

OPTIMISATION OF THE LIQUID CHROMATOGRAPHY MASS SPECTROMETRY TRIPLE-QUADRUPOLE AND LOW-COST MICROEXTRACTION ANALYSIS FOR CARBAMAZEPINE

(Pengoptimuman Kromatografi Cecair Spektrometer Jisim Tiga-Caturkutub dan Analisis Pengekstrakan Mikro Kos Rendah bagi Karbamazepin)

Siti Sabrina Kasri^{1,2}, Hannis Fadzillah Mohsin³, Wan Nurhayati Wan Hanafi¹, Wardah Tahir⁴, and Chia Chay Tay^{1,5*}

¹Faculty of Applied Sciences,

Universiti Teknologi MARA, Shah Alam, Selangor, 40450, Malaysia

²Centre of Foundation Studies,

Universiti Teknologi MARA, Cawangan Selangor, Kampus Dengkil,

Dengkil 43800, Selangor, Malaysia

³Faculty of Pharmacy,

Universiti Teknologi MARA Cawangan Selangor,

Puncak Alam, 42300, Selangor, Malaysia

⁴School of Civil Engineering,

College of Engineering,

Universiti Teknologi MARA, Shah Alam, 40450, Selangor, Malaysia

⁵myBioREC, School of Civil Engineering,

College of Engineering,

Universiti Teknologi MARA, Shah Alam, 40450, Selangor, Malaysia

*Corresponding author: taychiay@uitm.edu.my

Received: 25 September 2022; Accepted: 18 December 2022; Published: 19 April 2023

Abstract

Sensitivity and reliability have become focal points in the analysis of contaminants in wastewater. The optimisation using liquid chromatography mass spectrometry triple-quadrupole (LCMS-QQQ) and carbamazepine microextraction parameters were investigated. The optimisation of gas flow, nebuliser, fragmentor voltage, and collision energy in LCMS-QQQ were examined. Carbamazepine microextraction parameters for extraction methods, extraction tools, various solvents, and solvent volume ratios were investigated. The optimised LCMS-QQQ conditions were 11 L/min gas flow, 25 psi nebuliser, 80 V fragmentor voltage, as well as 35 eV (179.1 m/z) and 15 eV (194.1 m/z) collision energy. For microextraction analysis, liquid-liquid extraction (LLE) yielded a higher carbamazepine average recovery percentage of 96±26%–100±10% than solid phase extraction (SPE) at 4±0%–8±1%. The sonicator and separatory funnel similarly showed a high average recovery percentage of carbamazepine at 96±26%–100±10%, but the sonicator saves time and human resources. The average recovery percentage for carbamazepine in solvents at 100±10% was higher compared to water at 26±4%. Statistically, there was no significant difference between the various solvents used in LLE. The optimised solvent volume-ratio LLE was 1.25. This study is vital for the analysis of carbamazepine in the environment and the development of an emerging pollutants monitoring database.

Keywords: carbamazepine, liquid chromatography mass spectrometry triple-quadrupole, optimisation, microextraction

Abstrak

Sensitiviti dan kebolehpercayaan telah menjadi tumpuan dalam analisis bahan cemar dalam air sisa. Oleh itu, pengoptimuman menggunakan kromatografi cecair spektrometri jisim tiga-caturkutub (LCMS-QQQ) dan parameter pengekstrakan mikro karbamazepin telah dikaji. Pengoptimuman aliran gas, nebuliser, voltan serpihan, dan tenaga perlanggaran untuk LCMS-QQQ telah diperiksa. Parameter pengekstrakan mikro karbamazepin untuk kaedah pengekstrakan, alat pengekstrakan, pelbagai pelarut, dan nisbah isipadu pelarut telah disiasat. Keadaan optimum LCMS-QQQ ialah aliran gas 11 l/min, nebuliser 25 psi, voltan serpihan 80 V, serta tenaga perlanggaran 35 eV (179.1 m/z), dan 15 eV (194.1 m/z). Untuk analisis pengekstrakan mikro, pengekstrakan cecair-cecair (LLE) menghasilkan purata peratusan pemulihan karbamazepin yang lebih tinggi pada $96 \pm 26\%$ – $100 \pm 10\%$ daripada pengekstrakan fasa pepejal pada $4 \pm 0\%$ – $8 \pm 1\%$. Sonikator dan corong pemisah menunjukkan keputusan yang sama, purata peratusan pemulihan yang tinggi bagi karbamazepin pada $96 \pm 26\%$ – $100 \pm 10\%$ tetapi sonikator menjimatkan masa dan sumber manusia. Purata peratusan pemulihan untuk karbamazepin dalam pelarut pada $100 \pm 10\%$ adalah lebih tinggi berbanding dengan air pada $26 \pm 4\%$. Pelbagai pelarut yang digunakan dalam LLE tidak menunjukkan perbezaan yang signifikan secara statistik. Nisbah isipadu pelarut LLE yang optimum ialah 1.25. Kajian ini adalah penting untuk analisis karbamazepin dalam alam sekitar dan pembangunan pangkalan data pemantauan bahan pencemar yang muncul.

Katakunci: karbamazepin, kromatografi cecair spektrometri jisim tiga-caturkutub, pengoptimuman, pengekstrakan mikro

Introduction

Carbamazepine is a pharmaceutical compound used as an anti-epileptic drug for suppressing seizures. It is a tricyclic compound that is persistent in the environment. The compound is not completely metabolised in the liver [1]. A total of 1% is excreted through urine and faeces into the sewage system and enters the wastewater treatment plants [2]. Current treatments in wastewater treatment plants are unable to remove carbamazepine because it is not designed to degrade stable contaminants, including carbamazepine [3]. Consequently, treated wastewater with carbamazepine flows into the water stream, accumulates, and causes adverse effects on human health, aquatic organisms, and the environment [4]. Long-term exposure to low concentrations of carbamazepine disrupts the human endocrine system [5]. Carbamazepine also disturbed the growth and development of zebrafish embryos and larvae [6]. In the environment, plants uptake carbamazepine, which accumulates and exposes human and aquatic organisms to risk [7]. Therefore, developing a method for isolating and analysing trace carbamazepine concentrations in wastewater is critical for improving water quality and safety for humans, aquatic organisms, and the environment.

Liquid chromatography mass spectrometry triple-quadrupole (LCMS-QQQ) has been used for the analysis of pharmaceutical compounds, industrial

chemicals, and pesticides in the environment due to its high sensitivity and selectivity. Hence, it is suitable for screening and quantifying the trace concentration of carbamazepine in wastewater. Kruglova et al. [8] reported the detection of trace carbamazepine at $0.4 \pm 0.1 \mu\text{g L}^{-1}$ using ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS). In the study by He et al. [9], $53.8 \mu\text{g L}^{-1}$ carbamazepine was analysed using LCMS-QQQ. Although the published methods of detection and quantifying carbamazepine can be used as a reference in the analysis of carbamazepine, optimisation of the method needs to be conducted. This is because assay performance and optimal conditions vary among different instruments and sample matrices [10].

The wastewater containing carbamazepine also has a high concentration of organic matter, microorganisms, and inorganic compounds [11]. Therefore, carbamazepine requires pre-treatment through microextraction before LCMS-QQQ analysis. The most widely used carbamazepine microextraction methods are liquid-liquid extraction (LLE) and solid-phase extraction (SPE). LLE involves the partitioning of compounds based on the solubility of the mixture in two different solvents [11]. Meanwhile, SPE uses a solid phase and a liquid phase to isolate and concentrate compounds from a solution [12]. In comparison to SPE, which requires expensive equipment and accessories,

LLE is a low-cost microextraction method suitable for research with limited funding [11, 12]. In LLE, microextraction tools, types of solvents, and solvent volume ratio are important optimisation parameters in determining the highest average recovery percentage of carbamazepine for high sensitivity and reliable results.

The objectives of this study are to optimise LCMS-QQQ through gas flow, nebuliser, fragmentor voltage, and collision energy, and to optimise microextraction methods, microextraction tools, types of solvents, and solvent volume ratio.

Materials and Methods

Chemicals and standard solutions preparation

The pharmaceutical compound carbamazepine $\geq 98\%$ purity used was purchased from Sigma Aldrich. HPLC and LCMS grade acetonitrile, methanol, ethanol, and acetone were used in the microextraction and LCMS analysis of carbamazepine. A 500 mgL^{-1} carbamazepine stock solution was prepared by dissolving 5 mg of carbamazepine in 10 mL of methanol.

LCMS-QQQ set-up for carbamazepine analysis

A liquid chromatography system (Agilent 1200 Series, Agilent Technologies, USA) equipped with a quaternary pump, an autosampler, a column thermostat, an inline degasser, and a Zorbax Extend-C18 (100 x 2.1 mm, 1.8

μm) column was coupled to the triple quadrupole (QQQ) mass spectrometry (Agilent 6410, Agilent Technologies, USA) with an electrospray ionisation interface (ESI) MS source. The column oven and flow rate were set to 40°C and 0.2 mL/min , respectively. A $2 \mu\text{L}$ of 1 mg/L^{-1} carbamazepine was injected into the system. A binary gradient mobile phase consisting of ultrapure water (UPW) and acetonitrile (ACN) was prepared and identified as A and B solvents. Both solvents were added with 0.1% formic acid. The gradient profile was set up at initial conditions of $20\%:80\%$ (A:B, v/v) and brought to $95\%:5\%$ (A:B, v/v) for the first 2 minutes, changed to $20\%:80\%$ (A:B, v/v) for 5 minutes, and restored to $95\%:5\%$ for 7 minutes. Ionization was performed using the ESI positive mode ion polarity. During optimisation, two mass transitions for carbamazepine, $237.3 \rightarrow 179.1 \text{ m/z}$ and $237.3 \rightarrow 194.1 \text{ m/z}$ were set in multiple-reaction monitoring mode (MRM) [13]. Samples of 1 mgL^{-1} carbamazepine were prepared in triplicates. This experiment used a single factor at a time optimisation design. The optimisation parameters of carbamazepine for gas flow, nebuliser, fragmentor voltage, and collision energy were investigated according to Table 1. Identification and quantitation analysis were performed using Mass Hunter Quantitative Analysis software (Version B.03.01, Agilent Technologies, USA).

Table 1. Optimisation parameters of carbamazepine using LCMS-QQQ

Optimisation Parameters			
Gas Flow (L/min)	Nebuliser (psi)	Fragmentor Voltage (V)	Collision Energy (eV)
10.5	20	80	5
11.0	25	100	10
11.5	30	120	15
	35	140	20
	40		25
			30
			35
			40

The optimised parameters were used to construct the calibration curve. A series of desired carbamazepine standard solutions at 0.001 mgL^{-1} to 1 mgL^{-1} were prepared in triplicates using the 500 mgL^{-1} prepared carbamazepine stock solution. The limits of detection

(LOD) and quantification (LOQ) were calculated as in Equations 1 and 2:

$$LOD = 3.3 \left(\frac{SD}{s} \right) \quad (1)$$

$$LOQ = 10 \left(\frac{SD}{S} \right) \quad (2)$$

where *SD* is the standard deviation of the response and *S* is the slope of the calibration curve.

Microextraction optimisation

The optimisation design used for microextraction optimisation was a single factor at a time, and the samples were prepared in duplicate. The microextraction methods used were LLE and SPE, followed by optimisation of the microextraction tools, and solvent versus water as in Table 2. The optimisation was further examined with various solvent combinations and solvent volume ratios as shown in Table 3. The methods were adopted from Mohamad-Nasir et al. [11]. In LLE (A), 2 mL of the 0.5 mgL⁻¹ carbamazepine standard solution were added to 11 mL of methanol and

9 mL of acetonitrile. The samples were mixed for 5 minutes. For SPE (B), the cartridges were equilibrated using 2 mL of methanol. A total of 2 mL of the 0.5 mgL⁻¹ carbamazepine standard solution was eluted into the cartridge, followed by 11 mL methanol and 9 mL acetonitrile. Next, samples extracted from LLE and SPE were rotavapor (IKA, Model: RV06-ML, Germany), filtered with a 0.22 µm syringe filter, and analysed using LCMS-QQQ. The LLE method was repeated under similar conditions, using the microextraction tools of a separatory funnel and sonicator for 5 minutes. Then, the experimental designs for A and B were repeated using methanol and water. After that, various solvent combinations and solvent volume ratios were examined (Table 3). The recovery percentage and average recovery percentage of carbamazepine were calculated using Equations 3 and 4, respectively.

Table 2. Optimisation of carbamazepine microextraction on microextraction methods, tools, and types of solvents

Solvents (mL)	A = LLE				B = SPE			
	A1		A2		B1		B2	
MeOH:ACN	11	9	11	9	11	9	11	9
MeOH:dH ₂ O	11	9	11	9	11	9	11	9

Note: A1-Separatory funnel, A2-Sonicator, B1-Select HLB, B2-Envi 18, MeOH-Methanol, ACN-Acetonitrile, dH₂O-Deionised water

Table 3. Optimisation of carbamazepine microextraction using various solvent combinations and solvent volume ratios

Solvents (mL)						Solvent Volume Ratios
MeOH:ACN	MeOH:EtOH	MeOH:ACE	MeOH:ACN	MeOH:EtOH	MeOH:ACE	
8.60	11.40	8.60	11.40	8.60	11.40	0.75
9.00	11.00	9.00	11.00	9.00	11.00	0.82
10.00	10.00	10.00	10.00	10.00	10.00	1.00
11.10	8.90	11.10	8.90	11.10	8.90	1.25
12.00	8.00	12.00	8.00	12.00	8.00	1.50
13.34	6.66	13.34	6.66	13.34	6.66	2.00

Note: MeOH-Methanol, ACN-Acetonitrile, EtOH-Ethanol, ACE-Acetone

$$\text{Recovery percentage, \%} = \frac{\text{Response reading of extracted samples}}{\text{Response reading of control}} \times 100 \% \quad (3)$$

$$\text{Average recovery percentage, \%} = \frac{\text{Total recovery percentage}}{\text{Number of replicates}} \times 100 \% \quad (4)$$

Results and Discussion

LCMS-QQQ set-up for carbamazepine analysis

Table 4 shows the results of the optimisation parameters of LCMS-QQQ for carbamazepine analysis, such as gas flow, nebuliser, fragmentor voltage, and collision energy. The optimised parameters were 11 l/min for gas flow, 25 psi for the nebuliser, 80 V for fragmentor voltage, and 35 eV (179.1 m/z) and 15 eV (194.1 m/z) for collision energy. These conditions are selected

because they provide the highest abundance, sharp peak shapes, and consistent retention times as shown in Figure 1. The highest abundance reflects the highest count of carbamazepine [14]. While sharp peak shapes signify the purity of carbamazepine, they reduce faulty results [15]. These outcomes lead to better sensitivity. Consistent retention time indicates the reliability of the optimisation of LCMS-QQQ.

Table 4. Optimisation parameters for carbamazepine analysis for LCMS-QQQ

Optimised Conditions		Abundance
1. Gas flow (l/min)		
10.5		104922
11.0		125130
11.5		110205
2. Nebuliser (psi)		
20		89732
25		97177
30		93617
35		96736
40		88544
3. Fragmentor voltage (V)		
80		68978
100		74817
120		80052
140		78013
4. Collision energy (eV)		
	m/z 179.1	m/z 194.1
5	ND*	4356
10	ND*	12092
15	ND*	15905
20	ND*	15423
25	882	11660
30	1765	7472
35	2060	3673
40	ND*	1556

*ND: Not detected

Figure 1 shows the spectrum of counts versus acquisition time for carbamazepine concentrations. In this study, the retention time for carbamazepine was at the range of 6.01–6.20 minutes for carbamazepine at concentrations from 0.001 mgL⁻¹ to 1 mgL⁻¹. From these carbamazepine concentrations, the average retention

time was found to be 6.16 ± 0.0222 minutes. He et al. [9] reported an 8-minute short retention time for carbamazepine using a C₁₈ column with a particle size of 2.6 µM. Generally, high particle size results in complete separation of carbamazepine in a minimum amount of time [17]. In this study, carbamazepine retention time

was shorter than He et al. [9], although they used a C18 column with a smaller particle size of 1.8 μM . This is due to the fact that optimising the LCMS-QQQ for

carbamazepine analysis is vital for achieving high sensitivity, saving time, and decreasing the volume of solvents used in the LCMS-QQQ.

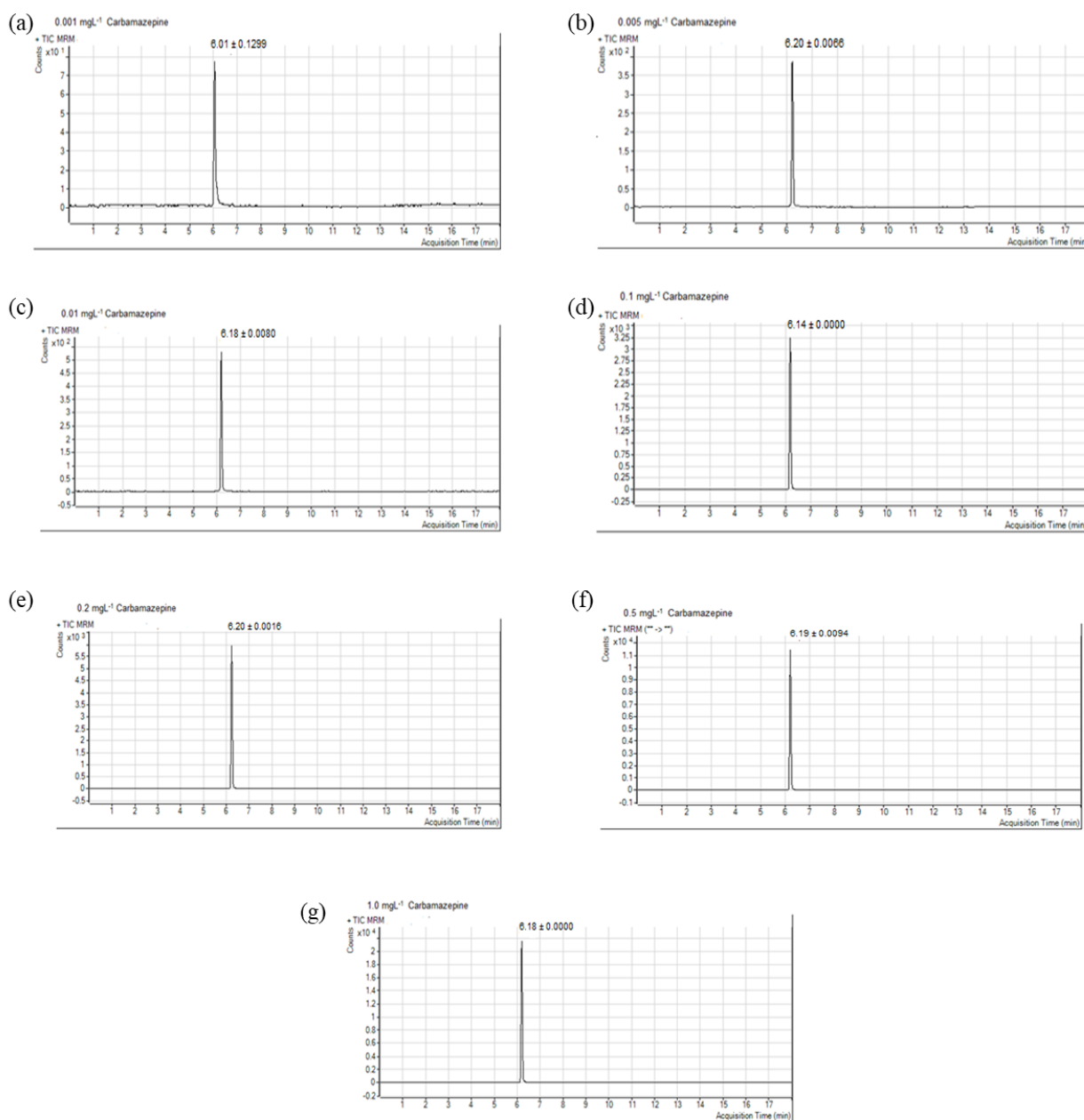


Figure 1. Carbamazepine spectrum of counts versus acquisition time for carbamazepine concentrations: (a) 0.001 mgL⁻¹ carbamazepine; (b) 0.005 mgL⁻¹ carbamazepine; (c) 0.01 mgL⁻¹ carbamazepine; (d) 0.1 mgL⁻¹ carbamazepine; (e) 0.2 mgL⁻¹ carbamazepine; (f) 0.5 mgL⁻¹ carbamazepine; (g) 1.0 mgL⁻¹ carbamazepine

Figure 2 shows a graph of the calibration curve for carbamazepine. The equation of the graph was $y = 79068x$, and the value for the correlation coefficient (R^2) was 0.9940. High R^2 represents an excellent linear relationship. The linearity of the calibration curve signifies the suitability of the chromatographic method over a wide range of concentrations for the studied pharmaceutical [18]. From this study, the LOD and LOQ obtained were at 0.04 mgL^{-1} and 0.13 mgL^{-1} . These results are highly reliable, as the retention time for

carbamazepine concentrations was less than 15%. Previous studies for carbamazepine analysis using LCMS-QQQ for the type of column, LOD, LOQ, and linearity are shown in Table 5. The use of column C18 with a particle size of $1.8 \mu\text{m}$ improved the efficiency of separation, resulting in better LOD, LOQ, and linearity. This confirms that the optimisation of LCMS-QQQ conducted in this study is suitable for the analysis of carbamazepine.

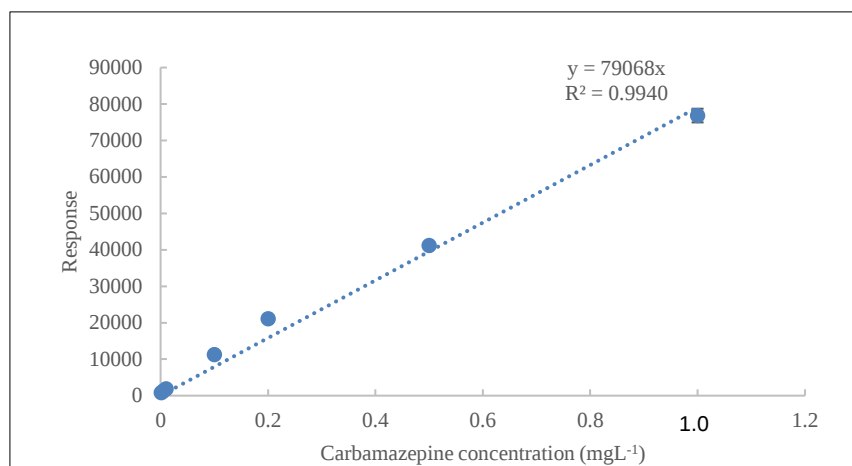


Figure 2. Calibration curve of carbamazepine

Table 5. Type of column, LOD, LOQ, and linearity of carbamazepine

Application	Type of Column and Size	LOD	LOQ	Linearity (R^2)	Reference
Quantification and screening CBZ in surface and drinking water	C ₁₈ 100 x 2 mm, 3 μM	0.2 ngL^{-1}	*NM	0.9900	[17]
Forensic toxicology cases	C ₁₈ 100 x 2.1 mm, 1.7 μM	0.12 mgL^{-1}	0.30 mgL^{-1}	0.9989	[18]
Quantification and screening CBZ in wastewater	C ₁₈ 100 x 2.1 mm, 1.8 μM	0.04 mgL^{-1}	0.13 mgL^{-1}	0.9940	This study

*NM: Not mentioned

Microextraction optimisation

Figure 3 depicts a graph of the microextraction methods LLE and SPE with the average carbamazepine recovery percentage. The average recovery percentage of carbamazepine in LLE for A1 and A2 at $96 \pm 26\%$ and $100 \pm 10\%$ respectively, higher than SPE for B1 and B2 at $8 \pm 1\%$ and $4 \pm 0\%$, respectively. Mohamad-Nasir et al. 2021 also support these results, where the 98.9% average recovery percentage of carbamazepine was higher than SPE at 16.1% [11]. The high average recovery percentage of carbamazepine in LLE was due to the direct methanol interaction with carbamazepine through mixing with the second solvent, acetonitrile [19]. Meanwhile, the low average recovery percentage of carbamazepine in SPE is associated with carbamazepine that is slightly polar, resulting in poor elution on the sorbent [21]. The use of a separatory

funnel (A1) and sonicator (A2) yielded high average recovery percentages of $96 \pm 26\%$ and $100 \pm 10\%$, respectively. Vigorous mixing increases the interaction of methanol molecules with carbamazepine. Sonicator offers the advantages of less time and human resources. For LLE (A1 and A2), solvent methanol and acetonitrile yield a higher average recovery percentage of carbamazepine at $96 \pm 26\%$ – $100 \pm 10\%$ than solvent methanol and water at $36 \pm 12\%$ – $26 \pm 4\%$. In addition, Zhang et al. [20] also supported the findings that carbamazepine has low solubility in water at 0.00001 (mole fraction). The average recovery percentages of carbamazepine in SPE for solvent methanol and acetonitrile and solvent methanol and water were neglected due to their low average recovery percentages. This further confirms the effectiveness of the LLE method.

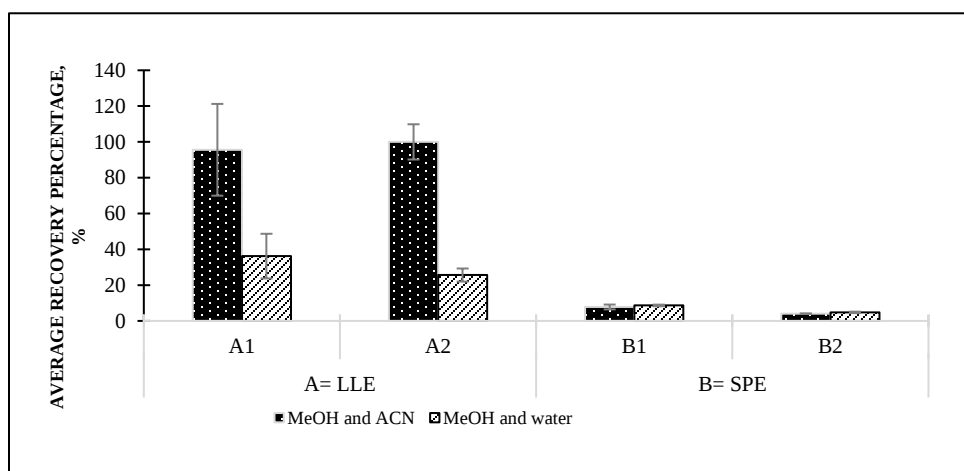


Figure 3. Average recovery percentage of carbamazepine for microextraction methods. A1: Separatory funnel; A2: Sonicator; B1: Select HLB; B2: Envi 18

Figure 4 illustrates the use of various solvents and the solvent volume ratio in LLE with the average recovery percentages of carbamazepine. The average recovery percentages of carbamazepine in methanol and acetonitrile, methanol and ethanol, and methanol and acetone were $100 \pm 13\%$, $100 \pm 1\%$, $100 \pm 12\%$, respectively. The optimum solvent volume ratios for methanol and acetonitrile, methanol and ethanol, and methanol and acetone were all at 1.25. An increase in

solvent polarity resulted in an increase in carbamazepine affinity. Thus, methanol in combination with these solvents at the optimum solvent volume ratio caused maximum interaction of carbamazepine and prevented excessive use of solvents. Overall, microextraction optimisation is important in the carbamazepine analysis because it prevents contamination that damages the analytical column of LCMS-QQQ, improves accuracy, and minimises the risk of false results.

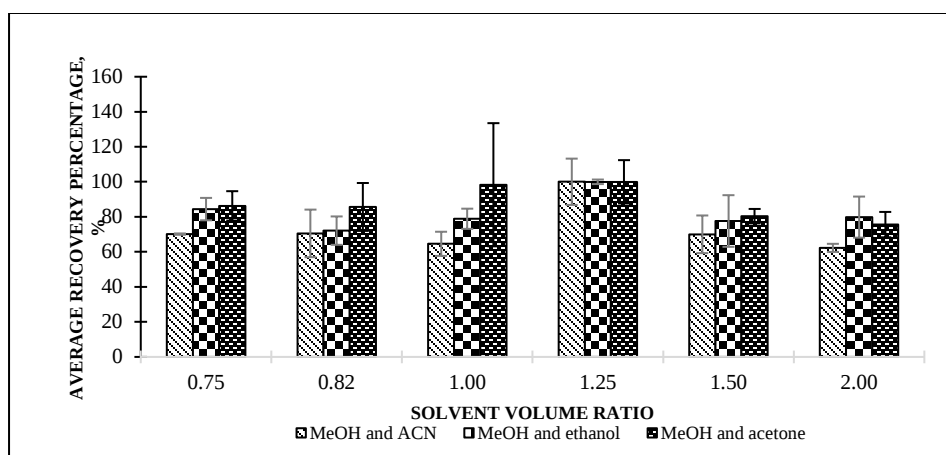


Figure 4. Average recovery percentage of carbamazepine for solvent type and solvent volume ratio

Conclusion

The optimisation parameters of LCMS-MS and microextraction methods for carbamazepine analysis were successfully investigated. The optimum gas flow, nebuliser, fragmentor voltage, and collision energy were at 11 l/min, 25 psi, 80 V, 35 eV (179.1 m/z), and 15 eV (194.1 m/z). For the microextraction methods, the LLE resulted in a higher average recovery percentage of carbamazepine at $96 \pm 26\%$ – $100 \pm 10\%$ compared to the SPE at $4 \pm 0\%$ – $8 \pm 1\%$. The use of a sonicator and a separatory funnel yielded a high average recovery percentage at $96 \pm 26\%$ to $100 \pm 10\%$. But, a sonicator has the advantage of saving time and human resources. The use of solvents resulted in a higher average recovery percentage of carbamazepine at $100 \pm 13\%$ compared to water at $26 \pm 4\%$. For various solvents used in LLE, methanol and acetonitrile, methanol and ethanol, and methanol and acetone have average recovery percentages of carbamazepine of $100 \pm 13\%$, $100 \pm 1\%$, and $100 \pm 12\%$, respectively. The optimal solvent volume ratio for various solvents in LLE was 1.25. The low-cost microextraction method LLE is suitable to be used in the study as it offers the advantages of simple apparatus, easy operation conditions, short time, and minimum resources used. This ensures reliable and consistent results in carbamazepine analysis for monitoring databases and policy development for emerging pollutants.

Acknowledgement

This work was supported by the University Teknologi MARA under grant 600-IRMI/FRGS 5/3 (355/2019). The authors would like to thank the UiTM Pharmacy Analytical Laboratory (UiPAL), the Faculty of Pharmacy, and the Faculty of Applied Sciences of University Teknologi MARA for providing laboratory facilities.

References

1. Zhang, Y., Geißen, S.-U. and Gal, C. (2008). Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere*, 73(8): 1151-1161.
2. Paltiel, O., Fedorova, G., Tadmor, G., Kleinstern, G., Maor, Y. and Chefetz, B. (2016). Human exposure to wastewater-derived pharmaceuticals in fresh produce: A randomized controlled trial focusing on carbamazepine. *Environmental Science & Technology*, 50(8), 4476-4482.
3. Rissardo, J. P. and Fornari Caprara, A. L. (2019) Carbamazepine associated urinary incontinence: a case report and literature review. *International Journal of Medical and Health Development* 24:114-117.
4. Brezina, E., Prasse, C., Meyer, J., Mückter, H. and Ternes, T. A. (2017). Investigation and risk evaluation of the occurrence of carbamazepine, oxcarbazepine, their human metabolites and transformation products in the urban water cycle. *Environmental Pollution*, 225: 261-269.

5. Street, M., Angelini, S., Bernasconi, S., Burgio, E., Cassio, A., Catellani, C., Cirillo, F., Deodati, A., Fabbrizi, E., Fanos, V., Gargano, G., Grossi, E., Iughetti, L., Lazzeroni, P., Mantovani, A., Migliore, L., Palanza, P., Panzica, G., Papini, A.M., Parmigiani, S., Predieri, B., Sartori, C., Tridenti, G. and Amarri, S. (2018). Current knowledge on endocrine disrupting chemicals (edcs) from animal biology to humans, from pregnancy to adulthood: Highlights from a national Italian meeting. *International Journal of Molecular Sciences*, 19(6), 1647.
6. Qiang, L., Cheng, J., Yi, J., Rotchell, J. M., Zhu, X. and Zhou, J. (2016). Environmental concentration of carbamazepine accelerates fish embryonic development and disturbs larvae behaviour. *Ecotoxicology*, 25(7): 1426-1437.
7. Dordio, A. V., Belo, M., Martins Teixeira, D., Palace Carvalho, A. J., Dias, C. M. B., Picó, Y., and Pinto, A. P. (2011). Evaluation of carbamazepine uptake and metabolization by *Typha spp.*, a plant with potential use in phytotreatment. *Bioresource Technology*, 102(17): 7827-7834.
8. Kruglova, A., Ahlgren, P., Korhonen, N., Rantanen, P., Mikola, A. and Vahala, R. (2014). Biodegradation of ibuprofen, diclofenac and carbamazepine in nitrifying activated sludge under 12°C temperature conditions. *Science of The Total Environment*, 499, 394-401.
9. He, Yonetani, T., Asada, Y., Echigo, S. and Itoh, S. (2019). Simultaneous determination of carbamazepine-n-glucuronide and carbamazepine phase I metabolites in the wastewater by liquid chromatography-tandem mass spectrometry. *Microchemical Journal*, 145: 1191-1198.
10. Stolker, A. Linda, A. M., Niesing, W., Fuchs, R., Vreeken, R. J., Niessen, W. M. A. and Brinkman, U. A. T. (2004). Liquid chromatography with triple-quadrupole and quadrupole-time-of-flight mass spectrometry for the determination of micro-constituents - A comparison. *Analytical and Bioanalytical Chemistry*, 378(7): 1754-1761.
11. Mohamad-Nasir, N., Abdul-Talib, S., Lokman, N. F., Hashim, S. N. and Tay, C. C. (2021). Efficient and low-cost extraction methods for pharmaceutical compounds of carbamazepine and caffeine. *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies*, 12(9):1-9.
12. Dugheri, S., Marrubini, G., Mucci, N., Cappelli, G., Bonari, A., Pompilio, L.T. and Arcangeli, G. (2020). A review of micro-solid phase extraction techniques and devices applied in sample pretreatment coupled with chromatographic analysis. *Acta Chromatographica*, 33(2): 99-111.
13. Daniele, G., Fieu, M., Joachim, S., Bado-Nilles, A., Beaudouin, R., Baudoin, P., James-Casas, A., Andres, S., Bonnard, M., Bonnard, I. and Vulliet, E. (2017). Determination of carbamazepine and 12 degradation products in various compartments of an outdoor aquatic mesocosm by reliable analytical methods based on liquid chromatography-tandem mass spectrometry. *Environmental Science and Pollution Research*, 24(20): 16893-16904.
14. Tuli, L., Tsai, T.-H., Varghese, R. S., Cheema, A. and Ressom, H. W. (2010). Using a spike-in experiment to evaluate analysis of LC-MS data. *2010 IEEE International Conference on Bioinformatics and Biomedicine Workshops*, 2010: 5703775.
15. Lange, V., Picotti, P., Domon, B. and Aebersold, R. (2008). Selected reaction monitoring for quantitative proteomics: A tutorial. *Molecular Systems Biology*, 4: 61.
16. Zenezini Chiozzi, R., Capriotti, A. L., Cavaliere, C., Ferraris, F., La Barbera, G., Piovesana, S. and Laganà, A. (2017). Evaluation of column length and particle size effect on the untargeted profiling of a phytochemical mixture by using UHPLC coupled to high-resolution mass spectrometry. *Journal of Separation Science*, 40(12): 2541-2557.
17. Pitt, J. J. (2009). Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *Clinical Biochemistry Reviews*, 30(1): 19-34.
18. Segura, P. A., MacLeod, S. L., Lemoine, P., Sauvé, S. and Gagnon, C. (2011). Quantification of carbamazepine and atrazine and screening of suspect organic contaminants in surface and drinking waters. *Chemosphere*, 84(8): 1085-1094.
19. Karinen, R., Vindenes, V., Hasvold, I., Olsen, K. M., Christophersen, A. S. and Øiestad, E. (2014). Determination of a selection of anti-epileptic drugs and two active metabolites in whole blood by reversed phase UPLC-MS/MS and some examples of application of the method in forensic toxicology cases. *Drug Testing and Analysis*, 7(7): 634-644.
20. Zhang, H., Zhu, Y., Qiao, N., Chen, Y. and Gao, L. (2017). Preparation and characterization of carbamazepine cocrystal in polymer solution. *Pharmaceutics*, 9(4): 54.
21. Al-Qaim, F., Mussa, Z., Yuzir, A., Tahrir, N., Hashim, N. and Azman, S. (2018). Transportation of different therapeutic classes of pharmaceuticals to the surface water, sewage treatment plant, and hospital samples, malaysia. *Water*, 10(7): 916.