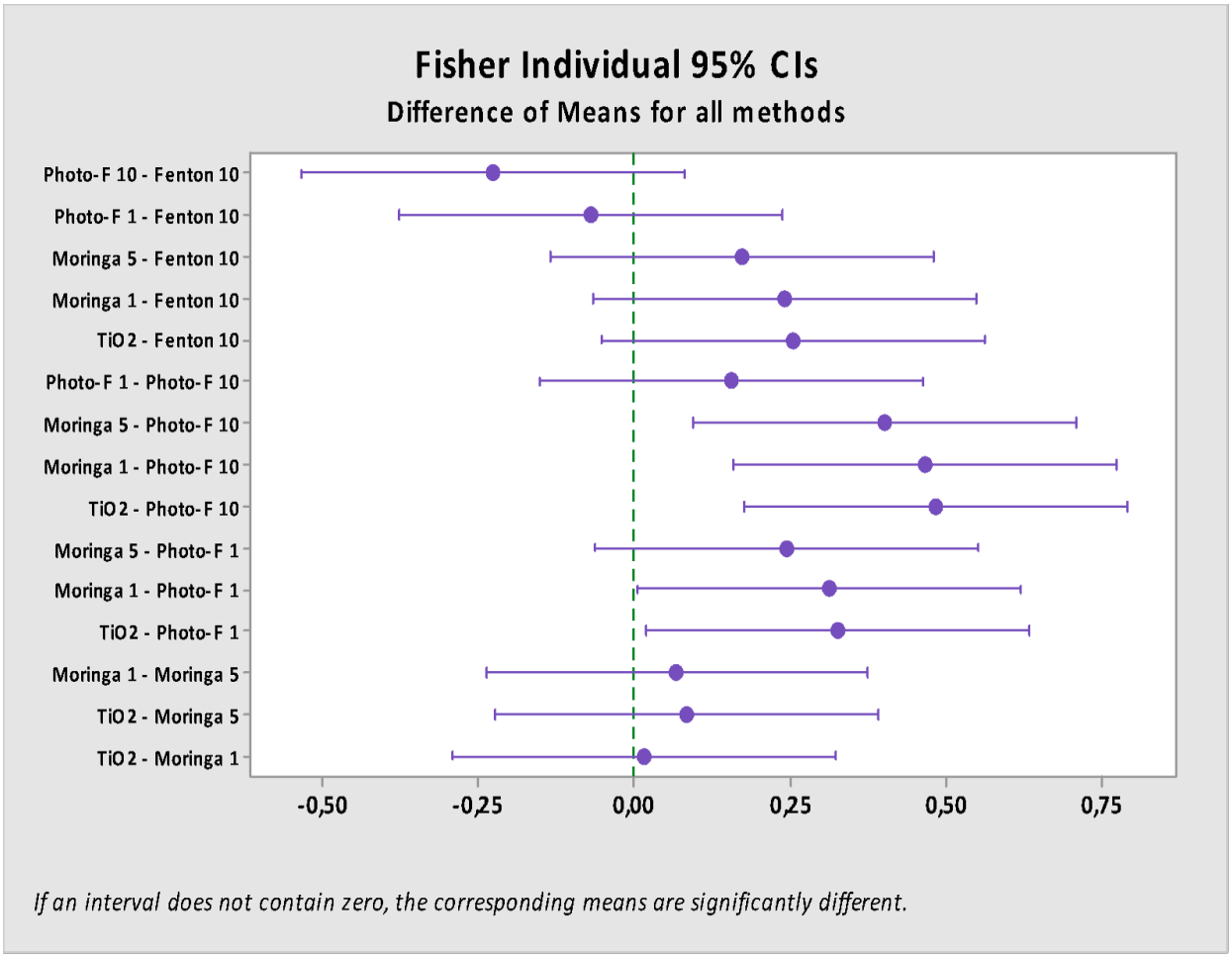

Figure 18. Fisher's test results. If an interval does not contain zero, the corresponding means are significantly different. Moringa (1 gram) and TiO_2 were found to be similar due to their similar caffeine removal.

4. Conclusions

- *Moringa oleifera* has been found to be effective in caffeine removal through the flocculation/coagulation process. It may be preferred over AOPs due to the feasibility of the process without much training.
- Higher caffeine removal was achieved with AOPs, and in the case of Fenton-like processes a dependency with Fe II concentration was found.
- The characterization process herein described was found to be reproducible, and stability of the calibration curve was found.
- Significant differences between the four methods were found through statistical analysis. However, TiO_2 and the lowest concentration of *moringa oleifera* were found to be similar in terms of caffeine removal.
- HPLC is an effective way of caffeine degradation monitoring due to its stability. The calculated LOD was found to be similar to the observed LOD (5 ppm). Method validation was achieved through repeatability and reproducibility in a 6 months period with two different caffeine sources.

5. Acknowledgments

I would like to thank the Collaboration Grant 2015 of Universidad San Francisco de Quito USFQ for financial support; with these funds HPLC filters were purchased. I am also grateful to my advisor David Egas for his support through all of this project, and to Andrea Landázuri, and Rodny Peñafiel for helpful experimental and editorial suggestions and comments.

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