Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 14, Issue 3, 2025, 131
https://doi.org/10.33263/LIANBS143.131

Development and Validation of MEPS-LC-MS/MS Method for Simultaneous Quantitation of Anti-convulsant Drugs from Biological Matrix in Forensic Cases

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Received: 21.01.2024; Accepted: 7.07.2024; Published: 6.09.2025

Abstract: A new, rapid, sensitive, accurate, and simple microextraction packed sorbent– liquid chromatography-tandem mass spectrometry (MEPS-LC-MS/MS) method was evolved, followed by its validation for simultaneous detection of 3 anti-convulsant drugs (ACDs), namely, lamotrigine (LMG), valproic acid (VA) and topiramate (TPM) in biological sample. Analytes were extracted from biological samples using a C-18 cartridge of MEPS, which has high extraction efficiency (70-120%). Gradient elution with a flow rate of 6 mL/min was used to carry out the chromatographic separation. The linear regression analysis of the calibrators conveyed a linear relationship of response and concentration with the value of the coefficient of regression (r^2) > 0.998 for the range of concentration from 5 µg/mL to 200 µg/mL. The validation of this method has followed ICH guidelines in terms of accuracy, recovery, selectivity, repeatability, precision, specificity, and robustness. LOD and LOQ obtained for LMG were 11.57 and 35.08 ng/mL, for VA were 12.82 and 38.84 ng/mL, and for TPM, it was 16.52 and 50.06 ng/mL. The reported values of LOD and LOQ were minimal compared to other research, which infers that the extraction of these drugs is in low amounts. This method can be used in forensic science laboratories and in clinical laboratories for analysis of selected drugs.

Keywords: anti-convulsant drug; lamotrigine; topiramate; valproic acid; LC-MS/MS; MEPS.

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Epilepsy/convulsions is a non-communicable chronic neurological disorder. According to the International League Against Epilepsy (ILAE), epilepsy can be defined as a disorder of the brain that is a result of continuous 2 seizures with a time gap of at least 24 hours. The affected rate of epilepsy has increased to 50 million (according to WHO), with a greater number of cases in developing countries. There are about 13 million disability-adjusted life years due to epilepsy [1].

Epilepsy, in ancient times, was termed 'The Falling Sickness', and its history can be traced back to the late Paleolithic period. The first case of epilepsy was reported around 3000 BC by Edwin Smith Papyrus [2]. There are many references to epilepsy in Hippocratic books, and 'phlegma' was considered as one of the causes of epilepsy [3][NO_PRINTED_FORM]. Since that time, there have been changes in the belief of the causes of epilepsy from superstitions to scientific reasoning. Nowadays, epilepsy is seen as one of the neurological disorders, but many individuals are still unaware of its cause [4,5].

From the beginning, many therapies have been developed for the treatment of epilepsy. Many belligerent therapies were used in ancient times, such as skull trephination (making a hole in the skull with the help of a drill), which was mostly used, followed by bloodletting and binding of limbs. Earlier therapies for epilepsy also included treatment with the use of flora and fauna. One of the treatments consists of seal genitals, hippopotamus testicles, and blood of the tortoise. Celsus recommended the blood of slain gladiators for the treatment of epilepsy [6].

Nowadays, there are many prescriptions available for epilepsy, which are collectively known as antiepileptic drugs (AEDs) or anticonvulsant drugs (ACDs), as well as antiseizure drugs. The very first pharmacological treatment for epilepsy was bromide salts, used in 1857, followed by potassium salts in the late 1800s. Alfred Huaptmann introduced phenobarbital in 1912 for the treatment of epilepsy. New treatments were then continued to be introduced in the market, such as phenytoin in 1938, Ethosuximide in 1958, Carbamazepine in 1965, Valproic acid (VA), and benzodiazepines (Diazepam, Lorazepam, Clonazepam) in the late 1960s. Many drugs have been introduced since the 1990s, such as Lamotrigine, Topiramate, Gabapentin, and many more [7].

These drugs are mainly classified into conventional and new anticonvulsant drugs. Conventional ACDs are the drugs that were discovered and used before the 1990s, such as phenobarbital, phenytoin, carbamazepine (CBZ), and valproic acid (VA). New ACDs are the drugs that were introduced after the 1990s, including lamotrigine (LMG), oxcarbazepine (OXC), topiramate (TPM), vigabatrin, zonisamide and many more [8].

With the increase in the cases of epilepsy, the use of ACDs is increasing according to their effectiveness. With the availability of these drugs in the market, these are being abused by people for the purpose of intoxication, leading to death. There are many cases of self-intoxication by overdosing or by taking the wrong medications. In one of the reviews done by Staniszewska et al., from 2002 to 2012 in Cracow, Poland, there were about 6% cases of ACDs, out of which the maximum were due to self-intoxication CMZ and VA [9]. In another case, a 41-year-old lady was found dead. Postmortem analysis of autopsy tissues was done using GC-MS. It was found that death was caused by intoxication of topiramate and citalopram [10].

The concentration of these drugs in the biological matrices is of crucial importance for forensic scientists. They can detect the quantity of drugs to know whether or not that particular drug is responsible for the mortality of an individual. Drugs can be extracted from a variety of biological samples, including blood, saliva, hair, urine, and tissues. The two matrices that are most frequently used for extraction are blood and urine. Other biological tissues are also being used for the extraction and detection of these drugs. In one of the research studies, bones were used to quantify the level of carbamazepine. The analyte was extracted using SPE and analyzed by GC-MS. This study promotes the utilization of bone matrix if other samples are not available for analysis [11].

There are many extraction techniques available for taking out psychotropic drugs from different biological matrices using many analytical tools. Dolatabadi et al. (2021) conducted high-performance liquid chromatography with ultra-violet spectroscopy (HPLC-UV) analysis after performing electro-membrane extraction for analysis of phenobarbitals and phenytoin from human biological samples, including plasma, saliva, and urine [12]. Claudia et al. performed UPLC-DAD on the plasma of patients with bipolar disorder for the detection of lamotrigine after extraction using the protein precipitation technique [13]. Feng et al. (2020) extracted various ACDs from urine using solid phase extraction (SPE), and quantification was

done using LC-MS/MS [14]. Mostafa et al. (2018) performed liquid-liquid extraction (LLE) for extracting VA from urine, followed by examination using gas chromatography- flame ionization detection (GC-FID) [15]. In other studies, LC-MS/MS analysis was done after LLE in urine samples for the detection of ACDs [16]. New extraction methods are being developed such as by advancing conventional methods such as ultrasound-assisted reverse-phase emulsification microextraction (UARPEME) [17], salting-out assisted LLE (SALLE) [18], dispersive liquid-liquid microextraction (DLLME) [19,20], QuECHERS [21,22], and many more. Various instrumental analysis is performed for measurement of these substances in biological matrices and these techniques include HPLC with Photodiode Array Detection (HPLC-DAD) [23–25], HPLC-UV [26], MS [27], GC-MS [18,19,28–31], GC-FID [17,32], LC-MS/MS [14,33–42], UHPLC-MS/MS [43], UPLC-MS/MS [44] microfluidic device [45] etc. The spectrophotometric method is an emerging approach to detecting these drugs in different samples. One of these methods was used for the concentration measurement of Phenytoin, Levetiracetam, and Pregabalin in breast milk samples [46]. Animal models have also been used to quantify AEDs to develop new methods for drugs. Qiu et al. used the LC-MS/MS method for quantification of VA, LMG, and valproate-glucuronide from rat plasma, which gave good results [31].

The present paper has been designed to give sensitive, accurate, efficient, fast, and authentic MEPS/LC-MS/MS quantification techniques for simultaneous screening of 3 ACDs- LMG, TPM, and VA (Figure 1) in biological matrix. Many methods have already been developed for some of the ACDs, but there is a lack of certainty and fidelity in those methods.

Figure 1. Chemical structure of selected ACDs.

The extraction method is an important step in analysis in case of complex matrices or when multiple drugs are present [47]. Microextraction packed sorbent (MEPS) is a modified and miniaturized SPE method that can be directly connected to the instruments for quantitative analysis. MEPS is a 4-step process: cartridge conditioning, sample loading, sorbent washing, and elution, followed by injection of eluent for instrumentation analysis (Figure 2) [48].

The process of MEPS includes 4 steps-conditioning, MEPS syringe was conditioned with methanol and water to remove all the impurities; sample loading, the sample was loaded in the sorbent by draw and eject method for about 4-5 times; sorbent washing, sorbent was washed using organic solutes such as isopropyl water to remove interfering components; and

elution, organic or inorganic solvents, and their mixtures are used to elute out the analyte of interest [49].

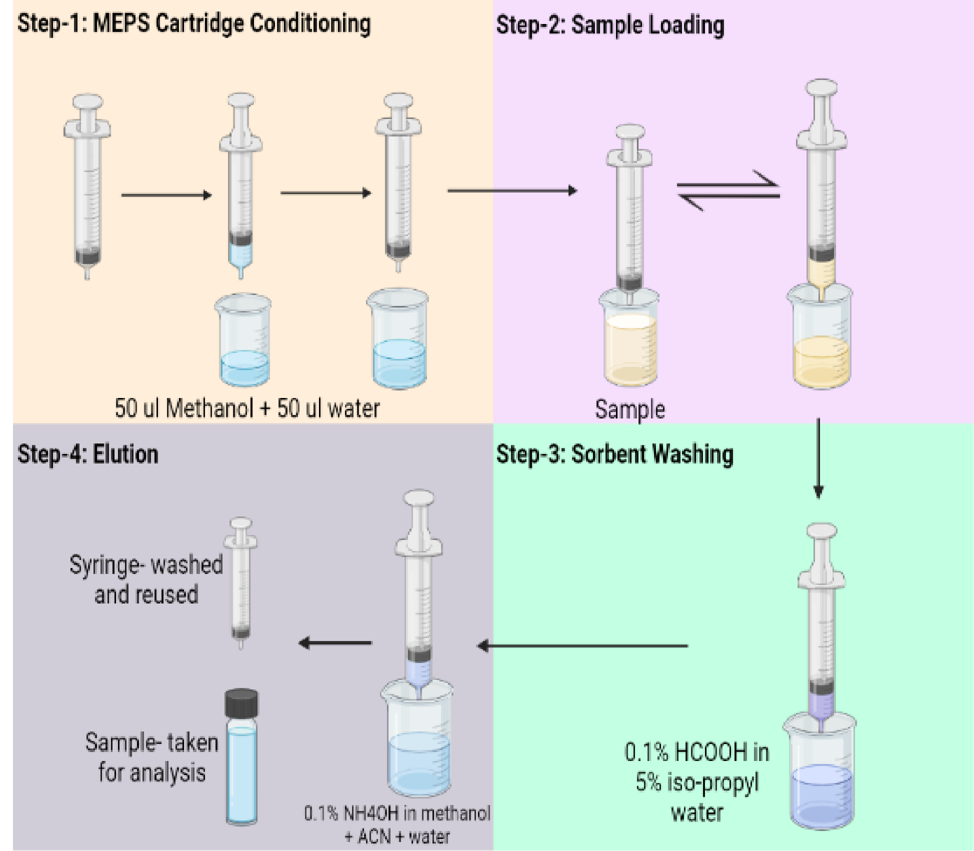

Figure 2. MEPS extraction procedure.The difference between MEPS and SPE is that a separate column for performing separation is present in SPE but not in MEPS. Also, the size of sorbent in MEPS is smaller as compared to that in SPE. The time taken by extraction in SPE is longer, which has been reduced by the MEPS method. The major advantage of the MEPS cartridge is that it is reusable and can be used about 100-120 times with biological samples, and with water, it can be used about 400 times [50]. MEPS has proved to be a very simple and efficient method when compared to conventional SPE and LLE as it can deal with smaller volumes of sample (10 μL) as well as solvent and gives results with accuracy and precision [51].

Also, those methods are highly costly and time-consuming. The developed extraction method in this paper has advantages over other methods with less run time. This method also has the benefit of detecting all of the target analytes in positive ion mode without switching in polarity, as seen in most studies. Simulated urine samples have been used in this study, ensuring reliability and accuracy similar to real samples [52]. The properties of the target ACDs of this study are depicted in Table 1.

Table 1. Physical properties of selected ACDs.

S.No.	Properties	Lamotrigine	Topiramate	Valproic acid
1.	Molecular formula	$C_9h_7cl_2n_5$	$C_{12}h_{21}no_8s$	$C_8h_{15}nao_2$
2.	Iupac name	6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine	2,3: 4,5-bis-O-(1-methyl ethylidene)-36-D-fructopyranose sulfamate	2-Propylpentanoic acid
3.	Common Name	Lamictal	Topamax	Epival
4.	Classification	New acids	New acids	Conventional acids
5.	Physical state	Solid	Solid	Liquid

S.No.	Properties		Lamotrigine	Topiramate	Valproic acid
6.	Appearance		White to off-white powder	White crystalline powder	Colorless
7.	Molecular weight (G/Mol)		256.09	339.36	166.19
8.	Boiling Point (°C)		503.1 ± 60.0	438.7	222
9.	Melting Point (°C)		218	123-125	N/A
10.	Solubility	In water	Sparingly dispersible in water	Dispersible in water	Dispersible in water
		Others	Dissolvable in organic solvents	Dissolvable in alkaline solutions such as NaOH and Na_3PO_4. Freely soluble in acetone, chloroform and acetone	Freely soluble in Naoh, methanol, alcohol, acetone, and ether. Sparingly soluble in HCl

N/A- Not available

2. Materials and Methods

2.1. Chemical reagent.

Analytical grade compounds and reagents were utilized, including methanol, lamotrigine, topiramate, valproic acid, acetic acid, isopropyl water, ammonium hydroxide, acetonitrile, and double distilled water, and were procured from Sigma Aldrich, St. Louis, MO, USA.

2.2. Stock solution.

A stock solution of 1 mg/mL in methanol was devised for all the acquired ACDs, including LMG, TPM, and VA. Working standard solutions of combined drugs were then prepared by diluting the stock solution with methanol. 6 calibrators of each drug were prepared, which were 5 ng/mL, 10 ng/mL, 20 ng/mL, 50 ng/mL, 100 ng/mL and 200 ng/mL.

2.3. Matrix.

2.3.1. Preparation of biological matrix.

Preparation of simulated urine: 13 components of urine were prepared from uric acid, creatinine, urea, magnesium sulfate heptahydrate, potassium oxalate, potassium chloride, trisodium citrate dihydrate, sodium dihydrogen phosphate dihydrate, sodium chloride, sodium phosphate dibasic dihydrate, ammonium chloride, sodium sulfate, calcium chloride [52].

2.4. MEPS extraction.

MEPS is a miniaturized SPE which is used for sample preparation. The following extraction process is given in Figure 2. The process includes 4 steps-conditioning, the MEPS syringe was conditioned by 50 µL methanol and 50 µL water; sample loading, 100 µL sample was loaded following draw eject method for about 4-5 times; sorbent washing, 100 µL of 0.1% HCOOH in 5% isopropyl water was used to wash the sorbent and to remove interfering components; and elution, 20-50 µL of 0.1% NH_4OH in methanol, acetonitrile and water in ratio 5:3:2 was used to elute out the analyte of interest.

The sorbent was then washed 4-5 times by100 µL 0.1% NH_4OH in methanol and acetonitrile (1:1) or 100 µL 0.1% HCOOH in isopropanol and water (1:1) and reused.

2.5. Instrumentation and LC-MS/MS condition.

By using an Agilent 6470 platform LC-MS/MS, extracted samples were examined. Mobile phase A was 5mM Ammonium format in 0.1% formic acid in water, and mobile phase B was composed of 0.1% formic acid in 100% methanol. The column was held at a temperature of 55°C, and the gas with the flow rate of 6 mL/min was used for analysis of 10 µL of injected sample using the gradient method. The gradient started with 95% of A and 5% of B, which then increased to 60% A and 40% B at 10 minutes, 40% A and 60% B at 12 minutes, 5% A and 95% B at 15 minutes, 90 % A and 10% B at 18 minutes and 95 % A and 5 % B at 20 minutes.

To get the most points across the different analyte peaks, the employed method was run in dynamic MRM mode.

2.6. Method validation.

This method was validated according to the International Council of Harmonisation for Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines (ICH Tripartite Guidelines)- Validation of Analytical Procedures: Text and Methodology (Q2(R1)).

Linearity between the response in terms of area under the peak and concentration was calculated by running independent runs for the above-mentioned 6 calibrations. The calibration curve was acquired using weighted least square linear regression (WLSLR) along with slope, intercept, and coefficient of regression (r^2) for which relative standard deviation (RSD) was calculated.

The limit of detection (LOD), also known as the smallest concentration of an analyte, may be identified using any of the methods without any intervention or disturbance, but that cannot be measured exactly. The limit of quantification (LOQ), also termed as the smallest amount of any drug that can be quantified with appropriate precision and accuracy on a day-to-day basis, was calculated by analyzing biological samples at 6 different concentrations. According to ICH guidelines, precision must be less than 20%, and accuracy must lie in the range of ±20%.

Both inter-day accuracy and precision were evaluated for 3 consecutive days using urine samples at 6 variable concentrations (5, 10, 20, 50, 100, and 200 µg/mL). In a similar manner, intraday accuracy and precision were estimated by 6 replicates for 3 concentrations (lower, middle, and upper concentration).

The absolute recovery of the analyte was calculated for all 6 concentrations by contrasting the analyte concentration that was determined by analysis with the compound that was spiked in the biological matrix.

The matrix effect is defined by its impact on the extraction and quantification of target analytes from biological matrices. It affects the ionization efficiency of the target analyte, leading to either its enhancement or depletion, due to which recovery ranges from 70 to 120 %. Table 2 gives the assay results of the analysis.

Table 2. Concentrations and linearity from the typical calibration curve.

Drug	Linear range (µg/mL)	Linear regression equation	Coefficient of regression (r^2)
LMG	5-200	Y=738.03X – 49.892	0.9991
VA	5-200	Y=1047.9X + 9044.6	0.9989
TPM	5-200	Y=363.31 X + 5043.4	0.9981

3. Results and Discussion

3.1. Optimization of sample preparation: MEPS condition.

Sample preparation is the most time-consuming and crucial step in the analysis and quantification of samples from any biological matrix. Traditional methods available for sample preparation are tedious, time-consuming, and less efficient. For this reason, researchers are working on advanced sample preparation techniques. In this paper, MEPS has been selected as a sample preparation technique as it has many advantages, such as rapidity in analyzing samples, minimal sample (about 10 µL) requirement, lesser manpower, minimal cost, and time efficiency. One of the advantages is that one syringe can be used about 100-120 times for sample preparation from different biological matrices with direct injection of analyte into the analytical instruments.

In MEPS, a C-18 cartridge has been used for efficient extraction. 4 stepped MEPS protocol starts with Cartridge conditioning, which is done with a mixture of organic solvents such as methanol, isopropanol, or acetonitrile and water. These organic solvents are used to remove impurities from the cartridge, if left, and to immobilize the target analytes on the sorbent. Elution of the analyte from the sorbent is also an important step, which is performed by pure organic solvent or a mixture of the former with acid or base. Acetonitrile, methanol, and water were used in combination with ammonium hydroxide, which resulted in more recovery when a mixture of methanol and acetonitrile was used alone.

3.2. Method validation.

A blank biological matrix was analyzed for any endogenous interferences with the targeted analyte at any retention time. Chromatograms of a blank and spiked biological matrix with the target analyte are shown in Figure 3. Retention times for LMG, VA, and TPM were found to be at 9.994, 7.452, and 11.986. The investigation found no evidence of endogenous interference.

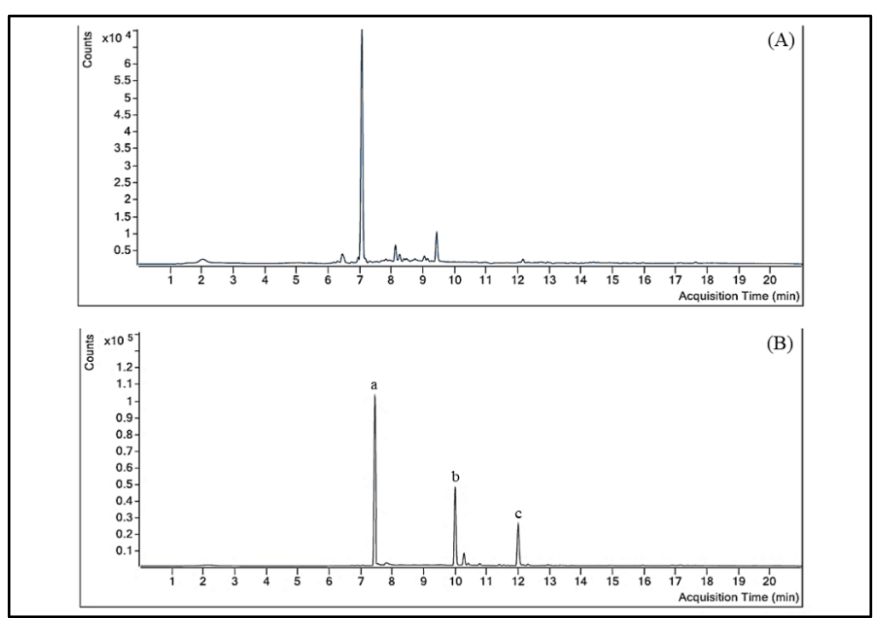

Figure 3. Chromatogram of **(A)** matrix blank; **(B)** biological matrix spiked with the standard of (a) VA, (b) LMG, and (c) TPM at 200 µg/mL.

For each of the three ACDs, linear calibration curves were obtained in the urine sample (Figure 4). WLSLR algorithm was used in the specific ranges for all the target molecules to obtain calibration curves. The curve thus obtained was linear with the coefficient of regression (r2) ≥ 0.9981. Table 2 contains the linear regression equation and values of the coefficient of regression for all 3 drugs.

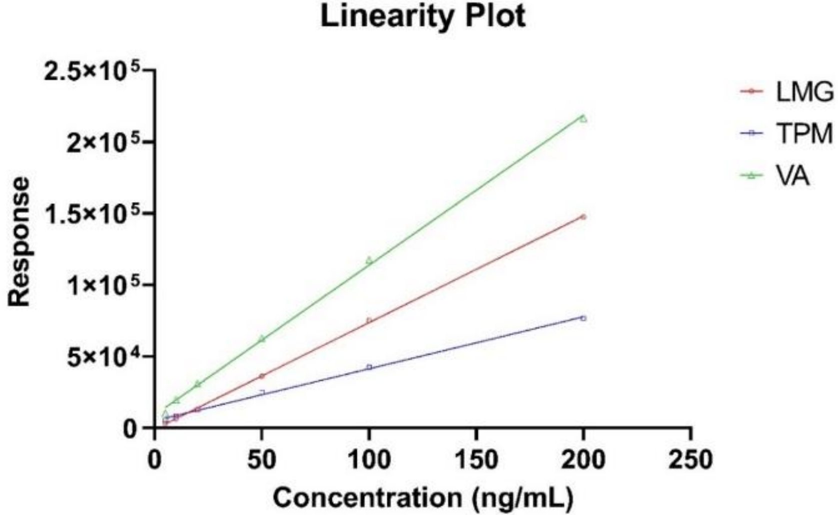

Figure 4. Calibration curve of 3 ACDs in urine sample.

As shown in Table 3, the LODs found were 11.57, 12.82, and 16.52 ng/mL for LMG, VA, and TPM. LOQs obtained for LMG, VA, and TPM were 35.08, 38.84, and 50.06 ng/mL from the biological matrix. The relative standard deviation (RSD) was under the acceptable range for all three drugs, i.e., less than 20%. For three days in a row, daily precision and accuracy were controlled, for which 6 standard concentrations were prepared. For three concentrations (low, middle, and high), intraday precision and accuracy were assessed and came under the admissible range. The recovery of all 3 analytes from the biological sample was between 70-120 %.

Table 3. LOD, LOQ, RSD, and recovery % for ACDs.

Drug	LOD (ng/mL)	LOQ (ng/mL)	RSD%	Recovery %
LMG	11.57713	35.08222	1.1594	94-104
VA	12.81933	38.84645	1.1325	74-120
TPM	16.51972	50.05976	1.1249	70-115

3.3. Implementation of chromatographic conditions: LC-MS/MS Conditions.

To obtain a consistent and symmetrical peak, LC-MS/MS conditions were improved and implemented. Mobile phase, flow rate, pH of buffer used, and column temperature are some of the important factors that affect the analysis of target analytes. Ammonium format was used as the mobile phase because it narrows down the peak compared to water, which broadens the peak. The addition of formic acid enhances the signal of LMG, VA, and TPM. Due to this reason, mobile phase A was used in 5 mM ammonium format in 0.1% Formic acid in water. The ESI-MS regulation was made in positive ion mode for all 3 ACDs, and their precursor and product ions are given in Table 4 with collision frequency.

Table 4. Precursor and production for ACDs.

S. No.	Compound	Retention time	Precursor ion	Product ion	Collision energy
1	LMG	9.994	257.1	157.0	12
2	VA	7.452	145.2	95.0	7
3	TPM	11.986	340.0	141.0	5

LC-MS/MS results in the form of chromatograms (acquisition time v/s counts) for simultaneous detection of 3 ACDs at 6 different concentrations (5 to 200 ng/mL) are given in Figure 5.

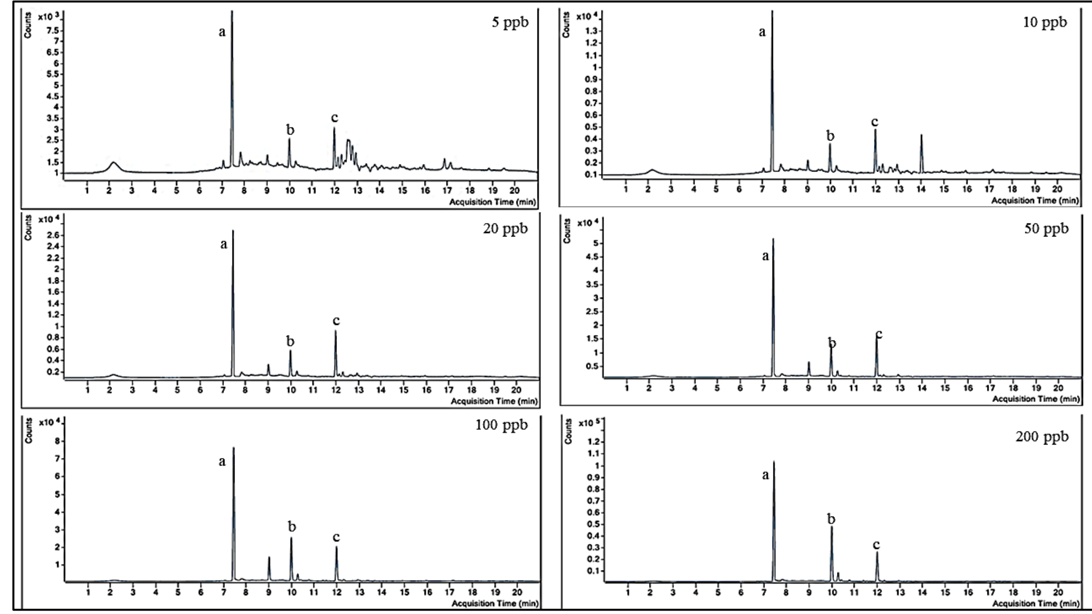

Figure 5. LC-MS/MS Chromatogram of (a) VA, (b) LMG, and (c) TPM at concentrations 5, 10, 20, 50, 100, and 200 µg/mL resp. in biological matrix.

Comparing the properties of the current method to those of previously presented methods for 3 ACDs- LMG, VA, and TPM, this method is a novel method for extraction and isolation of these 3 ACDs from biological samples using MEPS followed by analysis using LC-MS/MS. Nikolaou et al. (2015), in their SPE-GC-MS method, reported LOD as 0.15 µg/mL, LOQ as 0.50 µg/mL, and recovery as 97% for LMG in whole blood [29]. Shah and others (2013), in their SPE-HPLC method for dried blood spots, described LOQ to be 1 µg/mL for LMG [53]. Magafia et al. (2022) concluded LOD and LOQ for LMG as 2 X 10-2 µg/mL and 5 X 10-5 µg/mL when PPE-UPLC-DAD was used for the plasma sample [13]. Almeida and others (2020), from their study on TPM in aqueous solution, found LOD and LOQ to be 10.23 mg/ L and 30.99 mg/L using SALLE-GC-MS [18]. Mercolini and co-workers (2010) concluded LOD to be in the range of 0.1 to 0.3 µg/mL for TPM using an SPE-HPLC fluorescence detector [54]. Proenca and other scientists (2011) used SPE- UPLC-MS/MS for VA extraction from blood and described LOQ as 0.5 µg/mL and 95% recovery [55]. Shahdousti and co-workers (2007) conducted headspace liquid-phase microextraction followed by confirmation using GC-FID for the detection of VA and concluded LOD as 0.8 µg/mL [32]. Kumirska and other researchers (2019) worked on 20 pharmaceutical content in soil samples. Out of 20, 2 were ACDs- Valproic Acid and Primidone. They used QuEChERS and ultrasonic Assisted Extraction (UAE) followed by GC-MS for quantification. The recovery rate in the QuEChERS method was found to be 52 %, and in the AE, it was 100.70% [56]. In another study by Hassan et. at (2021), CBZ was extracted from human urine and wastewater samples using dispersive micro-SPE followed by HPLC-DAD for identification. The LOD of the

discussed method was found to be 0.51 µg/L [57]. Pashael and others (2022) used a common Liquid Extraction method for the extraction of 15 human pharmaceutical residues in fish and shrimp tissues, followed by their quantification using HPLC-MS/MS. LOD and LOQ of the used method were calculated as 0.046 µg/kg and 0.138 µg/kg [58]. Jiang et al. (2023) performed PPE coupled with UHPLC-MS/MS to concurrently detect 24 ACDs in human plasma samples. The interday accuracy measured was in the range of -1.07 to 13.69 %, and precision was found to be <6.70 % [43]. In another research by Yang et al. (2022), VA and its 6 metabolites were detected in human serum sample. The method followed extraction by PPE and detection by LC-MS/MS. Accuracy and precision for the used method were found to be in the acceptable range [59]. Zhou et al. (2022) developed the PPE-LC-MS/MS method for quantification of Levetiracetam, LMG, and 10-hydroxycarbazepine from a human plasma sample. The inter and intra accuracy and precision were within ±15% [36]. The LOD and LOQ we obtained in this research are the lowest as compared to the already published research studies.

4. Conclusions

ACDs use is increasing day by day as the cases of epilepsy are rising, leading to its abuse in forensic and clinical cases. A novel, highly responsive, and scrupulous MEPS-LC-MS/MS approach was developed and validated for simultaneous detection of three ACDs- lamotrigine, valproic acid, and topiramate from the biological matrix of volume 10 µL. The above-discussed method provides significant advantages as compared to already discussed methods in terms of simplicity, reliability, time efficiency, and high reproducibility with recovery in the range of 70- 120 % under the given conditions. The proposed method showed linearity in a concentration ranging from 5 to 200 µg/mL and has high accuracy, precision, and recovery rate for all three drugs. LOD obtained for LMG, VA, and TPM are 11.57713, 12.81933, and 16.51972 ng/mL. The suggested procedure can be used in forensic science laboratories to determine the presence of the discussed anti-convulsant drugs in various cases involving suicidal attempts, overdose cases, and many more, in less time with a low amount of samples, leading to more accurate and precise results which is not possible with conventional methods. The method can also be applied for therapeutic drug monitoring in clinical laboratories.

Author Contribution

All authors have read and agreed to the published version of the manuscript."

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This research received no external funding

Acknowledgments

Conceptualization, S.S. and J.S.; methodology, S.S.; software, S.S.; validation, J.S. and V.R.; formal analysis, S.S. and V.R.; investigation, S.S.; resources, S.S.; data curation, S.S. and V.R.; writing—original draft preparation, S.S.; writing—review and editing, S.S., V.R., and J.S.; visualization, S.S.; supervision, V.R.; project administration, J.S.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. WHO EPILEPSY A Public Health Imperative International League Against Epilepsy; 2019;
2. Gross, R.A. A Brief History of Epilepsy and Its Therapy in the Western Hemisphere. Epilepsy Res 1992, 12, 65–74, doi:10.1016/0920-1211(92)90028-R.
3. Walshe, T.M. On the Sacred Disease. In Neurological Concepts in Ancient Greek Medicine; Oxford University Press, 2016; pp. 43–60.
4. Patel, P.; Moshé, S.L. The Evolution of the Concepts of Seizures and Epilepsy: What's in a Name? Epilepsia Open 2020, 5, 22–35, doi:10.1002/epi4.12375.
5. Magiorkinis, E.; Diamantis, A.; Sidiropoulou, K.; Panteliadis, C. Highights in the History of Epilepsy: The Last 200 Years. Epilepsy Res Treat 2014, 2014, 1–13, doi:10.1155/2014/582039.
6. Gowers, W.R. A Manual of Diseases of the Nervous System; 3rd ed.; 1899;
7. Brodie, M.J. Antiepileptic Drug Therapy the Story so Far. Seizure 2010, 19, 650–655, doi:10.1016/j.seizure.2010.10.027.
8. Hakami, T. Neuropharmacology of Antiseizure Drugs. Neuropsychopharmacol Rep 2021, 41, 336–351, doi:10.1002/npr2.12196.
9. Staniszewska, A.; Dabrowska-Bender, M.; Czerw, A.; Olejniczak, D.; Juszczyk, G.; Bujalska-Zadrozny, M. Epidemiological Study of Acute Poisoning for Antiepileptic Drugs: A 2-Year Retrospective Study in Cracow, Poland. In Epileptology - The Modern State of Science; InTech, 2016.
10. Beer, B.; Libiseller, K.; Oberacher, H.; Pavlic, M. A Fatal Intoxication Case Involving Topiramate. Forensic Sci Int 2010, 202, doi:10.1016/j.forsciint.2010.04.035.
11. Fernández-López, L.; Mancini, R.; Rotolo, M.-C.; Navarro-Zaragoza, J.; Hernández del Rincón, J.-P.; Falcón, M. Carbamazepine Overdose after Psychiatric Conditions: A Case Study for Postmortem Analysis in Human Bone. Toxics 2022, 10, 322, doi:10.3390/toxics10060322.
12. Dolatabadi, R.; Mohammadi, A.; Nojavan, S.; Yaripour, S.; Tafakhori, A.; Shirangi, M. Electromembrane Extraction-high-performance Liquid Chromatography-ultraviolet Detection of Phenobarbital and Phenytoin in Human Plasma, Saliva, and Urine. Journal of the Chinese Chemical Society 2021, 68, 1522–1530, doi:10.1002/jccs.202100016.
13. Palacios-Magaña, C. V.; Romero-Tejeda, E.M.; Fajardo-Robledo, N.S.; González-Ortiz, L.J.; González-Mendez, J.G.; Pacheco-Moisés, F.P. Lamotrigine Extraction and Quantification by UPLC-DAD in Plasma from Patients with Bipolar Disorder. Int J Anal Chem 2022, 2022, 1–5, doi:10.1155/2022/3288646.
14. Feng, S.; Bridgewater, B.; Strickland, E.C.; McIntire, G. A Rapid LC–MS-MS Method for the Quantitation of Antiepileptic Drugs in Urine. J Anal Toxicol 2020, 44, 688–696, doi:10.1093/jat/bkaa095.
15. Mostafa, M.S.; Elshafie, H.S.; Ghaleb, S. A Rapid and Simple Procedure for Monitoring Valproic Acid by Gas Chromatography. Journal of Biological Research - Bollettino della Società Italiana di Biologia Sperimentale 2018, 90, doi:10.4081/jbr.2017.6359.
16. Anilanmert, B.; Çavuş, F.; Narin, I.; Cengiz, S.; Sertler, Ş.; Özdemir, A.A.; Açikkol, M. Simultaneous Analysis Method for GHB, Ketamine, Norketamine, Phenobarbital, Thiopental, Zolpidem, Zopiclone and Phenytoin in Urine, Using C18 Poroshell Column. Journal of Chromatography B 2016, 1022, 230–241, doi:10.1016/j.jchromb.2016.03.040.

17. Ismailzadeh, A.; Masrournia, M.; Es'haghi, Z.; Bozorgmehr, M.R. Microextraction and Gas Chromatography–Flame Ionization Determination of Five Antiepileptic Drugs in Biological Samples Using Amino Acid-Based Deep Eutectic Ionic Liquids. J Mol Liq 2020, 317, 113979, doi:10.1016/j.molliq.2020.113979.
18. Almeida, J.M.F. De; Silva, E.M.F.; Veríssimo, L.M.; Fernandes, N.S. Salting-out Assisted Liquid-Liquid Extraction Method Combined with GC-MS for the Determination of Topiramate in Aqueous Solutions: Development and Application of the Methodology. Sep Sci Technol 2020, 55, 2303–2312, doi:10.1080/01496395.2019.1624570.
19. Alcantara, G.K.S.; Calixto, L.A.; de Moraes, L.A.B.; Queiroz, R.H.C.; de Oliveira, A.R.M.; de Gaitani, C.M. Determination of Levetiracetam in Human Plasma by Dispersive Liquid-Liquid Microextraction Followed by Gas Chromatography-Mass Spectrometry. J Anal Methods Chem 2016, 2016, 1–12, doi:10.1155/2016/5976324.
20. Mashayekhi, H.A.; Abroomand-Azar, P.; Saber-Tehrani, M.; Husain, S.W. Rapid Determination of Carbamazepine in Human Urine, Plasma Samples and Water Using DLLME Followed by RP–LC. Chromatographia 2010, 71, 517–521, doi:10.1365/s10337-009-1456-6.
21. Matsuta, S.; Nakanishi, K.; Miki, A.; Zaitsu, K.; Shima, N.; Kamata, T.; Nishioka, H.; Katagi, M.; Tatsuno, M.; Tsuboi, K.; et al. Development of a Simple One-Pot Extraction Method for Various Drugs and Metabolites of Forensic Interest in Blood by Modifying the QuEChERS Method. Forensic Sci Int 2013, 232, 40–45, doi:10.1016/j.forsciint.2013.06.015.
22. Mizuno, S.; Lee, X.-P.; Fujishiro, M.; Matsuyama, T.; Yamada, M.; Sakamoto, Y.; Kusano, M.; Zaitsu, K.; Hasegawa, C.; Hasegawa, I.; et al. High-Throughput Determination of Valproate in Human Samples by Modified QuEChERS Extraction and GC-MS/MS. Leg Med 2018, 31, 66–73, doi:10.1016/j.legalmed.2018.01.002.
23. Ferreira, A.; Rodrigues, M.; Oliveira, P.; Francisco, J.; Fortuna, A.; Rosado, L.; Rosado, P.; Falcão, A.; Alves, G. Liquid Chromatographic Assay Based on Microextraction by Packed Sorbent for Therapeutic Drug Monitoring of Carbamazepine, Lamotrigine, Oxcarbazepine, Phenobarbital, Phenytoin and the Active Metabolites Carbamazepine-10,11-Epoxide and Licarbazepine. Journal of Chromatography B 2014, 971, 20–29, doi:10.1016/j.jchromb.2014.09.010.
24. Zhang, J.; Liu, D.; Meng, X.; Shi, Y.; Wang, R.; Xiao, D.; He, H. Solid Phase Extraction Based on Porous Magnetic Graphene Oxide/β-Cyclodextrine Composite Coupled with High Performance Liquid Chromatography for Determination of Antiepileptic Drugs in Plasma Samples. J Chromatogr A 2017, 1524, 49–56, doi:10.1016/j.chroma.2017.09.074.
25. Sabença, R.; Bicker, J.; Silva, R.; Carona, A.; Silva, A.; Santana, I.; Sales, F.; Falcão, A.; Fortuna, A. Development and Application of an HPLC-DAD Technique for Human Plasma Concentration Monitoring of Perampanel and Lamotrigine in Drug-Resistant Epileptic Patients. Journal of Chromatography B 2021, 1162, 122491, doi:10.1016/j.jchromb.2020.122491.
26. Lipecki, Y.; Haen, E. Simultaneous Quantification of 11 Antiseizure Medications and Metabolites in Serum for Therapeutic Drug Monitoring Using High-Performance Liquid Chromatography with Ultraviolet Detection: A Short Communication. Ther Drug Monit 2022, 44, 345–350, doi:10.1097/FTD.0000000000000908.
27. Hu, W.; Zhou, W.; Wang, C.; Liu, Z.; Chen, Z. Direct Coupling In-Tube Solid-Phase Microextraction with Mass Spectrometry Using Polymer Coated Open-Tubular Column for Rapid Analysis of Antiepileptic Drugs in Biofluids. Anal Chim Acta 2023, 1240, 340775, doi:10.1016/j.aca.2022.340775.
28. Conway, J.M.; Birnbaum, A.K.; Marino, S.E.; Cloyd, J.C.; Remmel, R.P. A Sensitive Capillary GC-MS Method for Analysis of Topiramate from Plasma Obtained from Single-Dose Studies. Biomedical Chromatography 2012, 26, 1071–1076, doi:10.1002/bmc.1749.
29. Nikolaou, P.; Papoutsis, I.; Dona, A.; Spiliopoulou, C.; Athanaselis, S. Development and Validation of a GC/MS Method for the Simultaneous Determination of Levetiracetam and Lamotrigine in Whole Blood. J Pharm Biomed Anal 2015, 102, 25–32, doi:10.1016/j.jpba.2014.08.034.
30. Mouskeftara, T.; Alexandridou, A.; Krokos, A.; Gika, H.; Mastrogianni, O.; Orfanidis, A.; Raikos, N. A Simple Method for the Determination of Lacosamide in Blood by <scp>GC</Scp> - <scp>MS</Scp>. J Forensic Sci 2020, 65, 288–294, doi:10.1111/1556-4029.14151.
31. Qiu, F.; Nie, S. Liquid Chromatography Coupled with Tandem Mass Spectrometry for Simultaneous Quantification of Valproate, Valproate-glucuronide and Lamotrigine in Various Biological Matrices of Rat. Biomedical Chromatography 2023, 37, doi:10.1002/bmc.5652.

32. Shahdousti, P.; Mohammadi, A.; Alizadeh, N. Determination of Valproic Acid in Human Serum and Pharmaceutical Preparations by Headspace Liquid-Phase Microextraction Gas Chromatography-Flame Ionization Detection without Prior Derivatization. Journal of Chromatography B 2007, 850, 128–133, doi:10.1016/j.jchromb.2006.11.013.
33. Linder, C.; Hansson, A.; Sadek, S.; Gustafsson, L.L.; Pohanka, A. Carbamazepine, Lamotrigine, Levetiracetam and Valproic Acid in Dried Blood Spots with Liquid Chromatography Tandem Mass Spectrometry; Method Development and Validation. Journal of Chromatography B 2018, 1072, 116–122, doi:10.1016/j.jchromb.2017.11.005.
34. Dwivedi, J.; Namdev, K.K.; Chilkoti, D.C.; Verma, S.; Sharma, S. An Improved LC-ESI-MS/MS Method to Quantify Pregabalin in Human Plasma and Dry Plasma Spot for Therapeutic Monitoring and Pharmacokinetic Applications. Ther Drug Monit 2018, 40, 610–619, doi:10.1097/FTD.0000000000000541.
35. D'Urso, A.; Cangemi, G.; Barco, S.; Striano, P.; D'Avolio, A.; de Grazia, U. LC-MS/MS-Based Quantification of 9 Antiepileptic Drugs From a Dried Sample Spot Device. Ther Drug Monit 2019, 41, 331–339, doi:10.1097/FTD.0000000000000600.
36. Zhou, S.; Li, R.; Chen, Z.; Ren, R.; Wang, X.; Dai, Q.; Wen, D.; Guan, Y.; Zhang, X.; Tang, S.; et al. LC–MS/MS Quantification of Levetiracetam, Lamotrigine and 10-hydroxycarbazepine in TDM of Epileptic Patients. Biomedical Chromatography 2022, 36, doi:10.1002/bmc.5393.
37. Yu, H.; Ren, X.; Liu, L.; Xiang, D.; Li, X.; Li, J.; Liu, D.; Gong, X. Simultaneous Determination of Eight Antiepileptic Drugs and Two Metabolites in Human Plasma by Liquid Chromatography/Tandem Mass Spectrometry. Acta Chromatogr 2023, 35, 161–169, doi:10.1556/1326.2022.01024.
38. Salzmann, L.; Spescha, T.; Singh, N.; Schierscher, T.; Bachmann, M.; Bauland, F.; Geistanger, A.; Risch, L.; Geletneky, C.; Seger, C.; et al. An Isotope Dilution-Liquid Chromatography-Tandem Mass Spectrometry (ID-LC-MS/MS)-Based Candidate Reference Measurement Procedure (RMP) for the Quantification of Lamotrigine in Human Serum and Plasma. Clinical Chemistry and Laboratory Medicine (CCLM) 2023, 61, 1930–1941, doi:10.1515/cclm-2022-0997.
39. Rajendran, K.; Anoop, K.; Nagappan, K.; Sekar, G.M.; Rajendran, S.D. Ultra-Performance Liquid Chromatography Coupled with a Triple Quadrupole Mass Spectrometric Method for the Quantification of Antiepileptic Drugs Methsuximide and Normesuximide in Human Plasma and Its Application in a Pharmacokinetic Study. Curr Pharm Anal 2022, 18, 228–239, doi:10.2174/1573412917666210211122311.
40. Adiraju, S.K.; Sumi, C.D.; Pandey, S.; Cotta, M.O.; Roberts, J.A.; Lipman, J.; Wallis, S.C.; Parker, S.L. Quantification of Levetiracetam in Plasma and Urine and Its Application to a Pharmacokinetic Study of Traumatic Brain Injury Patients. Bioanalysis 2023, 15, 31–42, doi:10.4155/bio-2022-0230.
41. Ma, D.; Ji, Z.; Cao, H.; Huang, J.; Zeng, L.; Yin, L. LC–MS3 Strategy for Quantification of Carbamazepine in Human Plasma and Its Application in Therapeutic Drug Monitoring. Molecules 2022, 27, 1224, doi:10.3390/molecules27041224.
42. Ji, Z.; Li, T.; Zhao, X.; Ma, W.; Li, Y.; Huang, J. Development and Validation of a Highly Sensitive and Rapid LC-MS3 Strategy to Determine Oxcarbazepine and Its Active Metabolite in the Serum of Patients with Epilepsy and Its Application in Therapeutic Drug Monitoring. Molecules 2022, 27, 5670, doi:10.3390/molecules27175670.
43. Jiang, R.; Zhang, D.; Zhao, Z.; Mei, S. Simultaneous Determination of 24 Antiepileptic Drugs and Their Active Metabolites in Human Plasma by UHPLC-MS/MS. J Pharm Biomed Anal 2023, 232, 115437, doi:10.1016/j.jpba.2023.115437.
44. Rmandić, M.; Stajić, A.; Jančić, J.; Samardžić, J.; Jović, N.; Malenović, A. Quantification of Zonisamide in Dried Blood Spots and Dried Plasma Spots by UPLC–MS/MS: Application to Clinical Practice. Molecules 2022, 27, 4899, doi:10.3390/molecules27154899.
45. Wentland, L.; Cook, J.M.; Minzlaff, J.; Ramsey, S.A.; Johnston, M.L.; Fu, E. Field-Use Device for the Electrochemical Quantification of Carbamazepine Levels in a Background of Human Saliva. J Appl Electrochem 2023, 53, 523–534, doi:10.1007/s10800-022-01785-9.
46. Noroozi, R.; Sohrabi, M.R.; Davallo, M. A Simple and Rapid Spectrophotometric Method Coupled with Intelligent Approaches for the Simultaneous Determination of Antiepileptic Drugs in Pharmaceutical Formulations, Biological, Serological, and Breast Milk Samples. Chemometrics and Intelligent Laboratory Systems 2022, 228, 104633, doi:10.1016/j.chemolab.2022.104633.

47. Rani, S.; Malik, A.K.; Singh, B. Novel Micro-Extraction by Packed Sorbent Procedure for the Liquid Chromatographic Analysis of Antiepileptic Drugs in Human Plasma and Urine. J Sep Sci 2012, 35, 359–366, doi:10.1002/jssc.201100789.
48. Pereira, J.; Câmara, J.S.; Colmsjö, A.; Abdel-Rehim, M. Microextraction by Packed Sorbent: An Emerging, Selective and High-Throughput Extraction Technique in Bioanalysis. Biomedical Chromatography 2014, 28, 839–847, doi:10.1002/bmc.3156.
49. Abdel-Rehim, M. Microextraction by Packed Sorbent (MEPS): A Tutorial. Anal Chim Acta 2011, 701, 119–128, doi:10.1016/j.aca.2011.05.037.
50. Abdel-Rehim, M. New Trend in Sample Preparation: On-Line Microextraction in Packed Syringe for Liquid and Gas Chromatography Applications. Journal of Chromatography B 2004, 801, 317–321, doi:10.1016/j.jchromb.2003.11.042.
51. Moein, M.M.; Said, R.; Abdel-Rehim, M. Microextraction by Packed Sorbent. Bioanalysis 2015, 7, 2155–2161, doi:10.4155/bio.15.154.
52. Sarigul, N.; Korkmaz, F.; Kurultak, İ. A New Artificial Urine Protocol to Better Imitate Human Urine. Sci Rep 2019, 9, 20159, doi:10.1038/s41598-019-56693-4.
53. Shah, N.M.; Hawwa, A.F.; Millership, J.S.; Collier, P.S.; McElnay, J.C. A Simple Bioanalytical Method for the Quantification of Antiepileptic Drugs in Dried Blood Spots. Journal of Chromatography B 2013, 923–924, 65–73, doi:10.1016/j.jchromb.2013.02.005.
54. Mercolini, L.; Mandrioli, R.; Amore, M.; Raggi, M.A. Simultaneous HPLC-F Analysis of Three Recent Antiepileptic Drugs in Human Plasma. J Pharm Biomed Anal 2010, 53, 62–67, doi:10.1016/j.jpba.2010.02.036.
55. Proença, P.; Franco, J.M.; Mustra, C.; Marcos, M.; Pereira, A.R.; Corte-Real, F.; Vieira, D.N. An UPLC–MS/MS Method for the Determination of Valproic Acid in Blood of a Fatal Intoxication Case. J Forensic Leg Med 2011, 18, 320–324, doi:10.1016/j.jflm.2011.06.003.
56. Kumirska, J.; Łukaszewicz, P.; Caban, M.; Migowska, N.; Plenis, A.; Białk-Bielińska, A.; Czerwicka, M.; Qi, F.; Piotr, S. Determination of Twenty Pharmaceutical Contaminants in Soil Using Ultrasound-Assisted Extraction with Gas Chromatography-Mass Spectrometric Detection. Chemosphere 2019, 232, 232–242, doi:10.1016/j.chemosphere.2019.05.164.
57. Hassan, A.A.; Tanimu, A.; Alhooshani, K. Iron and Cobalt-Containing Magnetic Ionic Liquids for Dispersive Micro-Solid Phase Extraction Coupled with HPLC-DAD for the Preconcentration and Quantification of Carbamazepine Drug in Urine and Environmental Water Samples. J Mol Liq 2021, 336, 116370, doi:10.1016/j.molliq.2021.116370.
58. Pashaei, R.; Dzingelevičienė, R.; Abbasi, S.; Szultka-Młyńska, M.; Buszewski, B. Determination of 15 Human Pharmaceutical Residues in Fish and Shrimp Tissues by High-Performance Liquid Chromatography-Tandem Mass Spectrometry. Environ Monit Assess 2022, 194, 325, doi:10.1007/s10661-022-09993-8.
59. Yang, X.; Jiang, Z.; Jiang, Y.; Ling, J.; Dong, L.; Zou, S.; Chen, R.; Hu, N. Determination of Valproic Acid and Its Six Metabolites in Human Serum Using LC–MS/MS and Application to Interaction with Carbapenems in Epileptic Patients. Biomedical Chromatography 2023, 37, doi:10.1002/bmc.5572.

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