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Quantitative determination of clozapine in plasma using an environmentally friendly technique

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ABSTRACT

A new method for the determination of clozapine in blood samples, a common antipsychotic drug, has been developed. It is based upon a dispersive liquid–liquid microextraction technique employed as the extraction method together with gas chromatography-mass spectrometry operating in selected ion monitoring (SIM). The method was fully validated including selectivity, sensitivity, accuracy, precision and recovery steps. The limit of quantification was set at 50 ng/mL. Intra and interday accuracy and precision values were lower than 17% at the concentration levels studied. Good recovery results were obtained for all concentrations. The proposed method was satisfactory applied to 10 plasma samples received at the Forensic Toxicology Service of the Forensic Science Institute of Santiago de Compostela, obtaining results within the therapeutic range. The results showed the usefulness of the analysis in any forensic toxicology laboratory and the posibility of determining clozapine in the presence of other drugs or drugs or abuse.

1. Introduction

Antipsychotic drugs (AP) are customarily used to control a wide range of severe psychiatric disorders including schizophrenia, bipolar disorder, severe anxiety and depression, behavioral disorders and dementia [1]. Available AP include both conventional (typical or first-generation antipsychotics), represented mainly by phenothiazine derivatives, butyrophenones and thioxanthenes, as well as new (atypical or second generation) antipsychotic medications. Both, first and second generation antipsychotic are known to cause serious side effects, whereas some of the atypical treatments have shown to provide greater tolerability than their classical counterparts [2]. Clozapine (Fig. 1), a tricyclic dibenzodiazepine derivative, is classified as an atypical antipsychotic with demonstrated efficacy in schizophrenia when standard antipsychotic drugs fail [3,4]. Schizophrenia causes psychosis and is associated with considerable disability and may affect all areas of life including personal, family, social, educational and occupational realms. It affects aproximately to 24 million people or 1 in 300 people (0.32%) worldwide [5]. The detection of AP is of considerable interest within forensic toxicology because of their potential abuse and their involvement in drug poisoning and suicidal behaviors. The AP are usually administered orally at relatively low daily doses and are widely metabolized. For all those reasons, sensitive analytical methods are needed. Different detection techniques, especially liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been mentioned in the scientific bibliography for the determination of antipsychotics in biological samples many times [2,6–14]. However, many laboratories do not have the opportunity to work with this technique, so other alternatives should be found. Gas chromatography-mass spectrometry is a common technique in any forensic toxicology laboratory [4,15,16]. Therefore, it is a valid alternative for determining drugs.

Sample preparation is an important preliminary step when dealing with biological samples which are usually embedded within complicated matrices at low concentrations. Pretreating the sample decreases the matrix effect, improves the method selectivity, protects analytical columns and enhances the sensitivity [17].

Usually, the determination of this drug in biological samples involves different extraction techniques stemming from the classical ones, such as protein precipitation, liquid–liquid extraction (LLE) or solid phase extraction (SPE) [4,16,18,19], to more current and innovative techniques, such as dried blood spot (DBS) or liquid phase microextraction (LPME) [2,14,15,20,21]. The former suffers from some drawbacks, like being environmentally unfriendly, needing large amounts of organic solvents, slow, need to purchase of SPE cartridges and being labour

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intensive. However, the development of microextraction techniques has gone some way to resolving some of these problems. Dispersive liquid–liquid microextraction (DLLME) is one of the most interesting alternative solvent-minimization sample preparation techniques which are applied in the field of forensic toxicology. It was developed by Assadi et al. in 2006 and is based on a ternary system of solvents in which the extraction and dispersion solvents are rapidly injected into the aqueous sample with a syringe [22]. After gentle stirring of the mixture, a turbid solution is formed, and the analytes are extracted into a droplet obtained by centrifugation. The steps required to develop this technique have been described in a previous article [23]. Its main advantage consists of shorter extraction times, comparing with other techniques. In addition, the extraction of a wide variety of analytes from complex matrices is achieved using a very small volume of extractant solvent. It is also much simpler and has recoveries comparable with or better that the traditional techniques and it is environment benign [24]. The method is also known to have some weak points, such as excessive agitation that can tend to break the drop or the need of an extractant solvent with a density larger than water for the formation of the drop at the bottom of the tube, achieving a simpler separation. Organic solvents capable of meeting these requirements are mainly chlorinated solvents [25]. To the best of our knowledge, there are few articles in the scientific bibliography concerning the optimization of this technique for the determination of clozapine, and just only one in blood [20,21].

The aim of this study was to develop a gas chromatography-mass spectrometry method, a poor used method compared to LC, for the quantitative determination of clozapine in blood using DLLME as a new extraction method, useful for reducing the working time and the amount of solvent used for analysis. The validation of the method was carried out according to the guidelines of the Food and Drug Administration (FDA) and allowed its application in both the therapeutic and toxic range [26]. The method was then applied to blood samples collected from deceased people, whose results were within the therapeutic range.

2. Materials and methods

2.1. Chemicals

Acetone, carbon tetrachloride and Trifluoroacetic anhydride were purchased from Merck® (Madrid, Spain) and sodium chloride and Ammonia from Panreac® (Barcelona, Spain). Clozapine and clozapine-d4, used as internal standard, were obtained from Cerilliant®. Working solution were prepared in methanol. Destilled water was processed through a Milli-Q water system (Millipore, Bedford, MA, USA).

2.2. Blood samples

Drug-free plasma samples (used for method validation) were obtained from the Blood Bank of Santiago de Compostela. Blood samples obtained from autopsies were stored in our laboratory at −20 °C for analyzed for real cases. Plasma was obtained from whole blood by centrifugation (14000 rpm, 5 min) and kept at 4 °C.

2.3. Sample preparation

Blood was centrifuged at 14000 rpm for 5 min to isolate the plasma from proteins. If this process is not enough, methanol (1 mL) is added to achieve protein precipitation by centrifugation (4000 rpm, 10 min). For the DLLME, an aliquot of 1 mL plasma was placed in a conical bottom glass tube, spiked with Clozapine-d4 (40 μL Sol. 10μg/mL; internal standard). The pH value of the plasma sample was alkalized by the dropwise addition of 1 mL amonnium solution (pH 8, 10 mM) and 0.15 g sodium chloride was also added to facilitate the movement of the analytes to the organic phase. Eight hundred microliters of acetone (as dispersive solvent) containing 150 μL CCl_4 (as extraction solvent) were injected rapidly into the sample solution by using 1 mL syringe, and then the mixture was gently shaken for several seconds. A cloudy solution that consists of very fine droplets of CCl_4 dispersed into the sample solution was formed. After centrifugation for 10 min at 4000 rpm, the droplet formed was collected by a 100 μL syringe and transfered to a 8 mL glass vial. The elution solvent was evaporated to dryness under a gentle stream of N_2 at 40 °C. Dry extracts were derivatized with 40 μL of

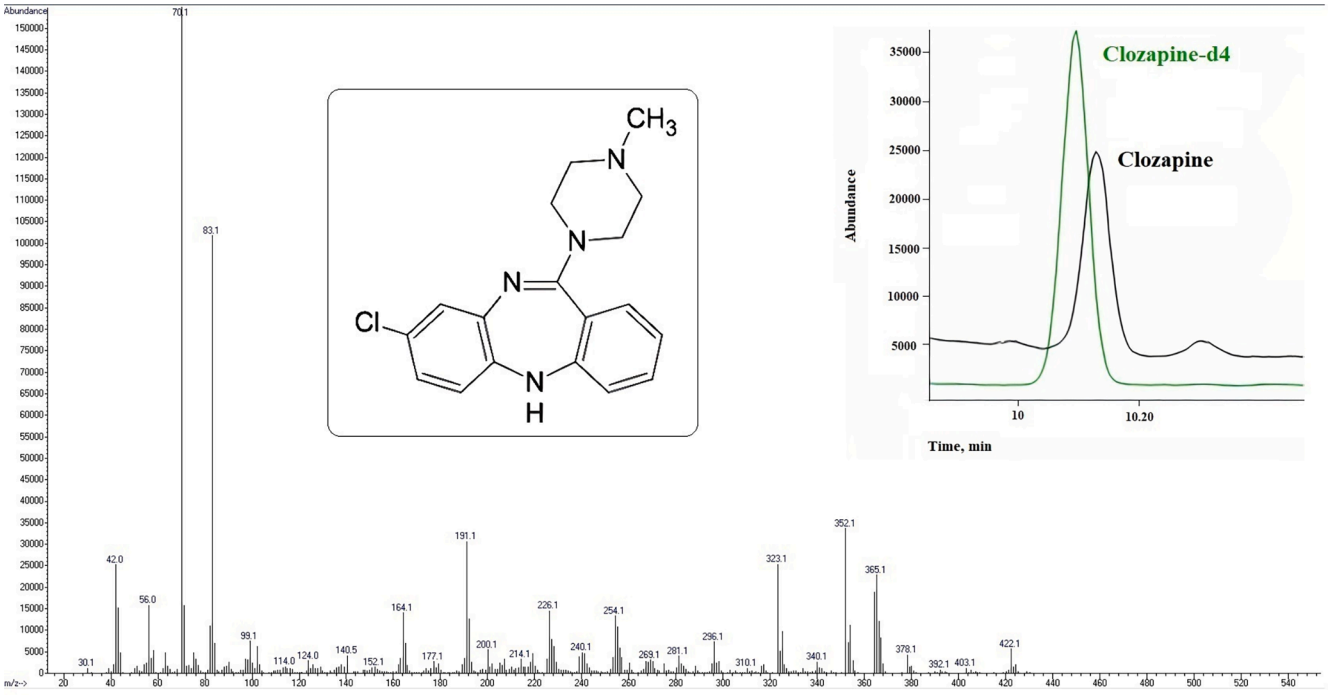

Fig. 1. Clozapine (chemical structure, mass spectra and chromatogram).

trifluoroacetic anhydride (TFAA) under 40 °C for 20 min, and then, they were evaporated again. Finally, the extracts were reconstituted in 40 μL ethyl acetate, vortexed and 1 μL of the extracts were injected into the GC–MS system.

2.4. Instrumentation

Analyses were performed using a gas chromatograph model 7890B from Agilent equipped with an Agilent 5977B mass spectrometer. The ionization source employed was electron impact ionization. Chromatographic elution was done with a HP-5 ms capillary column 30 m × 250 μm i.d. internally coated with 0.35 μm thick film of 5%-(phenyl)methylpolisiloxane, purchased from Agilent Technologies® (Las Rozas, Spain) with helium as carrier gas (1 mL/min). The injector temperature was set at 250 °C and a purge time of 2 min was used. Samples were injected in splitless mode. The following temperature program was applied: 1 min at 140 °C, then 40 °C/min up to 290 °C holding 7 min. All compounds were injected in full SCAN mode for their identification (mass spectra and retention time) and then, the analysis was performed in SIM (selected ion monitoring) mode to increase the sensibility of the method. The mass selective detector (MSD) was kept at 300 °C, the ion source at 230 °C and the quadrupole at 150 °C.

2.5. Identification of compounds

Initially, neat standards of Clozapine and Clozapine-d4 were injected and analyzed using the full scan mode of the GC/MS, which scanned from 50 to 550 amu (Figs. 1 and 2). Quantifier and qualifier ions used for each analyte were selected based on their abundances and mass-to-charge ratios (m/z). The ions selected for Clozapine were 83 (quantifier ion), 56 and 422 (qualifier ion) and for Clozapine-d4 were 87 (quantifier ion), 60 and 426 (qualifier ion). The retention time for Clozapine was 10.10 min and for Clozapine-d4 was 10.07 min. Upon selection of ion, the mass spectrometry was run in selected ion monitoring mode (Fig. 1).

2.6. Method of validation

The validation of this method was based upon the guidelines established by the Food and Drug Administration (FDA) [27] in terms of selectivity, linearity, limit of detection (LOD) and quantification (LLOQ), intra and interday precision and accuracy and recovery.

Selectivity was evaluated by analyzing N = 6 blank plasma samples of untreated people. Linearity was assessed by constructing six calibration curves at the 6 concentration levels between LLOQ (50 ng/mL) to ULOQ (800 ng/mL) analyzed from different days and covering the plasma therapeutic and toxic range. A linear regression model was applied based on the analyte versus IS peak area ratio. The LOD and LLOQ were calculated at a signal-to-noise ratio of 3 ($S/N = 3$) and 10 ($S/N = 10$), respectively. Precision was established through the evaluation of relative standard deviation (%RSD). Blank samples were fortified with three QC samples at low level (50 ng/mL), medium level (600 ng/mL), and high level (800 ng/mL), within the linear range of the calibration curve of the analyte. The analysis were carried out using 5 replicates of each QC sample on the same day (intraday accuracy), and 5 replicates on 5 different days (interday accuracy). Accuracy was calculated through relative error (RE, %), following the same schedule than precision. In order to meet the validation criteria, the error of accuracy and precision should not exceed 15% for each calibration standards, except for LLOQ, where 20% of error is accepted. Recovery was also studied in three different concentration levels (50, 600 and 800 ng/mL).

3. Results

3.1. Study of the derivatization process

In order to detect clozapine by GC/MS, it has been necessary to include a derivatization process, using Trifluoroacetic anhydride (TFAA) as a derivatizing agent. This process has been optimized by studying 4 factors: derivatizer volume, temperature and time in the stove and evaporator temperature. The results are shown in Fig. 3, with the best results being obtained by adding 40 μL TFAA for 20 min at 40 °C. The application of heat in the evaporation process was not a significant variable.

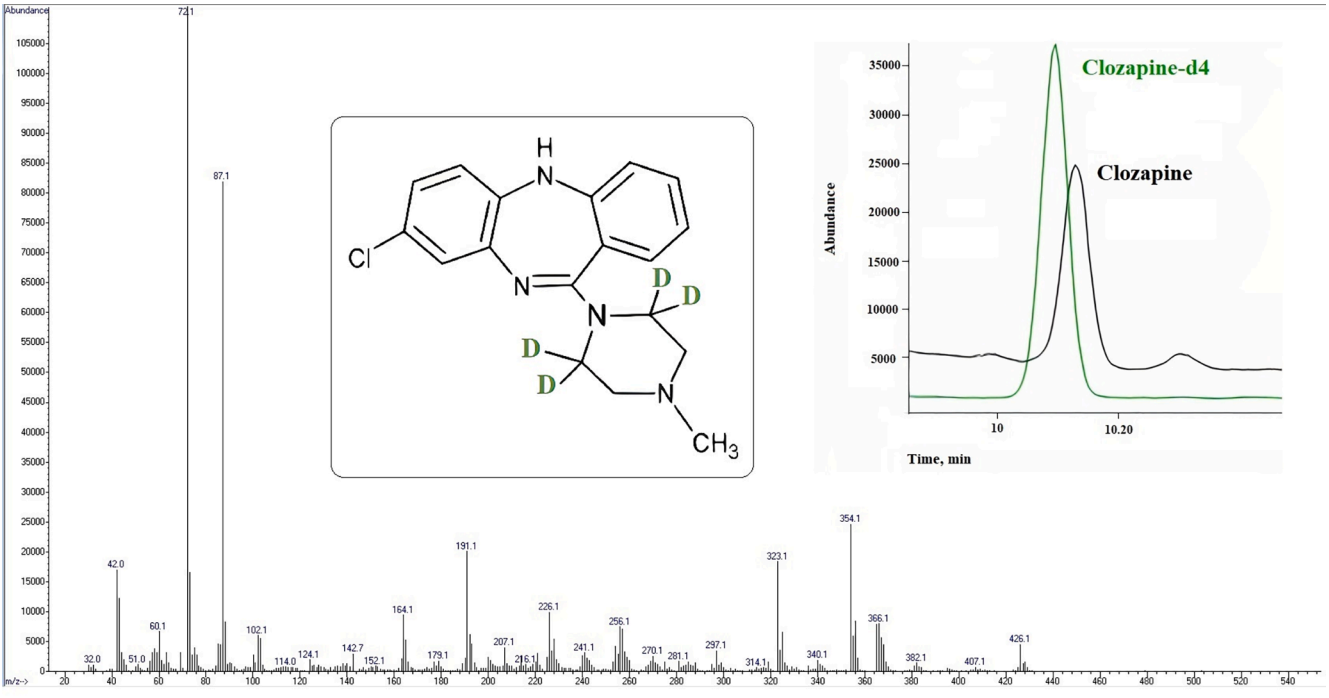

Fig. 2. Clozapine d-4 (chemical structure, mass spectra and chromatogram).

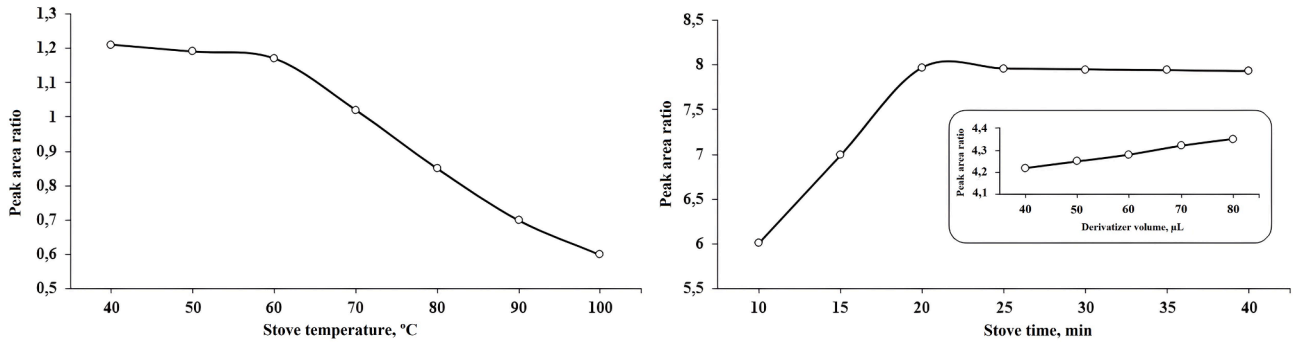

Fig. 3. Derivatization conditions (Stove temperature, time and derivatizer volume studies).

3.2. Selection of extraction techniques

Four extraction techniques (liquid–liquid extraction (LLE), solid phase extraction (SPE), membrane-assisted solvent extraction (MASE) and dispersive liquid–liquid microextraction (DLLME)) were evaluated and compared (Fig. 4). In all of them the samples were doped with the same amount of clozapine and IS, evaporated to dryness and finally derivatized with TFAA. DLLME provided the best results, although no significant difference was shown when compared with LLE or SPE. However, it has not been posible to extract clozapine using MASE. Finally, DLLME has been selected to determine the substance under study, since, in addition to offering a slight better response, it is a novel technique poorly used for the determination of clozapine in blood sample.

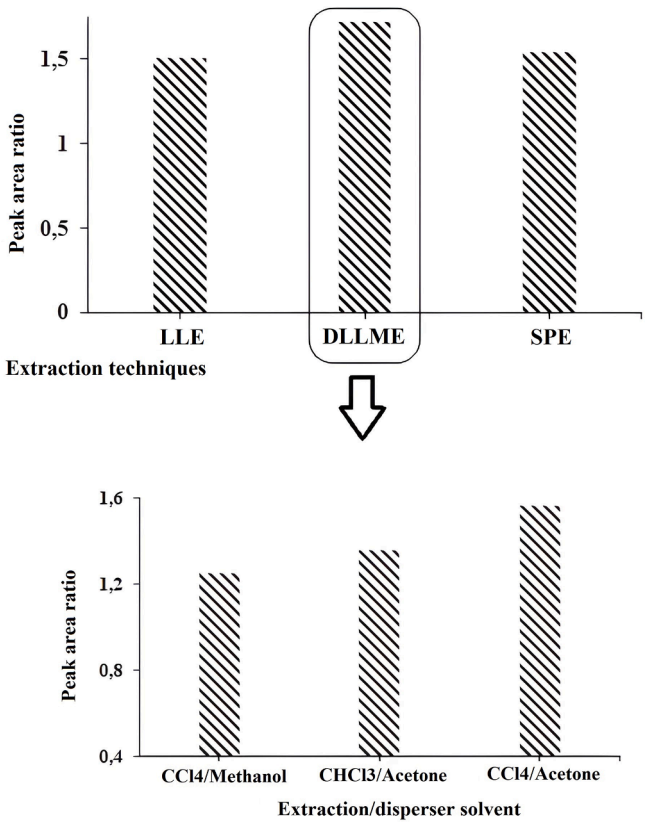

Fig. 4. Comparison between **different extraction techniques** and **optimization of extraction and disperser solvent** *(Extraction conditions: sample volume, 0.5 mL; buffer volume* pH *9, 1 mL; disperser solvent volume,* 800 µL*; extraction solvent volume,* 100 µL*).*

3.3. Study of DLLME

The recovery in DLLME can be improved by optimization of certain experimental variables, including extractant and disperser type, extractant and disperser volume, sample amount, pH of aqueous phase, ultrasound time, centrifugation time and ionic strength.

3.3.1. Study of the type of extraction and disperser solvent

These solvents have some common requirements that must be met. The extraction solvent must be inmiscible with water and miscible with the disperser solvent and must be able to dissolve the analyte of interest. Maximum extraction efficiencies are usually observed at lower extractant volumes (20–200 µL). However, disperser solvent has to be miscible with both, the extraction solvent and the aqueous sample. It is tipically denser than water (mainly chlorinated solvents), which will allow the formation of the settled phase at the bottom of a conical tube after the centrifugation step. A few hundreds microliters (200–1000 µL) are often enough to disperse the organic extractant in the sample. Low volumes of disperser solvent could not be sufficient to disperse the extraction solvent properly, and therefore, cloudy solution may not be formed completely. On the contrary, at high volume, the solubility of analytes in water increases by increasing the volumen of disperser solvent, so the extraction process is incomplete. The applicability of DLLME is mainly based upon the distribution coefficient (K) defined as the ratio between the analyte concentration in extraction solvent and the ratio between the analyte concentration in extraction solvent and sample solution, so K should be greater than 500 to achieve suitable application of DLLME. However, K has not always available, so K_{ow} can be used as an alternative parameter, indicating the lipophilicity of the analyte. [17,28,29].

In this study, all combinations of chloroform (density 1.47 g/mL), carbon tetrachloride (density 1.59 g/mL) and dichloromethane (density 1.32 g/mL) (as extraction solvents) and methanol and acetone (as disperser solvents) were applied to approach the best mixture of solvents. The obtained droplet was formed following three possible combinations, as shown Fig. 4. According to the obtained results, the mixture containing carbon tetrachloride and acetone resulted in the highest recovery percentage.

3.3.2. Study of pH

For the acid or alkaline analytes, the distribution coefficient could be increased by controlling the pH value of the sample solution, making the analytes existing in nonionic state [30]. Considering the basic character of clozapine (pKa value 7.5), the influence of the sample pH was an important parameter to study. Five samples, spiked with drugs, were basified at different pH values (6–12) with different buffers. The best conditions were achieved with amonium buffer, 10 mM, pH 8 (Fig. 5).

3.3.3. Study of sonication

The sonication can be used to assist the dispesion of the extraction solvent in the aqueous solution in order to reduce the extraction time. In

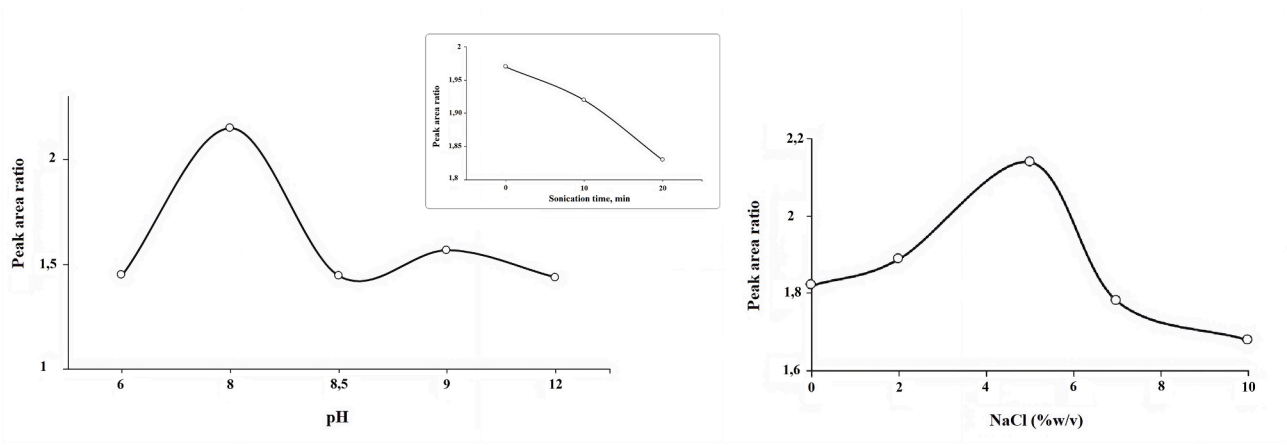

Fig. 5. Study of DLLME parameters: **pH** (Extraction conditions: sample volume, 0.5 mL; buffer volume, 1 mL; disperser solvent (acetone) volume, 800 μL; extraction solvent (CCl_4) volume, 100 μL), **sonication time** (Extraction conditions: sample volume, 0.5 mL; amonium buffer 10 mM pH 8 vol, 1 mL; disperser solvent (acetone) volume, 800 μL; extraction solvent (CCl_4) volume, 100 μL) and **ion strength** (Extraction conditions: sample volume, 0.5 mL; amonium buffer 10 mM pH 8 vol, 1 mL; disperser solvent (acetone) volume, 800 μL; extraction solvent (CCl_4) volume, 100 μL).

this study, different sonication times were evaluated. The results showed that greater extraction of clozapine was obtained by no sonication (Fig. 5).

3.3.4. Study of ionic strength

The solubility of the target analyte and organic extraction solvent in aqueous phase usually decreases with increasing ionic strength due to the salting out effect. In order to evaluate the influence of ionic strength on the recovery of DLLME, several experiments were performed by adding different amounts of NaCl (0–30% w/v) keeping the rest of the variables constant (Fig. 5). The highest percentages of salt gave rise to very dirty extracts, so they have not been taken into account. As shown in Fig. 5, increasing the amount of salt up to 5%, increases the peak area ratio of Clozapine. However, from 5% onwards, the higher the percentage, the worse the results. Based on these results, 5% w/v NaCl was chosen as the optimal salt concentration in the DLLME procedure.

3.3.5. Study of several variables with statistical package

To find the best conditions for DLLME, 16 experiments were performed using a 2 × 4 factorial design with four factors: extraction solvent volume, disperser solvent volume, buffer volume and sample volumen. The experimental design was achieved using StatGraphics Centurion statistical package (18.1.12 version; Manugistics, Rockville, MD, USA). It was performed randomly to avoid the influence of external conditions. In the experimental domain, different ranges for each variable were tested (sample volume: 0.1–1 mL; buffer volume: 0.1–1 mL; extraction solvent volume: 0.05–0.2 mL; disperser solvent volume: 0.5–1.5 mL). Using the Main Effects Plot (Fig. 6), the variability of each of the variables studied can be observed. Better results are obtained by increasing the volume of the sample, extraction solvent and buffer. However, it is posible to improve the results by reducing the volume of disperser solvent. The optimal conditions are as follows: 1 mL plasma was diluted with 1 mL of amonium buffer 10 mM, pH 8 and 150 μL CCl4 and 800 μL acetone were used as extraction and disperser solvent, respectively.

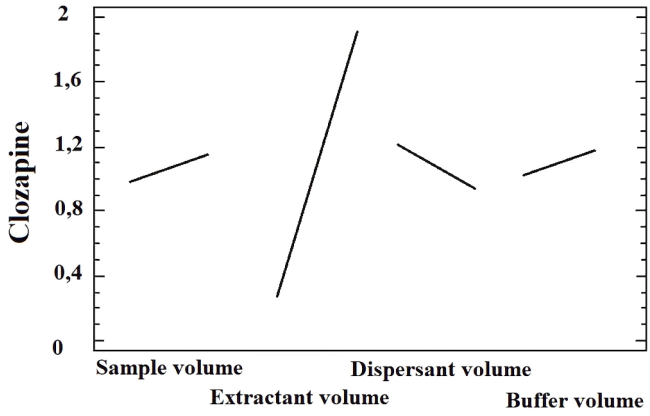

Fig. 6. Main Effects Plot for Clozapine *(Extraction conditions: 5% w/v NaCl; amonium buffer 10 mM* pH *8; acetone as disperser solvent and CCl_4 as extraction solvent)*.

3.4. Performance parameters and validation

Some analytical parameters as described in the *"Method of Validation"* section were determined under the optimal conditions to evaluate the proposed DLLME-GC/MS method.

3.4.1. Selectivity

Blank blood samples from six individual sources were analyzed to ensure that there are no matrix effects throughout the application of the method. The extracted ion chromatogram of blank blood sample spiked with internal standard is shown in Fig. 1SM. The results showed no interfering peaks from endogenous substances at the retention time of the analyte of interest and IS.

3.4.2. Linearity

The calibration curve was created using a blank sample and spiked with clozapine at concentrations of 50–800 ng/mL, covering the therapeutic and toxic levels [31–34]. All standard samples were spiked with internal standard (Clozapine-d4; 400 ng/mL). The curve was obtained by fitting the ratio of the peak areas of Clozapine to that of IS versus concentrations. The correlation coefficient r^2 was not<0.99, demonstrating good linearity.

The LOD, defined as the lowest concentration giving a response of at least three times relationship S/N, was 25 ng/mL. The lowest standard on the calibration curve, LLOQ, should be accepted as the limit of quantification if the analyte response is at least 10 times the response compared to blank response. It was set at 50 ng/mL.

3.4.3. Precision and accuracy

The results of intraday and interday precision (expressed in relative standard desviation, RSD) and accuracy (expressed in Relative Error, RE) are shown in Table 1SM. The intraday and interday precission were

all<14%, and the accuracy of intra and interday were in the range of 9–17%. The results met the aceptable criteria defined by FDA [26].

3.4.4. Extraction recovery

The extraction recovery of the Clozapine at three levels are presented in Table 1SM. All results were greater than 82%.

3.4.5. Method applicability

Our method was succesfully applied to 10 blood samples collected from deceased people, sent to our laboratory for analysis. All concentration results are presented in Table 1, as well as information regarding each sample donor. All the concentrations detected were within the therapeutic range. The method has been applied to a small number of cases, but enough to prove the usefulness of the analysis in any forensic toxicology laboratory.

4. Discussion

Antipsychotics are an increasingly prevalent class of drugs due to the increase in the diagnosis of mental illnesses [1]. For this reason, good quality analytical methods are essential in order to monitor the blood levels of these drugs, especially those that present a greater risk to the patient. Clozapine is a commonly used atypical antipsychotic with demonstrated efficacy in schizophrenia and bipolar disorder [35].

We are aware of the fact that LC is a state-of-the-art technique in any laboratory, especially when combined with tandem mass spectrometry. There is no doubt that this fact also applies to the objective of this work, the determination of clozapine in a forensic toxicology laboratory [1,2,11,21,35,36]. On the other hand, although scientific literature does not frequently report on the application of GC–MS for this purpose [3,4,20] since GC is limited by the thermolability of the compounds (often required chemical derivatization that increase the time of the analysis), our reseach group has successfully applied to determine this analyte. It is important to note that GC–MS is an affordable technique, compared to LC, present in many laboratories and, in this case, it has provided results that are as precise and reliable as those obtained with other techniques.

The sample preparation step in an analtical process typically consists of an extraction procedure that results in the isolation and enrichment of components of interest from a sample matrix. Regarding this, LLE, SPE, DBS or microextraction in packed sorbent (MEPS) [2,4,8,11,14–16,35,37] were used in the literature for analysis of Clozapine in biological samples, but all of them suffered from some disadvantages such as long extraction time, large sample volumes, excess consumption of organic solvents, multiple extraction steps to increase analyte recovery and cleaner extracts and excesive SPE cartridges. In order to improve these problems, DLLME has been considered in our work, as it can provide less consumption of organic solvents and higher recoveries due to the large contact surface area of the extraction solvent. Subsequently, different variables of the selected technique were optimized (type and volumes of solvents, amount of salt, pH adjustment and the amount of sample). In addition, after an exhaustive bibliographic review collected in Table 2, it has been observed that the technique selected to carry out this study, DLLME, is a technique that is rarely used for the extraction of the analyte under study. It has also been posible to verify the good recovery data obtained in comparison with the different studies reviewed. X. Chen et al. [20] have introduced a variation of the conventional DLLME, using a lower density solvent as the extraction solvent and the ultrasound energy for assisting in the emulsification process. However, the total run time is longer than that optimized in our method and the recoveries achieved were lower than those published in this article. The second article in the scientific bibliography that has used DLLME for the extraction of Clozapine was published by J. Chen et al. [21], but urine was used in this case.

More sensitive approaches were reported, with LLOQ lower than ours, but these methods used more sensitive analytical techniques, as LC–MS/MS or GC–MS/MS [8,11,14,16,35]. Our limits proved to be adequate for monitoring this compound since they cover the therapeutic range, the usual range of the real cases analyzed. Regarding those authors who have used GC–MS and have managed to improve the sensitivity, the recoveries have been considerably affected [4,20], or large volumes of derivatizer with longer derivatization times or greater total run time have been used [3,4].

Finally, DLLME was demonstrated as a satisfactory microextraction technique for the determination of Clozapine in blood samples, with good validation results.

Table 1
Real cases information (M: male; F: female; Bzd: benzodiazepines; Mtd: methadone; Atp: antidepressants; Qtp: Quetiapine; Valp A: Valproic Acid).

Real cases	Age (sex)	Etiology of death	Prescribed clozapine	Other substances found	Clozapine concentration, ng/mL
1	45 (M)	Natural	Yes	Bzd	**226.7**
2	59 (M)	Accidental	Yes	–	**372.3**
3	59 (F)	Natural	Yes	Qtp, Valp A, Topiramate, Biperiden	**<LLOQ**
4	47 (M)	Accidental	Yes	Norclozapine	**58.15**
5	37 (M)	Overdose	Unknown	Cocaine	**187.2**
6	53 (F)	Accidental	Yes	Mtd, Bzd, Atp	**310.1**
7	42 (M)	Overdose	–	Cocaine, opiates	**73.2**
8	39 (F)	Natural	Yes	Bzd	**331.2**
9	51 (M)	Accidental	Yes	Bzd	**271.9**
10	59 (F)	Natural	Yes	Bzd	**190.5**

5. Conclusions

The present results show that the reported methods are able to determine AP not only at toxic concentrations but also within therapeutic levels in postmortem cases. Therefore, it is important to come up with a sensitive method to cover the therapeutic range in which these treatments are usually applied. In this work, several extraction methods have been tested, being DLLME the one that showed the most satisfactory results. A fully validated method is described for the determination of Clozapine in blood using the former extraction method, as an alternative extraction procedure to those more conventional, employing GC/MS as a detection technique. This miniaturized extraction procedure revealed to be simple, preventing the high use of organic solvents and associated to a rapid extraction procedure. The proposed method was optimized, being successfully applied to real cases, and allowing recoveries between 82 and 112%. The method validation results demonstrated good linearity, high sensitivity, precision and accuracy for extraction of Clozapine from real samples. It demonstrated that the method can be successfully implemented in the toxicology laboratory for routine analysis. The authors want to thank Dr. J. Rajeev for permission to include his image on the DLLME in the graphical abstract [38].

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Table 2
Analytical figures of merit of available methodologies for the determination of clozapine in biological fluids through preconcentration and chromatography. SPE (solid phase extraction); DBS (dried blood spot); PPT (protein precipitation); LLE (liquid–liquid extraction); UA-LDS-DLLME (ultrasound-assisted-low-density solvent dispersive liquid–liquid microextraction); MEPS (microextraction in packed sorbent); LC-MS/MS (liquid chromatography-tandem mass spectrometry); GC–MS/MS (gas chromatography-tandem mass spectrometry); HPLC-UV (high performance liquid chromatography-ultraviolet detector); HPCE-DAD (high performance capillary electrophoresis-diode array detector).

References	Biological matrix	Extraction technique	Detection technique	Other data of interest
P. Proenca et al., 2020	Plasma	SPE	LC-MS/MS	▪ Calibration range: 5–500 ng/mL ▪ Accuracy and precisión < 15%
C. Ruggiero et al., 2020	Plasma	DBS	LC-MS/MS	▪ LLOQ = 5.70 ng/mL ▪ Accuracy and precisión < 10%
D. Koller et al., 2019	Plasma	PPT; μ-SPE	LC-MS/MS	▪ Calibration range: 0.5–1000 ng/mL Total run time: 9 min ▪ Accuracy and prec. < 15% (except for LLOQ; < 20%)
D. Caramelo et al., 2019	Oral fluid	DBS	GC–MS/MS	▪ Calibration range: 10–400 ng/mL ▪ Total run time: 25 min ▪ Recovery: 82.7–94.4% Accuracy and precision < 15%
T. Rosado et al., 2018	Plasma and oral fluid	SPE	GC–MS/MS	▪ Calibration range (blood): 20–600 ng/mL ▪ Total run time: 14.25 min ▪ Derivatization: 65 μL MSTFA with 5% TMCS (domestic microwave oven, 2 min) ▪ Recovery: 76–95%
I. Miroshnichenko et al., 2018	Plasma	LLE	LC-MS/MS	▪ Calibration range: 1–1000 ng/mL Total run time: 11 min ▪ Recovery: 92–99% ▪ Accuracy and prec. < 15% (except for LLOQ; < 20%)
X. Chen et al., 2017	Plasma	UA-LDS-DLLME	GC–MS	▪ Calibration range: 5–500 ng/mL ▪ Total run time: 25 min ▪ Recovery: 65–88% ▪ Accuracy and precision < 15%
V. Boumba et al., 2016	Plasma	SPE	GC–MS	▪ Calibration range: 25–800 ng/mL ▪ Total run time: 15.50 min ▪ Recovery: 40–50% ▪ Accuracy and precision < 15%
B. M. da Fonseca et al., 2013	Plasma	MEPS	GC–MS/MS	▪ Calibration range: 1–1000 ng/mL ▪ Total run time: 25 min ▪ Derivatization: 65 μL MSTFA with 5% TMS (85 °C, 45 min) ▪ Recovery: 70–84% ▪ Accuracy and prec. < 15% (except for LLOQ; < 20%)
J. Chen et al., 2011	Urine	DLLME	HPLC-UV	▪ LLOQ: 39 ng/mL ▪ Recovery: 98%
I.Vardakou et al, 2010	Plasma	SPE	GC–MS	▪ Calibration range: 3–600 ng/mL ▪ Total run time: 35 min ▪ Derivatization: 80 μL TFAA (52 °C, 45 min) ▪ Recovery: 82–90% ▪ Accuracy and precision < 10%
D. Thieme et al., 2007	Hair	Ultrasonification (55 °C, 3 h)	LC-MS/MS	▪ Calibration range: 1–5 pg/mg
M.A. Raggi et al., 2001	Plasma	SPE	HPCE-DAD	▪ Calibration range: 100–2000 ng/mL Total run time: 3 min ▪ Recovery: 89%
Our method	Plasma	DLLME	GC–MS	▪ Calibration range: 50–800 ng/mL ▪ Total run time: 12 min ▪ Recovery: 82–112% ▪ Accuracy and prec. < 15% (except for LLOQ; < 20%)

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2022.107612.

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