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Efficient and Low-cost Extraction Methods for Pharmaceutical Compounds of Carbamazepine and Caffeine

Najihah Mohamad-Nasir[1], Suhaimi Abdul-Talib[2], Nurul Fariha Lokman[2], Saiyidah Nafisah Hashim[3], Chia Chay Tay[1,2*]

[1] Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, 40450, MALAYSIA.
[2] myBioREC, Faculty of Civil Engineering, Universiti Teknologi MARA, Shah Alam, Selangor, 40450, MALAYSIA.
[3] Faculty of Applied Sciences, Universiti Teknologi MARA, Tapah, Perak, 35400, MALAYSIA.
**Corresponding Author (Tel: +60123625809, taychiay@uitm.edu.my, taychiay@gmail.com)*

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Abstract

This study focuses on determining the efficient extraction methods between Liquid-liquid Extraction (LLE) and Solid Phase Extraction (SPE) for carbamazepine and caffeine. Optimization on methanol volume was investigated and evaluated through average recovery percentage. Overall, the analytical average recovery percentage of carbamazepine and caffeine was greater than 80% for LLE, while the average recovery percentage for the SPE method was very low (5-35%). Optimum methanol volume for carbamazepine and caffeine extraction was determined at 11 mL. In conclusion, the low-cost LLE method using 11 mL methanol was selected as the efficient method and optimum methanol volume for carbamazepine and caffeine extraction. Statistical analysis showed a significant difference between the methods. The finding sheds light on the reliable and consistent result in further instrumental analysis.

Disciplinary: Environmental and Pollution Sciences & Technology, Chemistry.

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1 Introduction

Carbamazepine and caffeine are pharmaceutical compounds widely used for medical purposes. Carbamazepine is prescribed for bipolar disorder, epilepsy, and schizophrenia treatment. Meanwhile, caffeine is used in combination with acetylsalicylic acid and paracetamol for migraine treatment [1, 2, 3]. The bioavailability of carbamazepine and caffeine after human consumption is

found to be excreted into wastewater. Since there are no treatment methods available, these compounds eventually flow into the water stream and cause negative effects on aquatic organisms and the environment [4]. It was reported that bluegill fish *Lepomis macrochirus* produced high cortisol due to stress factors when exposed to carbamazepine [5]. Caffeine resulted in the death of zebrafish embryos when caffeine concentration in water was higher than 300 mg/L [6]. To minimize such impacts on aquatic organisms and the environment, analysis and treatment of water and wastewater are addressed.

Analysis of water and wastewater contaminated with carbamazepine and caffeine is a crucial step before the treatment method is considered and decided. In general, current technologies for the analysis of carbamazepine and caffeine concentration are conducted using high-end instruments such as gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). Both GC-MS and HPLC provide high resolution and sensitivity analysis [7]. Since water and wastewater are aqueous solutions that contain mud, soil residue, and microorganisms, direct analysis is impossible as it damages the analytical column of GC-MS or HPLC [7]. Thus, eliminating the effects of contaminants to perform high sensitivity and resolution analysis requires careful precautions in sample handling. An extraction method needs to be conducted before analysis to separate the low molecular weight carbamazepine and caffeine from a mixture of solutions is done.

Liquid-liquid Extraction (LLE) and Solid Phase Extraction (SPE) are examples of extraction methods that are widely used in research. LLE is a solvent extraction method that separates compounds based on the solubility of the compound in two different solvents which are usually compound in an aqueous solution into an organic solvent. SPE is defined as an extraction method that uses solid and liquid phases to isolate a compound from a solution. Both LLE and SPE have been extensively used in the extraction of pharmaceutical compounds [8, 9, 10, 7, 11]. LLE is the low-cost conventional method that is advantageous for limited funding research. However, it requires tedious work and hours to perform. Meanwhile, SPE is the recently developed extraction method that is simple and time-saving. However, SPE requires expensive equipment and accessories. Since different pharmaceutical compounds prefer different extraction methods, a comparison of methods is conducted to determine the suitable extraction method before the main study is conducted. Lindsey *et al*. [9] conducted a study on the comparison of LLE and SPE for sulfonamide and tetracycline compounds. Another study by Bonnefous and Boulieu [8] performed a comparison study of LLE and SPE on diltiazem. In addition, a methanol volume comparison was conducted since different methanol volumes affect extraction efficiency. A previous study showed that different methanol volumes influenced the average recovery percentage of amitriptyline and nortriptyline [12]. However, observation on comparison of LLE and SPE and different methanol volumes using carbamazepine and caffeine is yet to be addressed. Hence, this study focuses on the comparison of LLE and SPE and the optimum methanol volume to be used in the determination of efficient extraction methods for carbamazepine and caffeine.

2 Method

2.1 Standard Solutions Preparation and Calibration Curve

A 100 mgL^{-1} carbamazepine standard solution was prepared by dissolving 0.005 g of carbamazepine in 50 mL methanol. A 25 mgL^{-1} carbamazepine standard solution was prepared by diluting the prepared stock solution of 100 mgL^{-1} carbamazepine standard solution. The 25 mgL^{-1} carbamazepine solution was transferred into an amber vial (Cronus™). The steps were repeated for 0.5, 1, 5, 10, 15, and 20 mgL^{-1} carbamazepine standard solutions. Each carbamazepine standard solution in amber vials was analyzed using GC-MS. All samples were prepared in triplicates.

A calibration curve for carbamazepine was conducted using GC-MS (Perkin Elmer™). The GC with column Elite 5ms (Perkin Elmer™) was operated at 250°C with helium as carrier gas at the pressure of 18.7 psi and 1.00 mLm^{-1} and 39.5 $cmsec^{-1}$ initial flow rate. Meanwhile, the MS was operated at 150°C. The peak response of carbamazepine was obtained from GC-MS result analysis. The response reading obtained from GC-MS for each concentration was used to construct graphs of response versus time and to determine retention time for carbamazepine. Graph of response versus prepared concentrations was also plotted. The procedures for the calibration curve were repeated by using caffeine with concentrations of 0.5, 1, 5, 10, 15, 20, and 25 mgL^{-1}.

2.2 Extraction Method Analysis

2.2.1 LLE Method

Standard solution for carbamazepine at the concentration of 10 mgL^{-1} was prepared in triplicates using methods mentioned in section 2.1. The 10 mgL^{-1} concentration was selected for extraction study because it can be detected by GC-MS. LLE method was conducted to extract the standard solution samples. Each separatory funnel was washed using 2 mL methanol. 2 mL of the 10 mgL^{-1} standard solutions were transferred into the separatory funnel followed by 9 mL methanol: 9 mL acetonitrile. The mixture was shaken vigorously three times to increase the contact time of pharmaceutical compounds with organic solvent and pressure was released after each shake. The extracted samples were rotavaped (Buchi™) at 226 mbar to remove acetonitrile and analysed using GC-MS using condition in section 2.1. The steps were repeated using methanol volume with a ratio of 11 mL methanol: 9 mL acetonitrile. This experimental design was repeated using caffeine.

2.2.2 SPE Method

A 10 mgL^{-1} standard solution for carbamazepine was prepared using methods mentioned in section 2.1. The 10 mgL^{-1} was selected since the concentration was analysed during the calibration curve. The standard solution triplicate samples were extracted using VisiPrep™ SPE Vacuum Manifold Solid Phase Extraction (SPE) using the specific cartridge for pharmaceuticals analysis, Supel Select HLB cartridges (Supelco™). Each SPE cartridge was equilibrated using 2 mL methanol. A 2 mL of the 10 mgL^{-1} standard solutions was eluted into the cartridge followed by 9 mL methanol: 9 mL acetonitrile. The cartridge was dried under negative pressure using a vacuum pump. The extracted samples were rotavaped (Buchi™) at 226 mbar to remove acetonitrile and analysed using

GC-MS under the condition mentioned in section 2.1 (Supelco Manual). The steps were repeated using different methanol volumes at a ratio of 11 mL methanol: 9 mL acetonitrile. The procedures for the SPE method were then repeated using caffeine.

2.3 Data Analysis

The response readings obtained from analysis of carbamazepine and caffeine standard solution samples were used to calculate the recovery percentage and average recovery percentage using the formula in Equations (1) and (2), respectively.

$$\text{Recovery percentage}, \% = \frac{\text{Response reading of extracted samples}}{\text{Response reading of control}} \times 100\% \quad (1)$$

$$\text{Average recovery percentage}, \% = \frac{\text{Total recovery percentage}}{\text{Number of replicates}} \times 100\% \quad (2)$$

The average recovery percentage was used to plot the graph of average recovery percentage versus extraction methods. The response readings were also analysed with SPSS 20 software using one-way ANOVA and T-test.

3 Result and Discussion

3.1 Carbamazepine Retention Time

Figure 1 shows the time profile for different carbamazepine concentrations. The average retention time for carbamazepine at concentrations of 1, 5, 10, 15, 20, and 25 mgL^{-1} was found to be 18.76±0.004 minutes. However, the retention time for 0.5 mgL^{-1} failed to be detected due to the GC-MS detection limit.

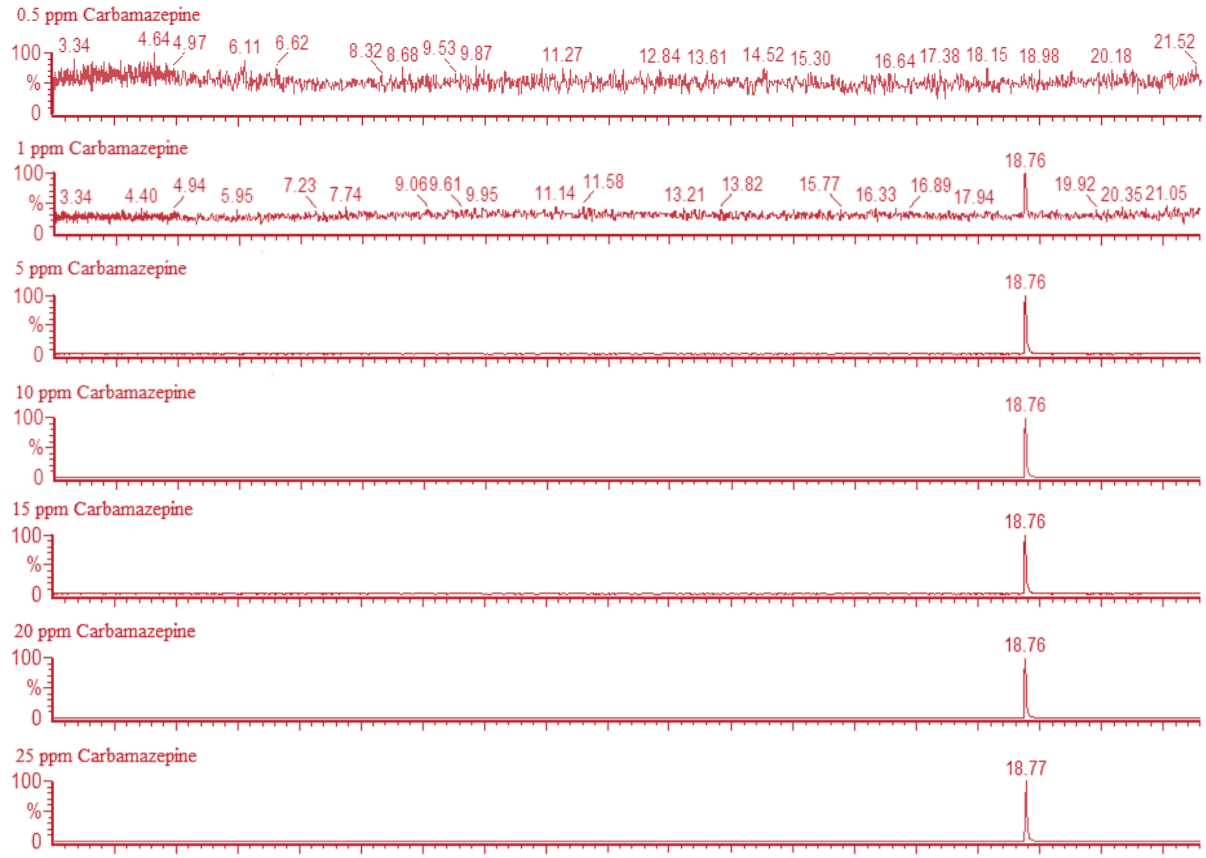

Figure 1: Carbamazepine time profile for different concentrations

3.2 Carbamazepine Calibration Curve

Figure 2 shows the calibration curve for carbamazepine, with y = 1051.3x and correlation coefficient (R^2) 0.9827. A high R^2 value represents excellent reliability and accuracy of the analysis.

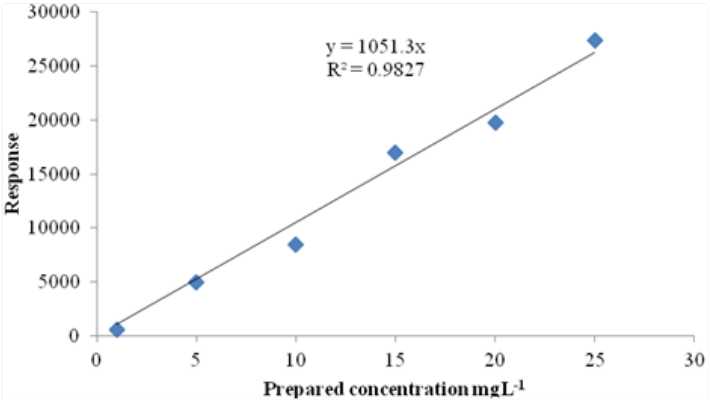

Figure 2: Carbamazepine calibration curve

3.3 Comparison of Extraction Method for Carbamazepine

Figure 3 shows the average recovery percentage of carbamazepine for both LLE and SPE methods. The average recovery percentage of carbamazepine for LLE with 9 mL methanol and 11 mL methanol were 90.0% and 98.9%, respectively. Meanwhile, the average recovery percentage of carbamazepine for SPE with 9 mL methanol was 16.1% and SPE with 11 mL methanol was 5.5%. In summary, the LLE method for carbamazepine showed a high average recovery percentage but the SPE method showed a low average recovery percentage. LLE method is designed for alkaline pharmaceutical compounds [10]. Carbamazepine is classified as a weak alkaline pharmaceutical compound; hence, the average recovery percentage obtained was higher when the LLE method was performed than the SPE method. A study conducted by Schneider *et al.* [14] also demonstrated a similar trend where the LLE method for carbamazepine, alimemazine, alprenolol, codeine, doxepine, and methadone resulted in high-intensity peaks and average recovery percentage.

For methanol volume, the addition from 9 mL to 11 mL using the LLE method increased the average recovery percentage. This circumstance was mainly due to the increase in the phase contact area. When methanol was mixed with the samples, more molecules were bound with carbamazepine, thus resulted in a higher response and average recovery percentage [15]. For SPE, when the methanol volume increased, the average recovery percentage of carbamazepine decreased. Yazdi *et al.* [12] supported this study as they reported that excessive methanol volume in the extraction of amitriptyline and nortriptyline also decreased the average recovery percentage.

Table 1 shows the one-way ANOVA result for LLE and SPE of carbamazepine. The one-way ANOVA result demonstrated that there were significant differences among the methods ($p<0.05$). The post hoc Tukey test result supported the one-way ANOVA result where all methods were significant to each other. Different letters in Figure 3 represent that there were significant differences between the methods based on the Tukey test result. For verification, the t-test result is shown in Table 2. The t-test result for carbamazepine supported the Tukey test result with all $p< 0.05$.

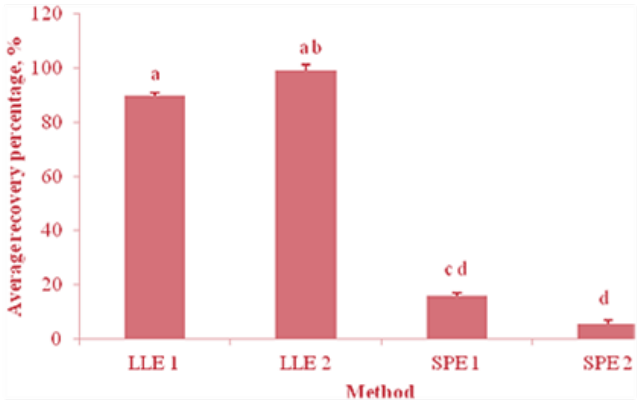

Figure 3: Carbamazepine average recovery percentage

Table 1: One-way ANOVA for LLE and SPE of carbamazepine

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	21303.82	3	7101.274	3192.803	1.19E-12	4.066181
Within Groups	17.7932	8	2.22415			
Total	21321.61	11				

Table 2: Carbamazepine t-test result

Method	Comparison method	P(T<=t) two-tail
LLE 1	LLE 2	3.67E-03
	SPE 1	6.27E-08
	SPE 2	1.11E-07
LLE 2	SPE 1	5.05E-07
	SPE 2	4.54E-07
SPE 1	SPE 2	2.82E-04

3.4 Caffeine Retention Time

Figure 4 demonstrates the response of different caffeine concentrations over time. The average retention time for caffeine was 17.24 ± 0.008 minute for concentrations of 1 mgL^{-1}, 5 mgL^{-1}, 10 mgL^{-1}, 15 mgL^{-1}, 20 mgL^{-1} and 25 mgL^{-1}. Similar to carbamazepine, the retention time for 0.05 mgL^{-1} was not detectable.

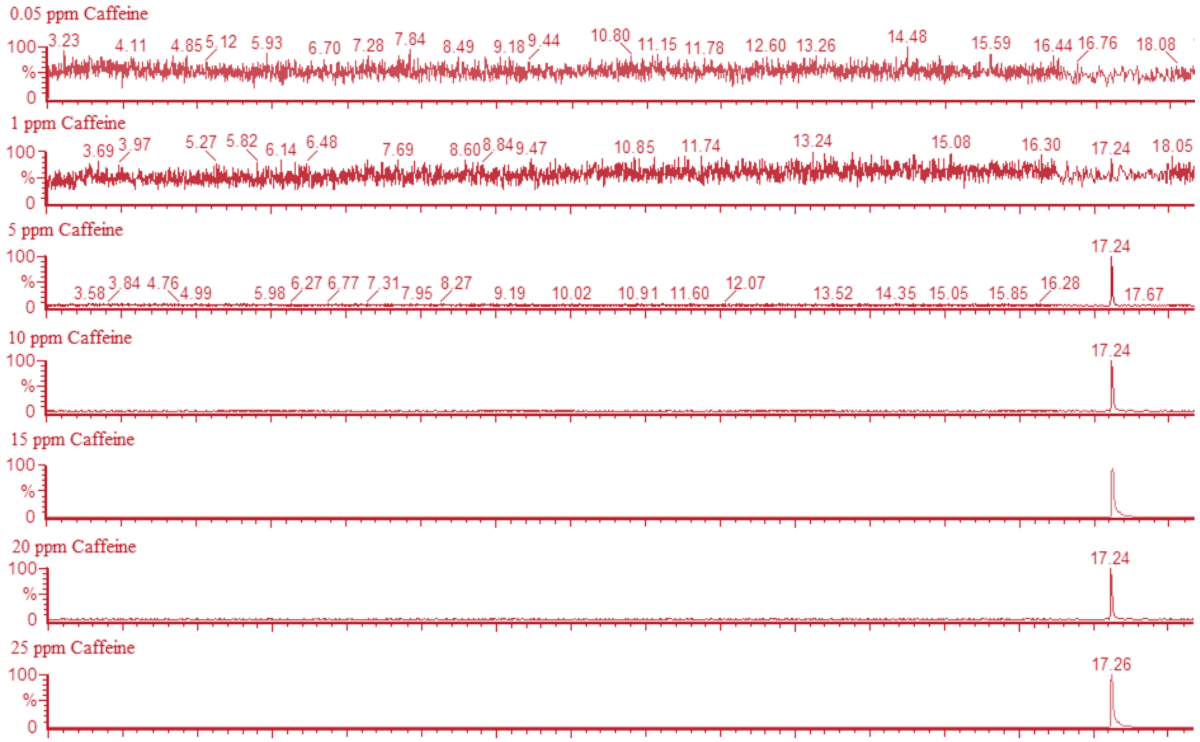

Figure 4: Caffeine retention time for each concentration

3.5 Caffeine Calibration Curve

Figure 5 shows the calibration curve for caffeine. The fit and correlation coefficient (R^2) of the graph are y = 171.72x and 0.9845, respectively. This signifies a strong linear relationship and high accuracy of the analysis.

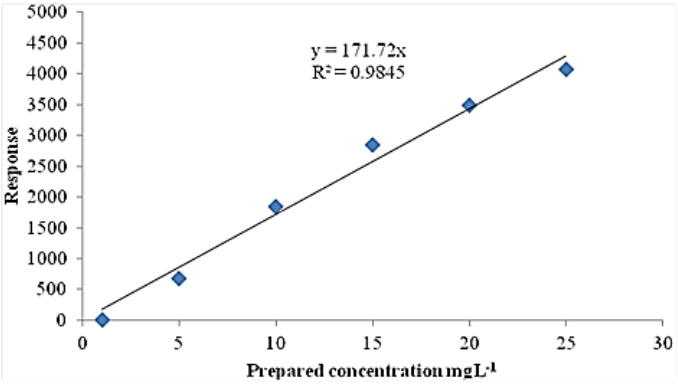

Figure 5: Caffeine calibration curve

3.6 Comparison of Extraction Method for Caffeine

Figure 6 demonstrates the average recovery percentage of caffeine for LLE and SPE methods. For LLE with 9 mL methanol, the average recovery percentage was 80.2% while LLE with 11 mL methanol was 93.6%. For the SPE method, the average recovery percentage of caffeine with 9 mL methanol and 11 mL methanol were 21.2% and 25.0%, respectively. In tandem with carbamazepine, a high average recovery percentage was obtained when the LLE method was performed when compared to the SPE method. For caffeine, both LLE and SPE methods resulted in a higher average recovery percentage when methanol volume was increased to 11 mL. Methanol molecules interacted with the caffeine molecules; hence, higher response and average recovery percentage were obtained [15]. Yazdi *et al.* [2] reported that the increase in methanol volume when not in excessive condition resulted in a higher average recovery percentage of amitriptyline and nortriptyline.

Table 3 shows the one-way ANOVA result of caffeine for LLE and SPE. From the result, the methods had significant differences where $p<0.05$. Tukey test results also showed that there were significant differences for all methods. Figure 6 that is labeled with different letters indicated a significant difference between the methods. Table 4 demonstrates the t-test result which further validated the Tukey test result ($p< 0.05$).

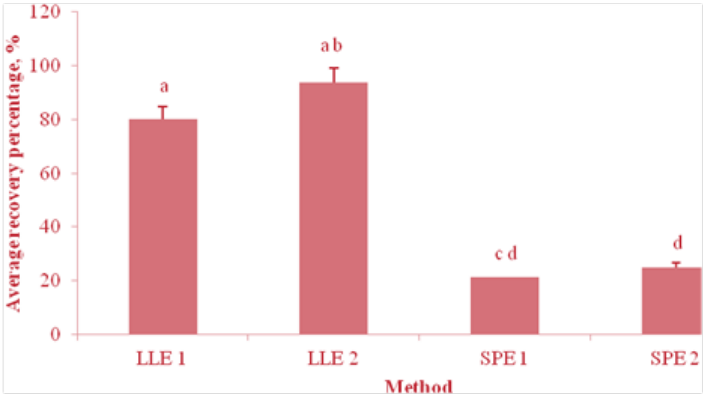

Figure 6: Caffeine mean recovery percentage

Table 3: One-way ANOVA for LLE and SPE of caffeine

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	17809.68	3	5936.561	432.0131	3.48E-09	4.066181
Within Groups	109.933	8	13.74162			
Total	17919.62	11				

Table 4: Caffeine t-test result

Method	Comparison method	P(T<=t) two-tail
LLE 1	LLE 2	3.27E-02
	SPE 1	1.92E-05
	SPE 2	1.16E-05
LLE 2	SPE 1	1.82E-05
	SPE 2	1.18E-05
SPE 1	SPE 2	2.82E-04

4 Conclusion

LLE method and SPE method were evaluated as potential efficient extraction methods for carbamazepine and caffeine in this study. The LLE method resulted in the highest average recovery percentage with 98.9% for carbamazepine and 93.6% for caffeine. Meanwhile, the SPE method was not suitable for carbamazepine where the average recovery percentage obtained for carbamazepine and caffeine were only 16.1% and 25.0% respectively. The optimum methanol volume was 11 mL for both compounds. The result obtained from one-way ANOVA, Tukey test, and t-test showed all methods were significant to each other. From this study, the LLE with 11 mL methanol was determined as an efficient extraction method for carbamazepine and caffeine. Since this study has limited funding, an efficient low-cost LLE method is an advantage for further subsequent biodegradation studies. Reliable and consistent results from low-cost analysis of carbamazepine and caffeine can be achieved in further study to safeguard the aquatic organisms and the environment.

5 Availability of Data And Material

Data can be made available by contacting the corresponding author.

6 Acknowledgement

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Najihah Mohamad-Nasir is a Master's postgraduate student in Biology who pursues her study in Universiti Teknologi MARA, Shah Alam. Her fields of interest are Bioremediation, Tissue Culture and Biology.

Dr.Suhaimi Abdul-Talib is an Assistant Vice-Chancellor (College of Engineering Studies), Universiti Teknologi MARA. He got a B.E. (Hons) from the University of Melbourne, an M.Sc. in Water and Environment Management from Loughborough University, U.K., and a PhD. from Universiti Teknologi Malaysia. His research interests include In-sewer Processes, Environmental Management, Pollution Control, Bioremediation, Water & Wastewater Engineering and Water Resource and Wastewater Service Management.

Dr.Nurul Fariha Lokman is a Senior Lecturer at Universiti Teknologi MARA (UiTM), Shah Alam and a Civil Engineer at Institute of Leadership and Development (ILD), UiTM Nilai. She earned a JENESYS-scholarship and further an MSc at Nagaoka University of Technology, Japan. Her honors a PhD from Universiti Kebangsaan Malaysia was about Detection of Pb-metals in Water Using Surface Plasmon Resonance (SPR) Sensors System. She actively conducts research on Kenaf-sorb Beads Originating from Kenaf Core for Adsorption of Heavy Metals in Aqueous Solution.

Saiyidah Nafisah Hashim is a staff at Universiti Teknologi MARA (UiTM) Tapah Perak. She obtains her Master of Science in Plant Biotechnology from UiTM. Currently, she is doing her PhD in Plant Biotechnology at UiTM Shah Alam. She focuses on the Elicitation of Flavonoid Compound from Clinacanthus Nutan's Tissue and cell.

Ts. Dr. Chia Chay Tay is an Associate Professor in the Environmental Technology Program, Faculty of Applied Sciences, Universiti Teknologi MARA. She is involved in multiple disciplines and multi-institutional research projects across science, engineering and social science focusing on the Crisis to Sustainability, especially in Water Technology Processes, Management and Social Interactions. Her interests include Solid Waste and Water Pollutants, Plastic Waste and Emerging Pollutants, Eco-friendly Treatment Technology, and the Human Aspect of Water Security.