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Quantification of antidepressants in oral fluid and plasma samples using microextraction by packed sorbent and analysis by gas chromatography-tandem mass spectrometry

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ABSTRACT

The consumption of antidepressants is extremely significant as they are a class of medications widely used in the treatment of numerous disorders and are therefore considered a public health problem throughout the world. The aim of this work was to develop and optimize two methodologies for the determination of selected antidepressants and metabolites (fluoxetine, venlafaxine, *O*-desmethylvenlafaxine, citalopram, sertraline, paroxetine), in 250 μL of sample (oral fluid and plasma) using microextraction by packed sorbent (MEPS) as the extraction technique and gas chromatography coupled to tandem mass spectrometry (GC–MS/MS) for analysis. The two methods were fully validated considering the internationally accepted criteria for bioanalytical procedures, presenting linearity within the studied range, with limits of quantification between 10 and 100 ng/mL, coefficients of determination (R^2) of at least 0.99 and precision and accuracy with acceptable values of coefficients of variation and relative errors for all antidepressants in study and for both specimens. Recoveries ranged between approximately 12 and 93 % for oral fluid samples and between approximately 28 and 101 % for plasma samples. To our best knowledge, the described methods are the first to be reported using MEPS and GC–MS/MS for the identification of antidepressants in oral fluid and plasma samples, proving to be sensitive, simple, fast and capable of being applied in routine clinical and forensic toxicology scenarios.

1. Introduction

Depression is predicted by the World Health Organization as the second cause of global disease and is considered a severe or chronic mental illness, which affects any individual regardless of gender, age and social or economic situation, generally associated with physical health problems and difficulty in accessing health services. This condition is characterized by mood changes, tiredness, sleep disturbances, loss of interest and desire, impairment of social and occupational functions and even suicidal ideation behaviors [1–6]. There are several possible therapies for treating this disorder, but the most effective treatment is the administration of antidepressants, which have also been prescribed for other mental health illnesses such as anxiety. The increase in the number of prescriptions for these medications has even led to several warnings from experts. Classic antidepressants have been replaced by second-generation antidepressants, due to their similar effectiveness and fewer side effects, as the first-line of treatment for this disorder [7–11]. Since this class of compounds is usually prescribed in combination with other medications, drug interactions may occur, which can be aggravated by the uncertainty of the dose to be administered, inter-individual differences and narrow therapeutic windows. For these reasons, therapeutic drug monitoring (TDM) for this class of

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compounds is of great interest, with special importance for patient safety and compliance, and treatment adherence [9,12,13]. Monitoring these drugs makes it possible to adjust and customize treatment for each patient-minimizing toxicity, reducing side effects and improving quality of life-avoiding lack of response or non-adherence to treatment, saving costs and resources. In addition, these drugs are also associated to excessive use and abuse, which results in clinical and forensic cases of accidental or voluntary overdose [7,14–16].

Currently, oral fluid has been increasingly implemented both in the clinical and forensic fields for drug determination in biological matrices, as an alternative to classic specimens as it has advantages such as ease of collection, lower risk of adulteration, and a smaller drug detection window that allows a better correlation with drug effects [17–20]. On the other hand, plasma is one of the most used samples in the determination of drugs in several contexts and in TDM, as it allows the correlation between the concentration of the detected substance and the clinical condition or symptoms of the individual. Similar to oral fluid, plasma also has a relatively short drug detection window [16,20,21].

For the identification, quantification and monitoring of these medications to be possible, it is particularly important that analytical methodologies are developed for the determination of antidepressants and metabolites in biological specimens. An important step to take into account in the development of an analytical method is the isolation and concentration of the compounds of interest from the biological matrix. The most commonly used procedures for extracting antidepressants are solid-phase extraction (SPE) [22–26] and liquid–liquid extraction [27–29]. Other sample preparation procedures include miniaturized techniques, such as solid-phase microextraction [30,31] and dispersive liquid–liquid microextraction [32,33], and also sampling techniques such as the dried blood [34,35] and dried saliva spots [36] approaches. Another important step is the choice of chromatographic technique, and numerous approaches involving gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS) [32,33,37,38] or tandem mass spectrometry (MS/MS) [34,39,40] detectors have been published. Time-of-flight mass spectrometry [30] or quadrupole time-of-flight mass spectrometry [41] have also been reported.

Microextraction by packed sorbent (MEPS) is a miniaturized system derived from the SPE technique, for which the same steps are followed and the same type of sorbents are commercially available and applied. The sorbent bed is integrated into a syringe, allowing manipulation of small amounts of biological sample and organic solvents. MEPS technique is known to be simple and fast, has the possibility of being coupled online, and allows the sorbent reuse, which constitutes an economic and environmental advantage [42,43]. To date, several studies have been published where the MEPS technique was used to extract antidepressants from oral fluid and plasma samples [44–50]. Additionally, reviews have been published that compile articles using MEPS with LC-MS GC–MS to determine antidepressants in biological fluids [51].

Regardless, none of them have studied all the antidepressants present in this work, and neither complemented this microextraction technique with GC–MS/MS analysis. Thus, the novelty of this article represents an added value for the determination of these analytes of interest. This is due to the robustness of the analysis through the use of highly sensitive equipment and the implementation of the MEPS technique, which offers innovative features such as the use of low volumes of sample and organic solvents, ease and speed of extraction, and the reuse of the sorbent—an important factor considering the demand for analyzing a large number of samples. Additionally, the technique provides satisfactory recoveries of the compounds under study. This paper reports two methodologies for the identification of some of the most prescribed antidepressants, namely the selective serotonin reuptake inhibitors (fluoxetine (FLX), norfluoxetine (NFLX), citalopram (CIT), sertraline (SRT) and paroxetine (PXT)) and selective serotonin-norepinephrine reuptake inhibitors (venlafaxine (VLX) and *O*-desmethylvenlafaxine (DVLX)), in only 250 μL of oral fluid and plasma samples and within the limits of their therapeutic range, using MEPS as extraction procedure and analysis by GC–MS/MS. This microextraction technique can be considered an alternative to classical techniques usually employed in routine laboratory analysis.

2. Material and methods

2.1. Reagents and standards

Analytical standards of citalopram (CIT), fluoxetine hydrochloride (FLX), norfluoxetine (NFLX), paroxetine (PXT) and venlafaxine hydrochloride (VLX) were acquired from Sigma-Aldrich, (St. Louis, MO, USA). Sertraline hydrochloride (SRT) was kindly donated by Pfizer (Groton, MA, USA) and *O*-desmethylvenlafaxine (DVLX) was provided by LGC-Standards (Teddington, London). Protriptyline (PTP), which was used as internal standard (IS), was purchased from Sigma-Aldrich (Lisbon, Portugal). Acetonitrile (Carlo Erba Reagents, Val-de-Reuil, France), ammonium hydroxide (J.T. Baker, Deventer, Netherlands), formic acid (Panreac Química SA, Barcelona, Spain), methanol (Merck Co, Darmstadt, Germany) and 2-propanol (Fischer Chemical, Loughborough, UK) were of analytical grade. Potassium dihydrogen phosphate (KH_2PO_4) was acquired from Panreac (Barcelona, Spain), N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and trimethyl chlorosilane (TMCS) were provided from Macherey-Nagel (Düren, Germany). The MEPS syringe (250 μL) and M1 cartridges (4 mg; 80 % C_8 and 20 % SCX), all from SGE Analytical Science, were purchased from ILC (Porto, Portugal).

All analytical standards were acquired at 1 mg/mL and the working solutions were prepared by diluting the starting solutions with methanol to the final concentrations for the two analyte mixtures. Mixture 1 contained FLX, VLX, DVLX, and NFLX at 2500 ng/mL, CIT at 1000 ng/mL, SRT at 1250 ng/mL, and PXT at 500 ng/mL, while mixture 2 contained FLX and VLX at 1250 ng/mL, DVLX and NFLX at 625 ng/mL, CIT at 250 ng/mL, SRT at 500 ng/mL, and PXT at 125 ng/mL. A working solution of the IS was prepared at a concentration of 50 μg/mL in methanol. All the above solutions were stored in the absence of light at 4 °C.

2.2. Biological specimens

Drug-free oral fluid and plasma samples used in all experiments for the present work were obtained from laboratory staff. Authentic oral fluid and plasma samples were obtained from patients under treatment with these antidepressants at Centro Hospitalar Cova da Beira. The samples were sent to Laboratório de Fármaco-Toxicologia (UBIMedical, Covilhã, Portugal) for analysis. All oral fluid samples were collected by spitting and without using any specific collection device. Blood samples were collected in EDTA vacutainers and further centrifuged at 2700 rpm for 10 min for plasma separation. Both kinds of specimens were stored refrigerated at −20 °C until analysis.

2.3. Gas chromatographic and mass spectrometric conditions

For chromatographic analysis, an HP 7890A gas chromatography system equipped with a model 7000B triple-quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany), along with an MPS2 autosampler and a PTV injector from Gerstel (Mülheim an der Ruhr, Germany) was used. Separation of the analytes was achieved using a capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) with 5 % phenylmethylsiloxane (HP-5MS) (J&W Scientific, Folsom, CA, USA).

The conditions of the chromatographic method were those used in a work previously published by the research group [36], with an initial oven temperature of 150 °C maintained for 1 min, and subsequently increased to 280 °C at 5 °C/min, maintained for 4 min, for a total runtime of 31 min. Furthermore, the detector temperature was set at 280 °C, the injection inlet temperature was set at 250 °C, the helium with a

constant flow rate of 0.8 mL/min was utilized as a carrier gas and nitrogen was used as a collision gas at a flow rate of 2.5 mL/min. Derivatized sample (2 μL) was introduced into the gas chromatography system via splitless injection mode and the mass spectrometry was conducted with a filament current of 35 μA and electron energy of 70 eV in the positive electron ionization mode. Data acquisition was performed in MRM mode (MassHunter WorkStation Acquisition Software rev. B.02.01, Agilent Technologies).

The choice of the transitions for each compound was made in order to obtain better selectivity and sensitivity for the antidepressants under study and less interference from the matrix. Additionally, the choice of ions for these transitions was based on the highest masses and most abundant mass peaks taking into account the most specific masses for each analyte, with the purpose of maximizing the signal-to-noise ratio in the biological matrix extracts. Table 1 shows the detection criteria such as retention times, quantifier transitions, qualifier transitions and respective collision energies selected for each antidepressant.

2.4. Sample preparation

For pretreatment of oral fluid and plasma specimens, 250 μL of sample was mixed with 500 μL of acetonitrile, centrifuged, decanted and evaporated. Following evaporation, the residue was dissolved with 1 mL of 25 mM phosphate buffer (pH 5), 25 μL of IS at 50 μg/mL was added and the sample was homogenized for 10 min by rotation/inversion movements.

The final extraction procedure for oral fluid samples using the MEPS technique was composed of the following steps: conditioning of the M1 sorbent with 250 μL of methanol followed by 250 μL of 0.1 % formic acid in water; sample loading was performed with 12 draw-eject cycles of 150 μL; the sorbent was washed with four cycles of 50 μL of 1 % formic acid in water; the sorbent was dried with four 50 μL draw-eject cycles of air; and the analytes were eluted with four cycles of 100 μL of 1 % ammonium hydroxide in methanol. For plasma samples, the final extraction procedure was as follows: conditioning of the sorbent with 250 μL of methanol and with 250 μL of 0.1 % formic acid in water; sample loading was performed with 12 draw-eject cycles of 150 μL; the sorbent was rinsed with four cycles of 50 μL of 1 % formic acid in water; the sorbent was dried with four 50 μL draw-eject cycles of air; and the analytes were eluted with six cycles of 100 μL of 1 % ammonium hydroxide in methanol. The resulting extracts of the preparation of either specimen were evaporated to dryness under a gentle stream of nitrogen and subsequently derivatized with 50 μL of MSTFA with 5 % TMCS for 2 min in a microwave oven (800 W). An aliquot of 2 μL of the derivatized extract was injected into the GC–MS/MS system for analysis.

In order to reuse the M1 sorbent after each extraction, sequential washing steps were carried out with four cycles (100 μL) of each: 1 % ammonia in methanol:acetonitrile (50:50, v/v) and 1 % formic acid in 2-propanol:water (10:90, v/v).

Table 1
Retention times, selected transitions and collision energy for the identification of the selected antidepressants.

Analytes	Retention time (min)	Quantifier transition (*m/z*)	Qualifier transitions (*m/z*)	Collision energy (eV)
FLX	14.05	261.1—219.1	381.5—116.1	20 (15)
VLX	15.69	134.1—119.1	178.8—134.1	10 (20)
DVLX	16.71	191.7—177.1	391.0—273.3	10 (15)
NFLX[b]	17.13	319.2—215.1	319.2—86.1	5 (15)
PTP[a]	20.85	333.9—333.9	–	5
CIT	21.08	237.2—220.2	237.2—208.2	20 (20)
SRT	23.08	333.0—73.1	346.8—73.2	20 (20)
PXT	24.52	247.9—154.2	247.9—140.2	20 (20)

[a] Internal standard; [b] Only for qualitative analysis. The values between brackets in the collision energy (eV) column correspond to the qualifier transition.

3. Results and discussion

3.1. Optimization of the extraction procedure

In order to minimize interferences from the biological matrix and improve the efficiency of MEPS procedures, the technique was optimized for each specimen, as it is one of the most important assessments to perform before validating an analytical method. Optimization of the extraction techniques was carried out with oral fluid and plasma samples spiked to the highest concentration of the calibration curve for each of the antidepressants under study. The selection of organic solvents or solvent mixtures used for the conditioning, washing and elution steps of the MEPS procedure was based on existing scientific literature [42], the chemical properties of the analysed antidepressants, and also on the experience and evaluations performed by the research group. The tests previously executed made it possible to define which solvents to be used, as well as their percentages and volume, in the various steps of the MEPS extraction procedure for these antidepressants. The choice of the M1 sorbent is based on the physicochemical properties of the antidepressants and the dilution of biological samples with the phosphate buffer at pH 5 is related to the compounds' pKa and to the pH of each of the samples.

In this sense, the final solvents used for the proposed MEPS technique were 250 μL of methanol and 250 μL of 0.1 % formic acid in water for conditioning, 1 % formic acid in water for washing and 1 % ammonium hydroxide in methanol for elution.

Subsequently, an analysis was adopted for each of the biological samples using the statistical tool Design of Experiments (DOE) (MINITAB, version 21). The DOE evaluates, in a rapid and multivariate manner, the factors that can significantly influence the recovery of the analytes. For this study, a two-level full factorial design with three factors (2^3) and a central point (n = 3) was performed. The evaluated factors were: number of sample draw-eject cycles (strokes) (4 to 12 × 150 μL); number of washing cycles (4 to 8 × 50 μL); and number of elution cycles (4 to 6 × 100 μL). DOE was evaluated with blank oral fluid and plasma samples fortified with the compounds under study; the IS was added only after extraction.

The results obtained from the pareto charts for the oral fluid showed that the only factor with significant impact on the recovery of FLX, NFLX, SRT and PXT was the number of draw-eject cycles of the sample. From the main effects plots (Fig. 1 (A)), it can be concluded that the analytes demonstrate a better response when 12 strokes of 150 μL were implemented for sample loading. Regarding the remaining parameters, which have little effect on the extraction procedure, the automatic response optimizer showed that the best response for the number of washes was 4 cycles of 50 μL and that for the number of elutions was 4 cycles of 100 μL. Only DVLX had a slightly better response when 4 draw-eject cycles were used for sample loading, with no statistical difference between the extreme points. In general, the optimal response was observed when 12 draw-eject cycles of 150 μL were implemented for sample loading, 4 cycles of 50 μL for washing and 4 cycles of 100 μL of elution. For plasma, the results obtained from the pareto charts showed that the only factor with a significant impact on the recovery of all analytes, except for NFLX, was the number of draw-eject cycles of the sample. From the main effects plots (Fig. 1 (B)), it can be concluded for this sample as well that the analytes demonstrate a better response when 12 draw-eject cycles of 150 μL were adopted for sample loading. Concerning the remaining factors, which also have little effect on the extraction procedure, the automatic response optimizer showed that the best response for the number of washes was 4 cycles of 50 μL and that for the number of elutions was 6 cycles of 100 μL. In summary, the optimal response was observed when 12 strokes of 150 μL were implemented for sample loading, 4 cycles of 50 μL for washing, and 6 cycles of 100 μL of elution.

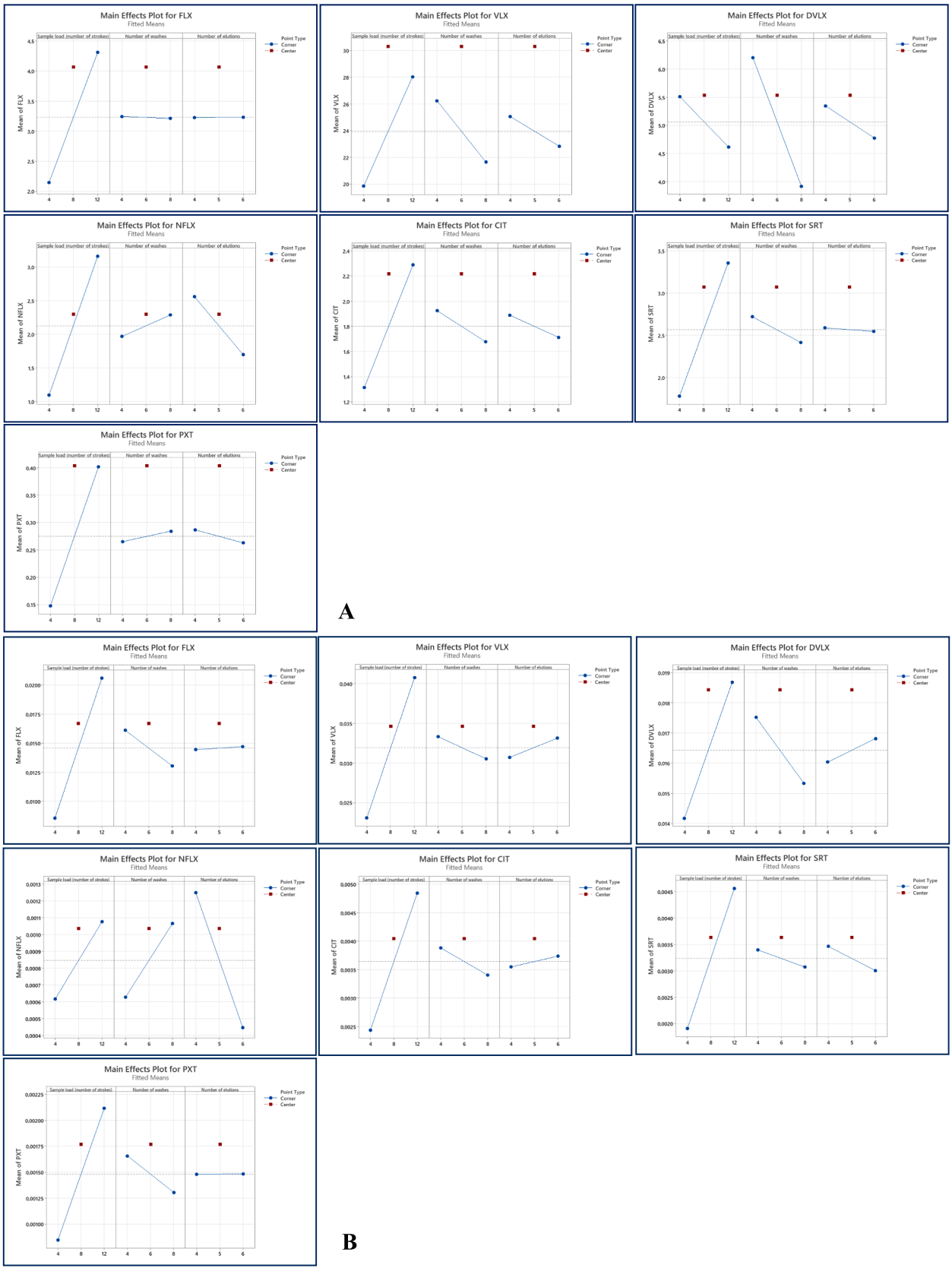

Fig. 1. Main effects plots of number of strokes, number of washes and number of elutions for the compounds under study, and for (**A**) oral fluid and (**B**) plasma samples.

Since the number of draw-eject cycles proved to be the most important condition in the recovery of the compounds under study, and the maximum number resulted in better responses, a univariate study was further performed to evaluate the impact on the recovery of the analytes if the number of strokes increased. Therefore, a subsequent evaluation of the number of strokes was carried out, keeping the remaining factors constant. The studied numbers of draw-eject cycles were 12, 14 and 16 (n = 3) for oral fluid and 12, 18 and 24 (n = 3) for plasma. The results of the univariate study, for oral fluid samples, revealed no significant differences between the number of strokes, with a Friedman's statistic of p = 0.097 for FLX, VLX, DVLX, CIT and SRT, p = 0.135 for NFLX and p = 0.264 for PXT. Regarding plasma samples, there were also no significant differences between the number of strokes evaluated, with a Friedman's statistic of p = 0.097 for FLX, VLX and SRT, p = 0.368 for DVLX, p = 0.135 for NFLX, p = 0.150 for CIT and p = 0.717 for PXT. For this reason, the lowest number of draw-eject cycles (12) was selected for both specimens, and the conditions optimized by the DOE studies were maintained.

3.2. Validation procedure

The methodologies were validated according to the guiding principles of the Food and Drug Administration (FDA) [52]. The validation for FLX, VLX, DVLX, CIT, SRT and PXT, in both biological samples, was carried out following a 5-day validation protocol, and the studied parameters included selectivity, linearity and limits, inter-day, intra-day and intermediate accuracy and precision, extraction recovery, stability and dilution integrity. FLX metabolite, NFLX, was not included in the validation procedures as it was not possible to obtain linearity. Therefore, this metabolite was evaluated qualitatively and only the results of the extraction optimization and recovery parameters are presented.

3.3. Selectivity

The selectivity of the developed methods, studied in order to verify the existence of interferences in the retention times and selected transitions for each antidepressant, was evaluated by analysis of pools of blank samples of oral fluid and plasma from different sources. For the identification of the analytes under study, positivity criteria included an absolute retention time within 2 % or ± 0.1 min of the retention time of the same antidepressant in the control sample and the presence of two transitions per compound. In order to guarantee suitable confidence in the identification of these antidepressants, the maximum allowed tolerances for the relative ion intensities between the transitions (as a percentage of the base peak) were as follows. Considering that the relative intensity in the control sample was greater than 50 %, an absolute tolerance of ± 10 % was accepted; if it was between 25 and 50 %, a relative tolerance of ± 20 % was used; for relative ion intensities between 5 and 25 %, an absolute tolerance of ± 5 % was permitted; and if this value was between 5 % or less, a relative tolerance of ± 50 % was allowed [53]. Therefore, the analytical methodologies would be considered selective if no antidepressant could be identified in the blank oral fluid and plasma samples.

All antidepressants were unequivocally identified in all spiked samples from both specimens, no interferences were observed in blank oral fluid and plasma samples. As such, both methods were considered selective. Figs. 2 and 3 show chromatograms of blank oral fluid and plasma samples and oral fluid and plasma samples spiked at the lower limit of quantification (LLOQ), respectively.

3.4. Calibration curves and limits

Linearity of the methods was established on fortified samples that were processed and analyzed using the proposed extraction procedure, in the range of 100–500 ng/mL for FLX and VLX, 50–500 ng/mL for DVLX, 20–200 ng/mL for CIT, 40–250 ng/mL for SRT, and 10–100 ng/mL for PXT. Seven calibrators with five replicates were used, and the calibration curves were obtained by plotting the peak area ratio between each antidepressant and the IS against the compound's concentration. PTP was chosen for IS because its chemical structure is similar to that of the studied analytes, which allows for better linearity, precision and accuracy, while minimizing compound losses during sample preparation. In addition, it is not commercialised in Portugal, which makes the possibility of it appearing in real samples very unlikely.

The criteria for acceptance of the calibration curve included a determination coefficient (R^2) of at least 0.99 and an accuracy (mean relative error (RE) (bias)) of the calibrators within ± 15 % of the nominal value, with the exception of the LLOQ, for which ± 20 % was considered adequate [52]. The calibration ranges were wide, and 1/x weighted least squares regressions had to be adopted to compensate for heteroscedasticity, for all analytes under study and for the two biological samples. The methodologies were linear within the adopted calibration intervals for all antidepressants, covering the respective therapeutic ranges. The LLOQ value was defined as the lowest concentration that could be assessed with adequate precision and accuracy, that is with a coefficient of variation (CV, %) of less than 20 % and an mean RE within a range of ± 20 % of the nominal concentration. The limits of detection (LODs) were determined as the lowest concentrations that showed a discrete peak clearly distinguishable from the blank sample and had a signal-to-noise ratio of at least 3. These limits were determined by the analysis of six replicates of fortified oral fluid and plasma samples and, for some antidepressants, were similar to the respective LLOQ. The data from the calibration curves and limits, for oral fluid and plasma samples, are shown in Tables 2 and 3, respectively.

Some articles published on this topic include the work developed by Chaves et al. [44], which developed a method to determine some of the antidepressants included in this study in plasma samples using MEPS (with a C_8 and strong cationic exchange sorbent, 2 mg), and analysis by LC with UV detection. The authors obtained LLOQ values of 20 ng/mL for FLX (our LOD for the compound), 10 ng/mL for CIT and SRT (a value between LOD and LLOQ in our method for those analytes), and 20 ng/mL for PXT (a value higher than ours); however, almost twice as much biological sample (400 μL) was used in their study. Using the same MEPS conditions as previously, Catai et al. [50] developed a methodology to determine the same antidepressants in again 400 μL of plasma samples, this time using non-aqueous capillary electrophoresis with spectrophotometric detection. However, they obtained LLOQ values higher than in the former study, namely 25 ng/mL for FLX and PXT, 30 ng/mL for CIT and 20 ng/mL for SRT. A methodology was developed by Souza et al. [47] with extraction by MEPS, using 200 μL of plasma, and analysis by LC-MS/MS. The authors prepared two hybrid silica monoliths functionalized with cyanopropyl or aminopropyl groups, which they used as sorbents for MEPS technique, obtaining an LLOQ of 0.05 ng/mL for FLX, SRT and PXT and 1 ng/mL for CIT; the method was applied to patient samples. More recently, Marasca et al. [49], developed a methodology to identify antidepressants in oral fluid using MEPS (C_2 sorbent) for sample pretreatment for their subsequent extraction by volumetric absorptive microsampling, along with analysis by LC with sequential spectrophotometric and spectrofluorimetric detection. Using 100 μL of sample, the authors obtained LLOQ values of 7 ng/mL for FLX and NFLX, 1 ng/mL for CIT, and 5 ng/mL for SRT. However, the methods described implemented liquid chromatographic approaches, and for one case with mass spectrometry detection, which is not accessible to all laboratories. Although the instrumentation used in our study is less sensitive, this did not impair the quantification of these antidepressants, since the aim of the present study was to develop two methods for quantifying these analytes in the context of therapeutic monitoring; therefore, their therapeutic ranges were considered during validation. Therefore, the LLOQs obtained were considered satisfactory.

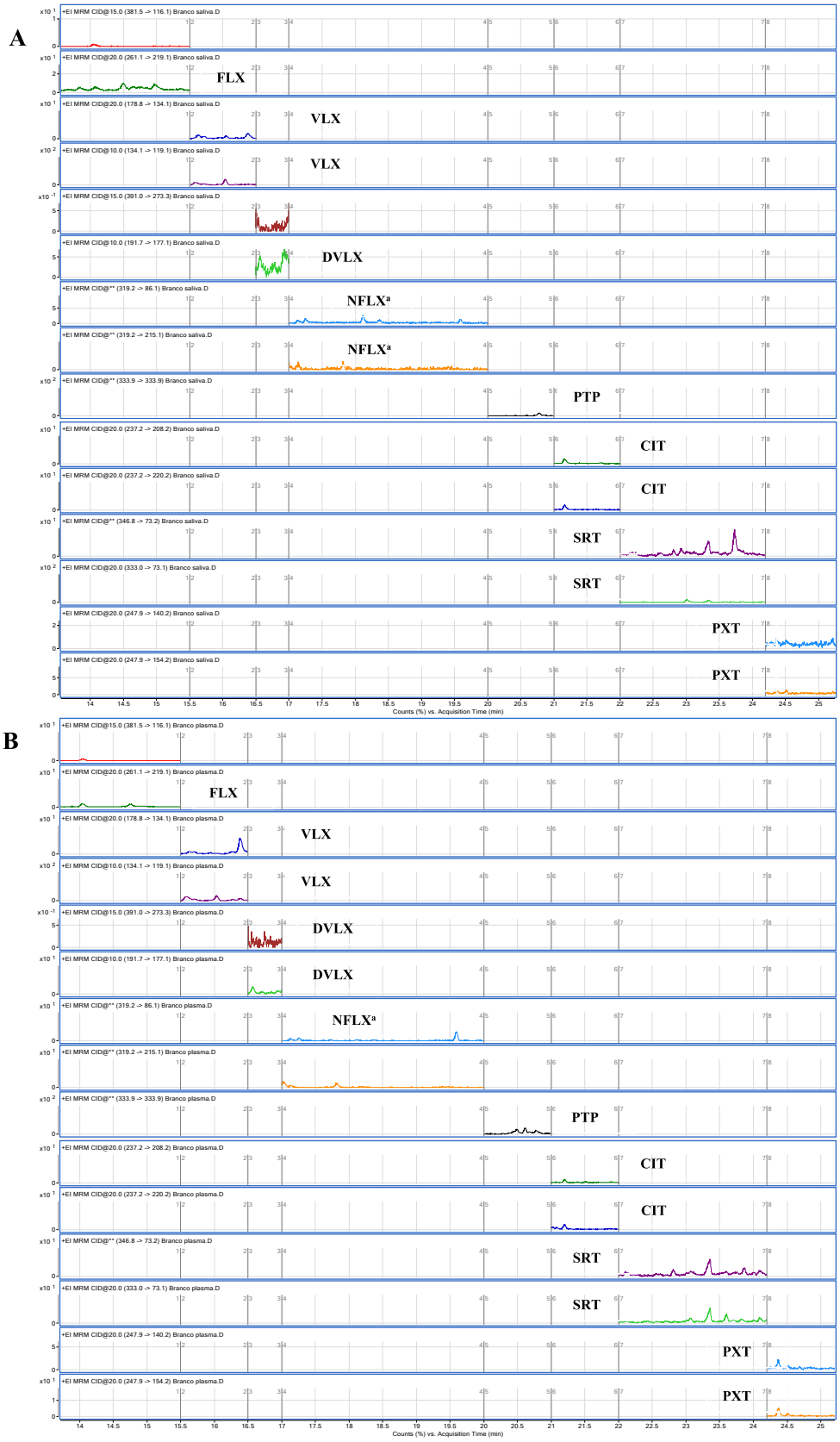

Fig. 2. Chromatograms of selected fragments obtained after extraction of a blank: (**A**) oral fluid sample and (**B**) plasma sample. [a]: Only for qualitative purposes.

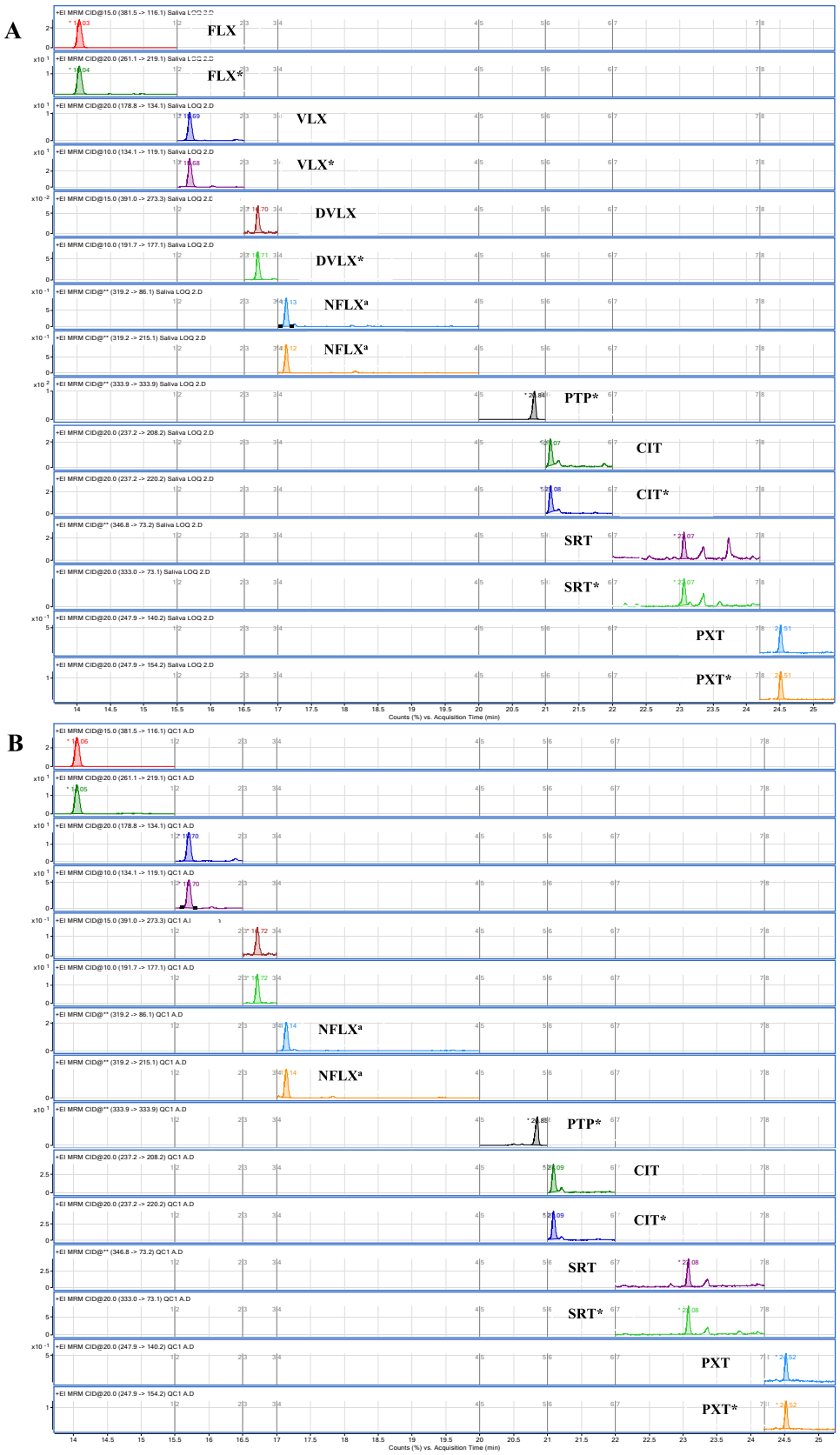

Fig. 3. Chromatograms of selected fragments obtained after extraction of: (**A**) oral fluid samples and (**B**) plasma samples, spiked at the LLOQ. Asterisk indicates quantifier transition. [a]: Only for qualitative analysis.

Table 2
Linearity data (n = 5) in oral fluid.

Analytes	Weight	Linear range (ng/mL)	Linearity		R^{2a}	LOD (ng/mL)	LLOQ (ng/mL)
			Slope[a]	Intercept[a]			
FLX	1/x	100–500	0.0031 ± 0.0008	0.0092 ± 0.0639	0.9909 ± 0.0009	20	100
VLX	1/x	100–500	0.0060 ± 0.0019	0.5854 ± 0.6463	0.9903 ± 0.0041	20	100
DVLX	1/x	50–500	0.0014 ± 0.0003	0.0925 ± 0.0715	0.9917 ± 0.0025	50	50
CIT	1/x	20–200	0.0025 ± 0.0009	0.0088 ± 0.0119	0.9947 ± 0.0023	20	20
SRT	1/x	40–250	0.0011 ± 0.0003	0.0309 ± 0.0404	0.9911 ± 0.0013	40	40
PXT	1/x	10–100	0.0036 ± 0.0008	−0.0266 ± 0.0064	0.9917 ± 0.0020	2	10

[a] : Mean values ± standard deviation.

Table 3
Linearity data (n = 5) in plasma.

Analytes	Weight	Linear range (ng/mL)	Linearity		R^{2a}	LOD (ng/mL)	LLOQ (ng/mL)
			Slope[a]	Intercept[a]			
FLX	1/x	100–500	0.0035 ± 0.0002	−0.1437 ± 0.0461	0.9894 ± 0.0037	20	100
VLX	1/x	100–500	0.0065 ± 0.0006	−0.0821 ± 0.0787	0.9905 ± 0.0038	20	100
DVLX	1/x	50–500	0.0027 ± 0.0002	−0.0073 ± 0.0100	0.9919 ± 0.0017	50	50
CIT	1/x	20–200	0.0018 ± 0.0001	−0.0092 ± 0.0042	0.9933 ± 0.0027	4	20
SRT	1/x	40–250	0.0015 ± 0.0000	−0.0050 ± 0.0048	0.9888 ± 0.0009	8	40
PXT	1/x	10–100	0.0012 ± 0.0003	−0.0030 ± 0.0019	0.9902 ± 0.0004	2	10

[a] : Mean values ± standard deviation.

3.5. Intra-Day, Inter-Day, and intermediate precision and accuracy

Taking into account FDA validation criteria, the precision of the methods was expressed in terms of CV (%) between measured concentrations, and the accuracy was evaluated in terms of the mean RE (%) between the measured and nominal concentrations. Concerning CV values, the established limit was ≤ 15 % for all concentrations, except for the LLOQ, for which values of ≤ 20 % were accepted; as for the mean RE, values within a ± 15 % interval were considered adequate for all concentrations, except for the LLOQ, for which a value in the range of ± 20 % was accepted.

For intermediate precision and accuracy, the four concentration levels of quality controls (QCs) were evaluated in triplicate (n = 15). In the case of oral fluid, CVs typically lower than 13 % were obtained, with accuracy within a range of ± 12 %; while for plasma, CV values lower than 14 % and mean RE values within a ± 11 % interval were obtained. The results are shown in Table 4.

Regarding intra-day precision and accuracy, four concentration levels were evaluated through the analysis of six replicates on the same day (n = 6). The CVs obtained for the oral fluid matrix were below 14 % at the concentrations studied, and the mean RE was also within the range of ± 14 %. For the plasma method, CV values below 12 % were normally obtained for all concentrations, except for the LLOQ for which values below 17 % were achieved, and the mean RE was within a ± 15 %

Table 4
Intermediate precision and accuracy (n = 15).

Analytes	Spiked (ng/mL)	Measured[a] (ng/mL)		CV (%)		RE[a] (%)	
		Oral Fluid	Plasma	Oral Fluid	Plasma	Oral Fluid	Plasma
FLX	100	100.72 ± 9.76	109.65 ± 5.48	9.69	4.99	0.72	9.65
	150	153.28 ± 13.17	143.38 ± 7.76	8.60	5.41	2.19	−4.41
	300	271.94 ± 15.98	294.05 ± 13.93	5.88	4.74	−9.35	−1.98
	500	517.32 ± 42.14	535.95 ± 25.23	8.14	4.71	3.46	7.19
VLX	100	95.13 ± 9.93	107.25 ± 5.94	10.44	5.53	−4.87	7.25
	150	150.69 ± 11.97	153.10 ± 11.25	7.95	7.35	0.46	2.07
	300	296.76 ± 34.60	294.82 ± 23.81	11.66	8.08	−1.08	−1.73
	500	514.39 ± 41.53	503.05 ± 22.55	8.07	4.48	2.88	0.61
DVLX	50	47.84 ± 6.07	55.50 ± 2.30	12.70	4.15	−4.32	11.00
	75	70.17 ± 9.09	78.16 ± 6.42	12.95	8.21	−6.44	4.21
	300	325.95 ± 24.85	317.06 ± 27.72	7.62	8.74	8.65	5.69
	500	471.79 ± 47.26	480.88 ± 24.87	10.02	5.17	−5.64	−3.82
CIT	20	20.61 ± 2.18	21.97 ± 1.37	10.58	6.24	3.03	9.86
	30	30.92 ± 2.31	29.45 ± 2.17	7.48	7.38	3.07	−1.82
	120	117.96 ± 13.06	115.53 ± 7.64	11.07	6.61	−1.70	−3.72
	200	198.26 ± 11.70	206.74 ± 11.85	5.90	5.73	−0.87	3.37
SRT	40	40.12 ± 3.89	41.88 ± 4.78	9.70	11.42	0.30	4.70
	60	62.24 ± 5.66	59.12 ± 6.19	9.09	10.47	3.73	−1.47
	150	139.24 ± 12.45	145.24 ± 12.36	8.94	8.51	−7.18	−3.17
	250	256.89 ± 27.02	264.96 ± 17.43	10.52	6.58	2.76	5.98
PXT	10	11.14 ± 0.44	10.60 ± 1.40	3.99	13.25	11.42	6.00
	15	15.54 ± 1.13	13.42 ± 0.79	7.30	5.91	3.62	−10.52
	60	58.33 ± 5.14	56.27 ± 7.68	8.81	13.65	−2.78	−6.21
	100	92.74 ± 7.33	111.03 ± 7.11	7.91	6.40	−7.26	11.03

All concentrations in ng/mL; relative error [(measured concentration − spiked concentration/spiked concentration) × 100]. CV − coefficient of variation; RE − relative error; [a]: Mean values ± standard deviation.

interval (Table 5).

Finally, inter-day precision and accuracy were evaluated at seven concentrations within a 5-day period (n = 5), for which CVs of less than 12 % were typically obtained, with a RE value within ± 10 %, for the oral fluid methodology; and CV values below 10 % and mean RE values within a range of ± 10 % were obtained, except for the LLOQ for which values were found within a ± 19 % interval, for the plasma matrix. The results are presented in Table 6.

3.6. Extraction recovery

In order to evaluate the absolute recoveries obtained with the two optimized MEPS procedures, two sets of oral fluid and plasma samples (n = 3) were prepared at low, medium and high concentrations: 100, 250 and 500 ng/mL for FLX and VLX; 50, 125 and 500 ng/mL for DVLX and NFLX; 20, 50 and 200 ng/mL for CIT; 40, 100 and 250 ng/mL for SRT; and 10, 25 and 100 ng/mL for PXT. One of the sets consists of the extract of a blank sample, fortified with the analytes of interest after extraction by MEPS (100 % recovery), while in the other the blank samples were spiked with the antidepressants under study before the extraction process. IS was added only after the elution step for both sets of samples and for both specimens. The recoveries were obtained by comparing the relative peak areas from the second set with those from the first set; the results are shown in Table 7.

The extraction efficiencies ranged between approximately 12 and 93 % for oral fluid samples, and between approximately 28 and 101 % for plasma samples. The lowest recovery values concern the metabolites; indeed, in the case of DVLX they varied between approximately 12 and 17 % for the oral fluid sample, while NFLX showed a recovery of approximately 28 % for the low plasma sample concentration. These results, although quite low, are acceptable particularly taking into consideration that a microextraction procedure is involved. For the remaining antidepressants and for both specimens, the recovery results can be considered satisfactory. In order to maximize extraction efficiency, the parameters influencing extraction were evaluated using an experimental design with considerable intervals, and these results were complemented with univariate studies, to achieve the best compromise between the speed of the procedure and recovery of the antidepressants. It should also be taken into account that, although some recoveries were low, they represent the absolute extraction of the antidepressants and did not affect the sensitivity of the methodologies, since, even using a low volume of 250 μL of biological sample, the compounds under study were detected and quantified with adequate precision and accuracy, and the intended LLOQs were achieved.

Among the papers published on this subject, and which include the study of recoveries from extraction procedures, is the aforementioned work by Marasca et al. [49], for which the authors have reported recoveries between 91 and 96 % for FLX, 88 and 91 % for NFLX, 91 and 95 % for CIT, and 90 and 95 % for SRT, using 100 μL of oral fluid. Magalhães et al. [46] developed a methodology to determine VLX and its metabolite, DVLX, in 100 μL of plasma samples, with extraction by MEPS (4 mg, solid-phase silica C_{18}) and analysis by high-performance liquid chromatography with fluorescence detection. The authors obtained recovery values between approximately 79 and 83 % for VLX and between 72 and 77 % for DVLX. The same authors also developed a method to identify FLX, NFLX and PXT in 500 μL of plasma specimens, also with extraction by MEPS (C_8) and analysis by liquid chromatography with fluorescence detection. For this methodology, recovery values were obtained between approximately 59 and 65 % for FLX, between 59 and 67 % for NFLX and between 70 and 77 % for PXT, for which they used twice the volume of the biological sample than the work presented [48]. Furthermore, the described methodologies present good sensitivity for the therapeutic concentration ranges of the antidepressants under study, and MEPS can be considered a powerful technique, presenting efficient extraction of the target analytes and using smaller sample and organic solvents volumes.

3.7. Stability

The stability of antidepressants was evaluated for different conditions and intervals between processed samples, short-term and freeze/thaw cycles and it was studied for both biological matrices at the concentrations of the four QCs (n = 3), at 100, 150, 300 and 500 ng/mL for FLX and VLX; at 50, 75, 300 and 500 ng/mL for DVLX; at 20, 30, 120 and

Table 5
Intra-day (n = 6) precision and accuracy.

Analytes	Spiked (ng/mL)	Intra-day					
		Measured[a] (ng/mL)		**CV (%)**		**RE[a] (%)**	
		Oral Fluid	Plasma	Oral Fluid	Plasma	Oral Fluid	Plasma
FLX	100	87.81 ± 3.84	112.11 ± 2.38	4.37	2.12	−12.19	12.11
	150	156.92 ± 9.30	148.49 ± 2.80	5.92	1.89	4.61	−1.01
	300	268.14 ± 10.53	305.63 ± 5.01	3.93	1.64	−10.62	1.88
	500	525.02 ± 17.04	536.10 ± 18.88	3.25	3.52	5.00	7.22
VLX	100	109.17 ± 8.47	97.21 ± 9.32	7.75	9.59	9.17	−2.79
	150	148.55 ± 19.49	164.83 ± 4.59	13.12	2.79	−0.97	9.89
	300	294.53 ± 37.87	299.01 ± 18.52	12.86	6.19	−1.82	−0.33
	500	522.94 ± 21.48	500.61 ± 25.65	4.11	5.12	4.59	0.12
DVLX	50	51.25 ± 5.54	55.92 ± 1.70	10.80	3.05	−0.73	7.67
	75	67.28 ± 5.62	83.08 ± 3.97	8.36	4.78	−10.29	10.78
	300	314.03 ± 32.33	317.62 ± 17.30	10.29	5.45	4.68	5.87
	500	518.75 ± 36.52	481.04 ± 31.62	7.04	6.57	3.75	−3.79
CIT	20	20.42 ± 2.75	20.04 ± 2.55	13.47	12.72	2.09	0.18
	30	30.03 ± 2.22	31.85 ± 0.64	7.40	2.00	0.11	6.16
	120	115.81 ± 14.64	115.89 ± 6.60	12.64	5.69	−3.49	−3.43
	200	195.55 ± 5.04	194.02 ± 13.32	2.58	6.86	−2.22	−2.99
SRT	40	35.97 ± 1.37	36.18 ± 2.57	3.81	7.10	−10.08	−9.56
	60	62.52 ± 5.10	66.61 ± 2.48	8.15	3.73	4.20	11.01
	150	144.28 ± 11.00	156.34 ± 2.95	7.63	1.89	−3.81	4.23
	250	262.44 ± 15.38	259.29 ± 16.35	5.86	6.31	4.97	3.72
PXT	10	11.35 ± 0.18	9.73 ± 1.62	1.59	16.61	13.54	−14.47
	15	15.65 ± 1.53	13.53 ± 0.71	9.79	5.24	4.32	−9.83
	60	54.69 ± 6.67	59.76 ± 7.08	12.20	11.85	−8.86	−0.40
	100	99.44 ± 6.09	101.78 ± 5.93	6.12	5.83	−0.56	1.78

All concentrations in ng/mL; relative error [(measured concentration − spiked concentration/spiked concentration) × 100]. CV − coefficient of variation; RE − relative error; [a]: Mean values ± standard deviation.

Table 6
Inter-day (n = 5) precision and accuracy.

Analytes	Spiked (ng/mL)	Inter-day					
		Measured[a] (ng/mL)		**CV (%)**		**RE[a] (%)**	
		Oral Fluid	Plasma	Oral Fluid	Plasma	Oral Fluid	Plasma
FLX	100	99.58 ± 4.05	109.60 ± 2.33	4.07	2.12	−0.42	9.60
	200	205.54 ± 10.26	185.52 ± 9.55	4.99	5.15	2.77	−7.24
	250	250.14 ± 16.57	240.69 ± 4.04	6.62	1.68	0.06	−3.73
	300	298.50 ± 15.28	287.16 ± 13.23	5.12	4.61	−0.50	−4.28
	350	340.06 ± 11.72	349.61 ± 12.41	3.45	3.55	−2.84	−0.11
	400	393.92 ± 12.56	405.40 ± 6.49	3.19	1.60	−1.52	1.35
	500	512.26 ± 19.37	522.02 ± 9.05	3.78	1.73	2.45	4.40
VLX	100	103.06 ± 7.95	103.25 ± 6.41	7.72	6.20	3.06	3.25
	200	191.56 ± 17.95	195.56 ± 15.57	9.37	7.96	−4.22	−2.22
	250	252.36 ± 15.69	243.07 ± 8.08	6.22	3.32	0.95	−2.77
	300	300.95 ± 11.43	300.19 ± 17.87	3.80	5.95	0.32	0.06
	350	343.73 ± 9.08	351.83 ± 18.25	2.64	5.19	−1.79	0.52
	400	400.48 ± 19.03	398.76 ± 15.41	4.75	3.86	0.12	−0.31
	500	507.86 ± 20.45	507.34 ± 22.35	4.03	4.41	1.57	1.47
DVLX	50	54.88 ± 2.89	54.86 ± 3.89	5.26	7.08	9.77	9.71
	100	90.91 ± 6.15	96.17 ± 9.45	6.77	9.83	−9.09	−3.83
	125	117.17 ± 9.41	112.92 ± 3.83	8.03	3.39	−6.26	−9.66
	300	325.90 ± 13.57	303.67 ± 21.06	4.16	6.94	8.63	1.22
	350	346.99 ± 15.93	364.12 ± 15.36	4.59	4.22	−0.86	4.04
	400	399.66 ± 10.70	397.32 ± 17.20	2.68	4.33	−0.08	−0.67
	500	489.48 ± 13.98	495.93 ± 23.52	2.86	4.74	−2.10	−0.81
CIT	20	21.88 ± 1.36	22.98 ± 0.69	6.22	2.99	9.40	14.91
	40	36.89 ± 1.71	36.39 ± 1.22	4.63	3.35	−7.78	−9.04
	50	48.57 ± 4.06	46.58 ± 1.90	8.36	4.07	−2.86	−6.84
	120	119.42 ± 3.76	115.82 ± 2.71	3.15	2.34	−0.48	−3.48
	140	137.06 ± 2.65	141.75 ± 7.32	1.93	5.17	−2.10	1.25
	160	165.83 ± 6.88	159.66 ± 4.35	4.15	2.73	3.64	−0.25
	200	200.35 ± 7.83	206.82 ± 7.98	3.91	3.86	0.17	3.41
SRT	40	39.16 ± 3.38	40.07 ± 2.90	8.64	7.23	−2.11	0.18
	80	81.20 ± 5.75	79.60 ± 7.34	7.08	9.23	1.50	−0.50
	100	104.35 ± 5.39	105.00 ± 5.73	5.17	5.46	4.35	5.00
	150	146.93 ± 7.31	140.32 ± 3.69	4.98	2.63	−2.05	−6.46
	175	168.89 ± 6.09	175.34 ± 14.60	3.60	8.33	−3.49	0.19
	200	200.10 ± 9.18	197.16 ± 7.23	4.59	3.67	0.05	−1.42
	250	254.37 ± 11.06	257.51 ± 9.61	4.35	3.73	1.75	3.00
PXT	10	10.58 ± 1.19	11.98 ± 0.30	11.25	2.54	5.77	18.27
	20	20.25 ± 2.30	17.62 ± 0.92	11.34	5.20	1.24	−11.90
	25	23.24 ± 1.86	22.64 ± 1.42	8.00	6.25	−7.05	−9.42
	60	57.74 ± 1.17	57.82 ± 3.56	2.03	6.16	−3.77	−3.64
	70	70.31 ± 3.07	67.23 ± 0.51	4.36	0.75	0.44	−3.96
	80	81.05 ± 3.74	83.67 ± 4.46	4.61	5.33	1.32	4.59
	100	101.61 ± 4.89	102.93 ± 2.59	4.82	2.52	1.61	2.93

All concentrations in ng/mL; relative error [(measured concentration − spiked concentration/spiked concentration) × 100]. CV − coefficient of variation; RE − relative error; [a]: Mean values ± standard deviation.

Table 7
Recoveries of antidepressants (n = 3).

Analytes	**Recoveries[a] (%)**					
	Low-spiked concentration		**Medium-spiked concentration**		**High-spiked concentration**	
	Oral Fluid	Plasma	Oral Fluid	Plasma	Oral Fluid	Plasma
FLX	71.41 ± 10.47	70.04 ± 7.43	77.67 ± 4.17	71.34 ± 2.23	83.40 ± 3.87	64.18 ± 2.85
VLX	69.69 ± 11.82	100.91 ± 2.17	57.90 ± 4.76	85.89 ± 9.50	72.79 ± 7.71	81.00 ± 3.43
DVLX	17.29 ± 3.30	59.99 ± 5.43	11.93 ± 1.38	45.40 ± 4.11	15.75 ± 2.12	41.32 ± 0.74
NFLX	47.41 ± 2.47	28.09 ± 5.48	42.14 ± 0.58	79.34 ± 15.38	58.24 ± 7.92	44.49 ± 8.63
CIT	86.47 ± 13.68	75.66 ± 5.32	83.49 ± 5.02	85.22 ± 8.64	92.90 ± 5.99	77.90 ± 0.85
SRT	82.33 ± 14.02	68.31 ± 3.34	86.42 ± 8.68	64.21 ± 2.56	92.39 ± 1.58	63.90 ± 1.29
PXT	86.89 ± 11.85	42.03 ± 7.71	52.21 ± 7.34	72.55 ± 8.21	71.89 ± 10.60	61.93 ± 1.26

[a] : Mean values ± standard deviation.

200 ng/mL for CIT; at 40, 60, 150 and 250 ng/mL for SRT; and at 10, 15, 60 and 100 ng/mL for PXT. The oral fluid and plasma samples prepared for stability studies were compared with samples prepared and analyzed on the same day, and they were quantified using the same calibration curve, to compare concentrations, calculate the respective mean RE value relatively to the theoretical concentrations and calculate the CV values. The analytes under study were considered stable if the criteria of CVs below 15 % and REs within a ± 15 % interval were both met.

To study the stability of the processed samples, the previously analyzed extracts were stored in the equipment's autosampler at room temperature for a period of 24 h, after which they were reanalyzed again. The results obtained allowed us to conclude that, for the oral fluid matrix, only FLX, SRT and PXT were stable at all concentrations studied; CIT was only stable at the two highest concentrations; and VLX and metabolite, DVLX, were not stable at any of the studied concentrations. The CV parameter presented values typically below 13 % and the mean RE values varied within a range of ± 11 %. For plasma samples, only FLX and PXT were stable at all concentrations; CIT was also only stable at the two highest concentrations; and VLX, DVLX and SRT were not stable at any of the studied concentration levels. Values below 15 % and values within a ± 15 % interval were obtained for the CV and mean RE parameters, respectively.

Short-term stability was assessed with samples spiked at the established concentrations, which were left at room temperature for 24 h, and then extracted and analyzed. For oral fluid, DVLX was not stable at any of the concentration levels; VLX was only stable at the two highest concentrations; and FLX, CIT, SRT and PXT were stable at all concentrations; with CVs typically lower than 14 % and a mean RE within a range of ± 15 %. For plasma, PXT was only stable at the two highest concentrations; while FLX, VLX, DVLX, CIT and SRT were stable at all concentration levels; with CVs lower than 12 % and a mean RE within a ± 15 % interval.

Regarding the stability after freeze/thaw cycles, samples spiked at the defined concentrations were stored at −20 °C for 24 h, after which they were thawed at room temperature and refrozen for 24 h more under the same conditions, and this cycle was repeated twice more before the samples were extracted and analyzed. All compounds under study were stable for at least three freeze/thaw cycles, and for both biological samples. For oral fluid, the CVs obtained were lower than 15 % and the mean RE was within the range of ± 15 % for all concentration levels; and the CVs obtained were typically lower than 12 % and the mean RE was within a ± 15 % interval for plasma.

The data obtained for stability indicate that these biological specimens should be preferably stored refrigerated, as at those particular conditions the stability of the analytes is not significantly affected.

3.8. Dilution integrity

This parameter was studied to enable providing a quantitative result for the compounds in those samples presenting concentrations higher than the method's upper limit of quantification (ULOQ). Two dilution factors (1:2 and 1:4) were tested for all compounds under study and for both specimens, spiked at concentrations that would fit within the calibration range after proper dilution. Each spiked sample was diluted with blank corresponding matrix, and subsequently the analytes' concentrations were determined by multiplication of the obtained value by the dilution factor employed.

The results showed CVs up to 12 % and RE values within the interval of ± 15 % for oral fluid, while CVs up to 15 % and RE values within the interval of ± 15 % were obtained for plasma. Consequently, even overly concentrated patient specimens could be adequately quantified after proper dilution.

3.9. Method applicability

The applicability of the developed methodologies was demonstrated by application to the determination of the analytes of interest in oral fluid and plasma samples belonging to patients under treatment with these antidepressants. Table 8 shows the obtained results for some of the analysed samples and Fig. 4 shows the chromatograms. Thus, the applicability of both methods was demonstrated, and they can be successfully implemented in routine analysis for identification and quantification of these antidepressants.

4. Conclusions

This work describes the optimization and validation of two analytical methods for the identification and quantification of five antidepressants and one metabolite (fluoxetine, venlafaxine, *O*-desmethylvenlafaxine, citalopram, sertraline and paroxetine), in oral fluid and plasma samples, using MEPS and GC–MS/MS.

The combination of this microextraction technique with tandem mass spectrometry resulted in a simple and rapid procedure, and the two methodologies that proved to be sensitive, selective and precise. Linearity was obtained within the range of 10–100 ng/mL for all antidepressants, with adequate accuracy and precision, and using only 250 μL of either oral fluid or plasma. The reduced sample volume required, associated with the sensitivity achieved by the methods described, provides an advantage, particularly when there is little sample availability, allowing multiple tests to be carried out on the same specimen. Taking into account the therapeutic concentration ranges of the analytes, acceptable recovery values were obtained for both specimens, between 12 and 93 % for oral fluid and between 28 and 101 % for plasma, the intended LLOQs were obtained and low LODs were achieved for some of the analytes.

This is the first report on the use of MEPS as an approach to preconcentrate these antidepressants from oral fluid and plasma samples and can be considered an alternative to the normally implemented SPE and LLE techniques, reducing the volume of biological sample and the volume of organic solvents, allowing sorbent reuse (around 40–50 extractions for oral fluid and 20–30 extractions for plasma samples) which constitutes an economic advantage, and enabling their automation. Furthermore, these results allow the routine use of these methodologies in the determination of these analytes in clinical and forensic toxicology analysis, and their application in real oral fluid and plasma samples has proven their potential in drug monitoring.

Table 8
Analysis of the patient oral fluid and plasma samples.

Oral Fluid Samples	Analytes	Prescribed Daily Dose (mg)	Concentration (ng/mL)
1	FLX/NFLX	n.a	324.57/Positive
2	VLX/DVLX	150	566.86/163.67
3	VLX/DVLX	n.a.	Not detected/72.75
4	CIT	n.a.	20.28
5	CIT	n.a.	52.36
6	PXT	30	24.69
Plasma Samples	**Analytes**	**Prescribed Daily Dose (mg)**	**Concentration (ng/mL)**
1	FLX/NFLX	20	132.08/Positive
2	VLX/DVLX	225	Not detected/292.38
3	CIT	10	31.25
4	SRT	100	77.34
5	PXT	30	109.19

n.a.: not available.

Informed Consent Statement

This study was approved by the Ethics Committee of the Centro Hospitalar Cova da Beira and conducted in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study (Protocol: "Monitorização terapêutica de fármacos antipsicóticos em doentes do foro psiquiátrico: contribuição para o estabelecimento de relações entre as concentrações terapêuticas em amostras biológicas e avaliação da adesão terapêutica" – CHCB/March 2012).

CRediT authorship contribution statement

Sofia Soares: Writing – original draft, Methodology, Investigation, Formal analysis. **Tiago Rosado:** Writing – review & editing, Supervision, Investigation, Formal analysis. **Mário Barroso:** Writing – review & editing, Validation, Supervision, Investigation, Conceptualization. **Eugenia Gallardo:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

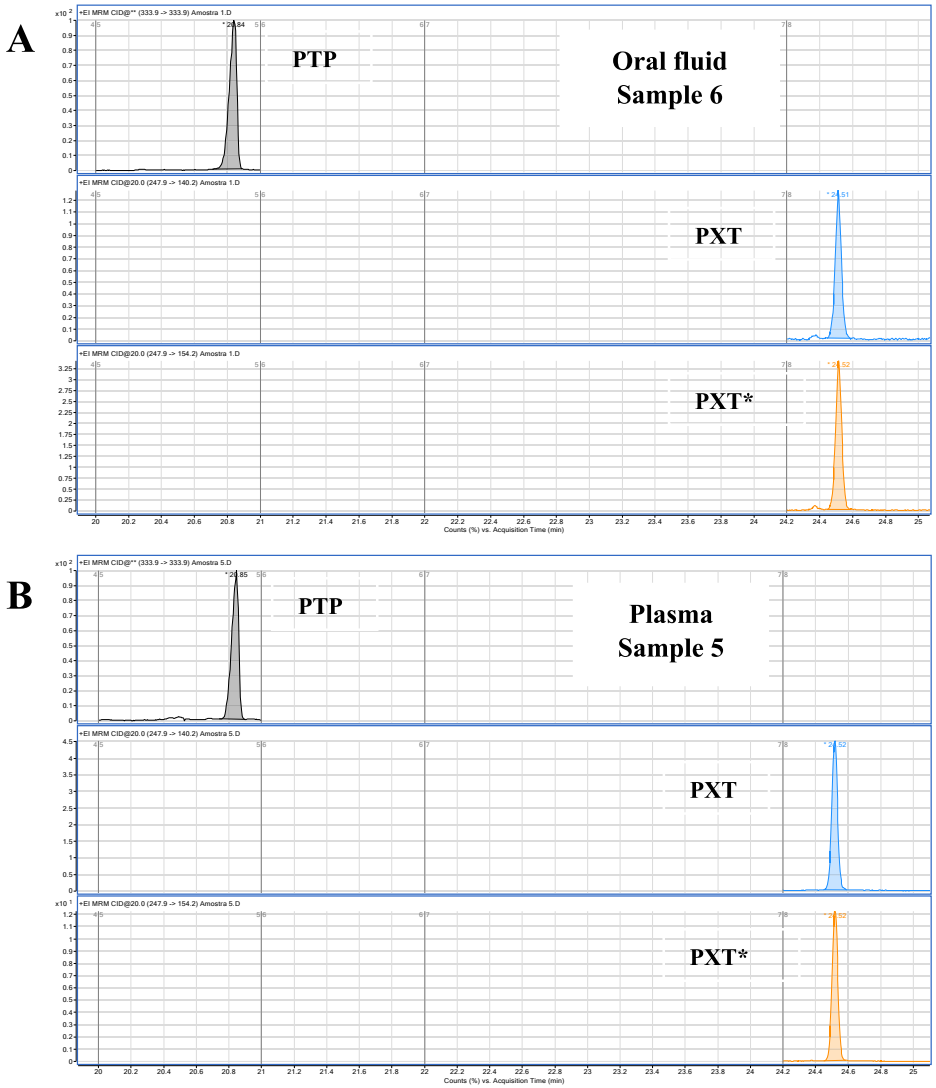

Fig. 4. Chromatograms obtained after analysis of patient specimens positive for antidepressants: (**A**) oral fluid sample from a PXT consumer; (**B**) plasma sample from a PXT consumer. Asterisk indicates quantifier transition.

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.

Data availability

Data will be made available on request.

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