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Determination of seven selected antipsychotic drugs in human plasma using microextraction in packed sorbent and gas chromatography–tandem mass spectrometry

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Abstract A method using microextraction by packed sorbent (MEPS) and gas chromatography–tandem mass spectrometry (GC-MS/MS) is described for the determination of seven antipsychotic drugs in human plasma. The studied compounds were chlorpromazine (CPZ), haloperidol (HAL), cyamemazine, quetiapine, clozapine, olanzapine (OLZ), and levomepromazine; promazine, protriptyline, and deuterated CPZ were used as internal standards. The validation parameters included selectivity, linearity and limits of detection and quantitation, intra- and interday precision and trueness, recovery, and stability and were studied according to internationally accepted guidelines. The method was found to be linear between the lower limit of quantitation and 1000 ng/mL, except for OLZ and HAL (200 ng/mL), with determination coefficients higher than 0.99 for all analytes, and extraction efficiencies ranged from 62 to 92 %. Intra- and interday precision ranged from 0.24 to 10.67 %, while trueness was within a ±15 % interval from the nominal concentration for all analytes at all studied levels. MEPS has shown to be a rapid procedure for the determination of the selected antipsychotic drugs in human plasma, allowing reducing the handling time and the costs of analysis. Furthermore, GC-MS/MS has demonstrated to be a powerful tool for the simultaneous quantitation of the studied compounds, enabling obtaining adequate selectivity and sensitivity using a sample volume of as low as 0.25 mL.

Keywords MEPS · GC-MS/MS · Antipsychotic drugs · Plasma

Introduction

Schizophrenia is a severe psychiatric disorder affecting approximately 1.5 % of the world's population, for which antipsychotic (AP) drugs are the treatment of choice. Indeed, AP medications are one of the fastest growing products in the pharmaceutical industry [1].

In routine clinical practice, first-generation (typical) APs were developed in the 1950s [2], and these included haloperidol (HAL), chlorpromazine (CPZ), cyamemazine (CYA), and levomepromazine (LVMP) [1, 3–6]. However, those APs presented severe side-effects, such as extrapyramidal symptoms due to their pharmacological action on D2 and D4 dopaminergic receptors [2]. A range of second-generation APs was developed in an attempt to reduce these side-effects, and risperidone, olanzapine (OLZ), clozapine (CLOZ), ziprasidone, quetiapine (QTP), and aripiprazole were introduced in therapy [1, 3–7]. The activity of these compounds on D2 and D4 receptors is considerably lower, and therefore extrapyramidal side-effects are not common [2].

AP drugs are usually administered at relatively low daily dosages. Therefore, their concentrations in plasma are normally low (picograms to nanograms per milliliter). In addition, they are frequently encountered in clinical and forensic toxicology scenarios [8]. So, reliable bioanalytical methods are needed to monitor efficiently the compounds in biological specimens, for an optimal supervision of patients and their

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therapy. This will help in avoiding medical complications, intoxications, lack of response, and noncompliance [9].

AP drugs are analyzed in biological matrices by several methods, such as gas chromatography (GC) coupled to either nitrogen-phosphorus detector [10], or mass spectrometric (MS) detection [11], high-performance liquid chromatography (LC) coupled to coulometric [12], MS [9, 13], tandem mass spectrometry (MS/MS) [4, 14], UV or diode array detectors [5, 6, 15–17] and capillary electrophoresis (CE) with electrochemiluminescence [18] or UV detection [19]. An excellent review has inclusively been published for AP determination in human samples by LC-MS-MS [20]. Sample pretreatment and clean-up is performed mostly by liquid-liquid (LLE) [1, 2, 15, 16, 18, 21, 22] and solid-phase extraction (SPE) [5, 6, 10, 13, 19, 23]. A method using on-line SPE and LC-MS-MS has been published, yet for the determination of antidepressant drugs [24]. These sample preparation techniques involve time and organic solvent consuming procedures, and are unsuitable in the field of emergency medicine, which demands rapid and sensitive detection. For this purpose, the use of microextraction techniques, such as microextraction in packed sorbent (MEPS) seems appealing. This recent sample preparation technique is based on the miniaturization of conventional SPE, using a gas-tight syringe as the extraction device [25, 26], which allows online coupling to analytical instruments. This packed syringe can be reused several times, and more than 100 extractions have been reported using plasma or urine samples [27–29]. MEPS is very promising because of its ease of use, speed, reduction of organic solvent volumes (being therefore more environmentally friendly) and the cost of analysis is minimal compared with conventional SPE procedures. This extraction technique has been used in bioanalysis, namely for the quantitation of antidepressants [30], risperidone [31], piperazines [25, 26], methamphetamine and amphetamine [26] in several biological specimens, using mainly liquid chromatographic procedures. A method for the determination of CLOZ in dried blood spots has also been published [12].

This paper describes for the first time the analysis of several APs (HAL, CPZ, CYA, LVMP, OLZ, CLOZ, and QTP) in plasma samples using a combination of MEPS and GC-MS/MS. The method was fully validated, allowing its application mainly in clinical scenarios where the compounds are involved.

Experimental

Reagents and standards

Analytical standards of OLZ, HAL, CLOZ, and CPZ, as well as its trideuterated analogue (CPZ-d$_3$) were purchased from LGC Promochem (Barcelona, Spain) in solution, at a concentration of 1 mg/mL. Promazine (PRZ; internal standard (IS)), protriptyline (PTP; IS), LVMP, and CYA were acquired from Sigma-Aldrich (Lisbon, Portugal). QTP (98 % purity) was kindly donated by AstraZeneca PLC (London, UK). It should be pointed that PRZ and PTP are not commercially available as therapeutic drugs in Portugal, and therefore their appearance in an authentic sample, impairing quantitative analysis, is highly unlikely to occur. Potassium dihydrogen phosphate (KH$_2$PO$_4$) was purchased from Panreac (Barcelona, Spain), acetic acid (50 % purity) from Sigma-Aldrich (Lisbon, Portugal), methanol from Merck (Darmstadt, Germany), and ammonium hydroxide (analytical grade) from J.T. Baker (Holland). Ultrapure water was obtained from a Milli-Q System (Millipore, Billerica, MA). N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and trimethylchlorosilane (TMS) were acquired from Macherey–Nagel (Düren, Germany). MEPS 250 µL syringe and barrel insert and needle M$_1$ (4 mg; 80 % C$_8$ and 20 % SCX) were purchased from ILC (Porto, Portugal).

Working solutions at 10, 1, and 0.1 µg/mL of CYA, LVPM, CLOZ, CPZ, and QTP and at 2, 0.2, and 0.02 µg/mL for HAL and OLZ were prepared by proper dilution of the stock solutions with methanol. A working solution of the IS at 2 µg/mL for CPZ-d$_3$ and 1 µg/mL for both PTP and PRZ was prepared also in methanol. All these solutions were stored light protected at 4 °C.

Biological specimens

Fresh human plasma was obtained from the excess supplies of the Instituto Português do Sangue (outdated transfusions). Authentic plasma samples belonging to patients under treatment with the studied compounds were collected at the Psychiatry Department of the local Hospital (Centro Hospitalar Cova da Beira, E.P.E., Covilhã, Portugal). All samples were stored at −20 °C until analysis.

Gas chromatographic and mass spectrometric conditions

Chromatographic analysis was performed using an HP 7890A GC system (Agilent Technologies, Waldbronn, Germany), equipped with a model 7000B triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany), a MPS2 autosampler and a PTV-injector from Gerstel (Mülheim an der Ruhr, Germany). A capillary column (30 m×0.25 mm I.D., 0.25 µm film thickness) with 5 % phenylmethylsiloxane (HP-5 MS), supplied by J & W Scientific (Folsom, CA), was used.

The oven temperature started at 120 °C for 2 min, followed by an increase of 20 °C/min to achieve the final temperature of 300 °C. The total separation time was 25 min. The temperatures of the injection port and the ion source were set at 250 and 280 °C, respectively. Helium was used as carrier gas at a constant flow rate of 0.8 mL/min.

The mass spectrometer was operated with a filament current of 35 μA and electron energy 70 eV in the positive electron ionization mode. Nitrogen was used as collision gas at a flow rate of 2.5 mL/min. Data were acquired in the multiple reaction monitoring mode, using the MassHunter WorkStation Acquisition Software rev. B.02.01 (Agilent Technologies).

The transitions were chosen for selectivity and abundance to maximize signal-to-noise ratio in matrix extracts. Table 1 resumes the precursor, product ions, collision energies, retention times, and dwell time selected for each analyte.

Sample preparation

The presented method was previously optimized by means of a multivariate approach (data not shown), in which all the factors (sample dilution, strokes, solvent composition, and volumes) were studied at two levels. None of the studied factors had a statistically significant influence on the response, and therefore the conditions which yielded the best apparent response were chosen (regardless of the lack of significance of the observed effect). The final conditions were as follows.

Twenty-five microliters of the IS solution was added to 0.25 mL of plasma previously diluted with 0.25 mL of 0.1 M KH_2PO_4 in a glass tube, and the sample was slightly vortex mixed for 30 s. Then, it was manually drawn through the sorbent and ejected in the same vial three times (strokes) at an approximate flow rate of 10 μL/s. The sorbent was washed with 100 μL of 5 % acetic acid and 100 μL of 10 % methanol in water to remove matrix-borne interferences. The analytes were eluted with 200 μL of 5 % ammonia in methanol. The extracts were evaporated to dryness under a gentle stream of nitrogen at room temperature, and the residues were dissolved in 65 μL of MSTFA with 5 % TMS. This derivatization reagent was chosen because it is the most widely used derivatizing agent in our laboratory, and therefore more compatible with other type of analyses that we perform. After derivatization at 85 °C for 45 min, the extracts were transferred to autosampler vials, and an aliquot of 2 μL was injected into the GC-MS/MS instrument in the split mode (1:5). After each extraction, the sorbent was washed sequentially with 5× 250 μL methanol and 4×250 μL water. This step aimed at decreasing memory effects, conditioning simultaneously the sorbent for the next extraction. Each sorbent could be used for about 100 extractions.

Validation procedure

The described method was fully validated according to the guiding principles of the Food and Drug Administration [32] and International Conference on Harmonization (ICH) [33]. This was done following a 5-day validation protocol, and the studied parameters included selectivity, linearity, calibration model, limits, intra- and interday precision and trueness, recovery, and stability.

Selectivity was studied by analyzing blank human plasma specimens from ten different origins. These samples were pooled and separated in 20 aliquots (ten analyzed as blanks and ten spiked with all the analytes), all spiked with the IS. Identification criteria for positivity included an absolute retention time within 2 % or ±0.1 min of the retention

Table 1 GC-MS/MS parameters (quantitation transitions are set in italics)

Time segment (min)	Retention time (min)	Analyte	Molecular weight	Internal standard	Transitions (m/z)	Collision energy (eV)	Dwell time (μs)
9.00	10.77	PRZ (1)	284.13	–	284.7 to *>238.4*	20	34.1
	10.97	PTP (2)	263.17	–	190 to *>189.1*	15	32.9
11.4	11.62	LVPM	328.16	1	328.9 to *>283.2*	20	15.6
					328.9 to >229.1	20	16.3
	11.51	CPZ-d$_3$ (3)	321.10	–	321.3 to *>272*	20	41.9
	11.53	CPZ	318.10	3	271.8 to >237.2	20	11.3
					271.8 to *>257.1*	20	10.0
	11.99	CYA	323.14	2	323.7 to *>277.2*	20	10.8
					323.7 to >100.3	20	12.5
	11.91	OLZ-N-TMS	384.18	2	301.8 to >285.1	15	14.2
					301.8 to *>286.1*	15	14.9
13.4	13.72	CLOZ	326.13	3	325.2 to *>105*	30	16.0
					325.2 to >104.7	30	14.2
14.2	14.43	HAL-2-TMS	519.22	2	296.9 to *>206.2*	10	24.6
					296.9 to >73	10	10.0
16.0	17.96	QTP-O-TMS	455.21	1	320.7 to >251.3	30	23.7
					320.7 to *>210.2*	30	20.8

time of the same analyte in the control sample and the presence of two transitions per compound. The maximum allowed tolerances for the relative ion intensities between the two transitions (as a percentage of the base peak) were as follows. If the relative ion intensity in the control sample was higher than 50 %, then an absolute tolerance of ±10 % was accepted; if this value was between 25 and 50 %, a relative tolerance of ±20 % was allowed; if it was between 5
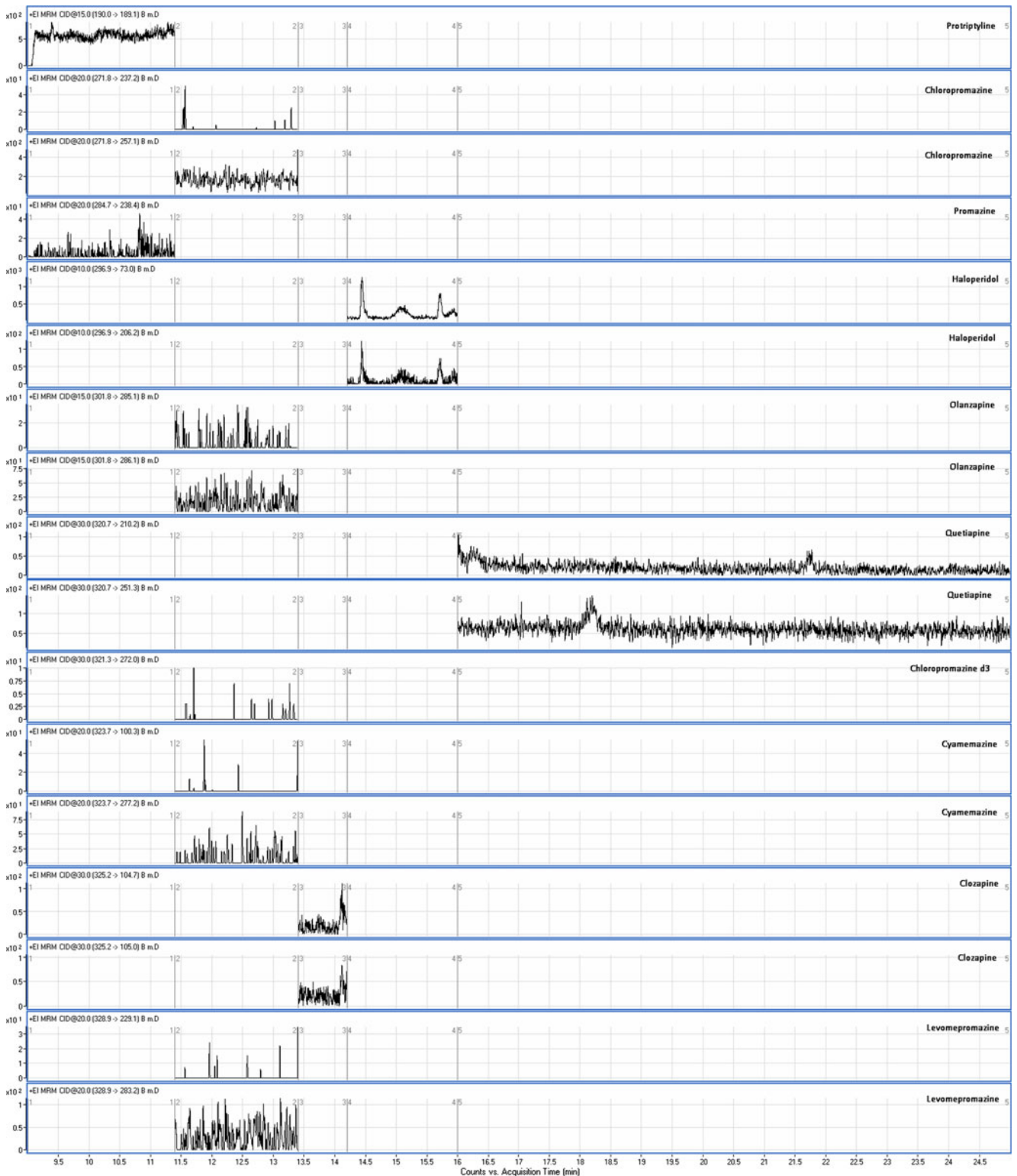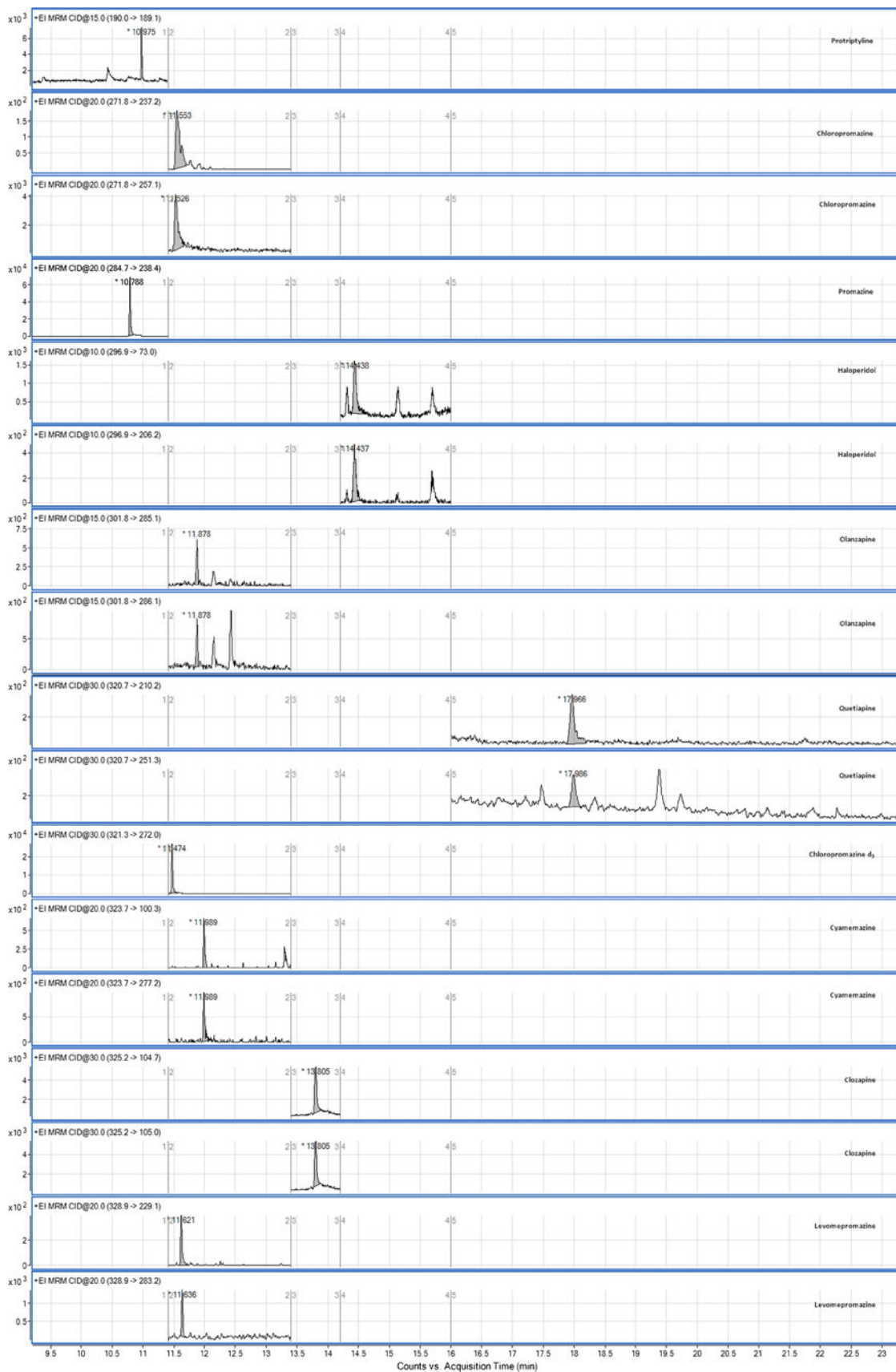

Fig. 1 Chromatogram of a blank sample

Fig. 2 Chromatogram of a spiked sample at the LLOQ for all compounds

and 25 %, an absolute tolerance of ±5 % was accepted and finally, for relative ion intensities of 5 % or less, a relative tolerance of ±50 % was used [34].

To evaluate the method's linearity, human plasma samples spiked at final concentrations ranging from 1 to 1,000 ng/mL for CPZ and CLOZ, 4–1,000 ng/mL for LVMP, QTP, and CYA, 0.8–200 ng/mL for OLZ, and 6–200 ng/mL for HAL were prepared and analyzed by the described procedure (eight calibrators evenly distributed and five replicates). Along with each calibration curve, a zero sample (blank sample with IS) and two quality control samples at medium (10 ng/mL for HAL and OLZ; 50 ng/mL for CPZ, CLOZ, LVMP, CYA, and QTP) and high (HQC, 100 ng/mL for HAL and OLZ; 500 ng/mL for CPZ, CLOZ, LVMP, CYA, and QTP) concentrations ($n=$ 3) were also analyzed. Calibration curves were obtained by plotting the peak–area ratio between each analyte and the IS against analyte concentration. The acceptance criteria included a determination coefficient of at least 0.99 and the calibrators' accuracy within a ±15 % (except at the lower limit of quantitation (LLOQ), where ±20 % was accepted)

The LLOQ was defined as the lowest AP concentration that could be measured precisely (coefficient of variation (CV) of less than 20 %) and accurately (measured concentration within ±20 % of the nominal concentration) and was determined by analyzing five replicates of spiked samples (independent from those of the calibration curve). The limits of detection (LOD) were defined as the lowest tested concentration fulfilling the abovementioned identification criteria which yielded a peak with a signal-to-noise ratio of at least 3 and were determined by analyzing five replicates of spiked samples.

Intra-day precision and trueness were evaluated at five concentration levels (4, 30, 60, 400, and 800 ng/mL for CPZ, CLOZ, LVMP, QTP, and CYA; 6, 12, 40, 80, and 160 ng/mL for HAL; and 0.8, 6, 12, 80, and 160 ng/mL for OLZ) using spiked samples prepared and analyzed as mentioned above (six replicates for each concentration). Interday precision and trueness were evaluated at a minimum of seven concentrations within a 5-day period. In addition, combined intra- and interday

precision was assessed by analysis of the quality control samples analysed in triplicate over the same 5-day period (15 measurements). For recovery studies, blank samples were spiked at three concentrations (50, 200, and 600 ng/mL for CPZ, CLOZ, LVMP, QTP, and CYA; 10, 40, and 120 ng/mL for HAL and OLZ) and were extracted by the described method ($n=3$), after which the IS was added. The obtained peak area ratios were compared with those obtained by spiking blank extracts with the same amount of the analytes and IS (neat standards). In order to study stability in processed samples, blank plasma was spiked with 4, 60, and 800 ng/mL for CPZ, CLOZ, LVMP, QTP, and CYA, 0.8, 12, and 200 ng/mL for OLZ and 6, 12, and 200 ng/mL for HAL, and extracted using the abovementioned procedure ($n=3$). However, after extraction the extract was left standing at room temperature in the autosampler for 24 h. Short-term stability was evaluated at the same concentration levels ($n=3$). Blank samples were spiked and were left at room temperature for 24 h. To study freeze and thaw stability, human plasma samples were spiked at the previously described concentrations, and were stored at −20 °C for 24 h. After this period they were thawed unassisted at room temperature, and then refrozen for 12–24 h under the same conditions. This freeze/thaw cycle was repeated twice more, and the samples were analyzed after the third cycle. During the entire stability procedure the analysed samples were compared with samples prepared and analyzed freshly during the same day.

The carry-over effect, of the MEPS extraction, was investigated by injecting the elution solution after the highest standard concentration and no memory effect was observed.

Results and discussion

Method validation

Selectivity

Using the abovementioned criteria for positivity, all the analytes were successfully and unequivocally identified in

Table 2 Linearity data ($n=5$)

	Weight	Linear range (ng/mL)	Linearity		R^2	LOD (ng/mL)
			Slope	Intercept		
CPZ	$1/x$	1–1,000	0.0482±0.0045	6.3225±0.2894	0.9995±0.0002	0.3
CLOZ	$1/x$	1–1,000	0.0056±0.0002	1.4415±0.8356	0.9990±0.0004	0.6
LVMP	$1/\sqrt{y}$	4–1,000	0.0042±0.0002	0.0045±0.0181	0.9996±0.0001	1
QTP	$1/x$	4–1,000	0.0290±0.0017	−0.0687±0.0372	0.9989±0.0007	1
HAL	$1/\sqrt{y}$	6–200	0.0194±0.0025	−0.0953±0.0144	0.9991±0.0004	0.8
OLZ	$1/x$	0.8–200	0.0397±0.0002	0.0106±0.0117	0.9993±0.0004	0.2
CYA	$1/y$	4–1,000	0.0175±0.0011	−0.0231±0.0200	0.9986±0.0013	1

all the spiked samples, whereas in the blank samples no analyte could be identified. Therefore, the method was considered selective for AP determination in plasma.

Representative ion chromatograms of a spiked (at the LLOQ) and a blank sample are shown in Figs. 1 and 2.

Calibration curves and limits

The method was linear within the adopted calibration ranges for all studied analytes; however, due to the wide calibration range and to compensate for heterocedasticity, weighted least squares regressions had to be adopted. Six weighting factors were evaluated for each analyte ($1/\sqrt{x}$, $1/x$, $1/x^2$, $1/\sqrt{y}$, $1/y$, and $1/y^2$), and the one which originated the best results was selected taking into account the data obtained during the assessment of the interday precision and trueness [35]. These factors were $1/x$ for CPZ, OLZ, CLOZ, and QTP, $1/\sqrt{y}$ for LVMP and HAL, and $1/y$ for CYA. By means of these weighted least squares regressions, linear relationships were obtained, and the calibrators' trueness (mean relative error (bias) between the measured and spiked concentrations) was within a ±15 % interval for all concentrations, except at the LLOQ (±20 %). Calibration data are shown in Table 2. The obtained LLOQs for the analytes were 0.8 ng/mL for OLZ, 1 ng/mL for CPZ and CLOZ, 4 ng/mL for LVMP, QTP and CYA, and 6 ng/mL for HAL. The LODs were 0.6 ng/mL for CLOZ; 0.3 ng/mL for CPZ; 1 ng/mL for QTP, CYA, and LVMP; 0.8 ng/mL for HAL; and 0.2 ng/mL for OLZ. These limits were considered satisfactory, particularly when compared with those obtained by other authors. For instance, Mercolini et al. [5] have obtained higher values (1 ng/mL for HAL, 1.5 ng/mL for CLOZ and QTP, and 2.5 ng/mL for CPZ), yet using HPLC-UV and a higher sample volume. Also using an HPLC-UV instrument, limits of 9 ng/mL for LVMP and of 20 ng/mL for CLOZ were obtained [6]. Other authors [11] utilized a sample volume of 0.9 mL, and analysis was performed by GC-MS, obtaining 3 ng/mL for CLOZ. Higher values were obtained also by de la Torre et al. [10], using GC-NPD and 2 mL of blood.

However, using LC-MS/MS, Saar et al. [2] have obtained similar values for some of the compounds, while for others lower limits were obtained, using 0.1 mL of sample.

Moreover, our limits are adequate for monitoring those compounds routinely since their concentrations in plasma usually fall within the dynamic range of the assay.

Intra- and interday precision and trueness

Regarding intra-day precision and trueness, the obtained CVs were in general lower than 8 % for all the compounds at all tested concentrations, presenting a mean relative error within a

±7 % interval. These results are presented in Table 3. The analysis of the interday precision and trueness yielded CVs generally lower than 11 % for all compounds at all concentration levels, while trueness was within a ±7 % interval. These data are presented in Table 4.) Concerning intermediate precision, the obtained CVs were always lower than 7 % for all analytes, and the relative error was within a ±5 % interval

Table 3 Intra-day precision and trueness ($n=5$)

Compound	Spiked	Measured	CV%	RE%
CPZ	4	4.26±0.15	3.62	6.51
	30	30.52±1.68	5.51	1.73
	60	61.99±1.68	2.71	3.32
	400	386.83±8.05	2.08	−3.29
	800	819.84±7.91	0.96	2.48
CLOZ	4	4.11±0.18	4.52	2.73
	30	31.35±2.24	7.16	4.49
	60	60.15±1.77	2.94	0.24
	400	403.36±6.74	1.67	0.84
	800	812.23±5.30	0.65	1.53
LVMP	4	3.84±0.16	4.20	−4.07
	30	31.19±0.43	1.38	3.98
	60	59.97±0.86	1.43	−0.05
	400	402.29±4.03	1.00	0.57
	800	807.20±8.51	2.30	0.90
QTP	4	4.20±0.09	2.24	5.00
	30	29.01±0.84	2.89	−3.30
	60	57.50±0.66	1.16	−4.17
	400	406.32±7.49	1.84	1.58
	800	798.57±14.67	1.83	−0.18
HAL	6	6.20±0.10	1.74	3.36
	12	12.68±0.22	1.73	5.73
	40	39.09±0.67	1.72	−2.26
	80	82.09±1.25	1.53	2.61
	160	163.03±1.53	0.94	1.90
OLZ	0.8	0.78±0.04	6.04	−1.96
	6	5.80±0.15	2.53	−3.41
	12	11.81±0.28	2.43	−1.62
	80	80.54±1.89	2.35	0.68
	160	161.67±3.85	2.38	1.05
CYA	4	3.93±0.09	2.44	−1.89
	30	29.90±0.90	3.01	−0.36
	60	60.05±2.88	4.80	0.09
	400	393.30±6.57	1.67	−1.68
	800	799.84 ±16.01	2.00	−0.02

All concentrations in nanograms per milliliter

CPZ chlorpromazine, *CLOZ* clozapine, *LVMP* levomepromazine, *QTP* quetiapine, *HAL* haloperidol, *OLZ* olanzapine, *CYA* cyamemazine, *CV* coefficient of variation, *RE* relative error ((measured concentration-spiked concentration/spiked concentration)×100)

Table 4 Inter-day precision and trueness ($n=5$)

Compound	Spiked	Measured	CV%	RE%
CPZ	1	1.07±0.05	5.39	6.85
	4	3.89±0.15	3.82	−2.65
	30	28.87±1.86	6.46	−3.78
	60	59.49±1.81	3.05	−0.85
	200	202.23±3.09	1.53	1.11
	400	398.41±2.14	0.54	−0.40
	600	590.05±1.41	0.24	−1.66
	800	810.99±5.14	0.63	1.37
	1,000	989,19±8.44	0.85	−1.08
CLOZ	1	0.99±0.03	3.84	1.44
	4	4.07±0.33	8.28	0.51
	30	28.45±1.55	5.44	−5.49
	60	60,98±4.12	6.77	2.29
	200	205.75±5.30	2.58	3.18
	400	401.75±7.04	1.75	0.83
	600	589.33±7.77	1.32	−3.47
	800	805.17±9.67	1.20	0.67
	1,000	999.40±9.81	0.98	0.27
LVMP	4	4.22±0.45	10.67	0.78
	30	28.06±1.05	3.77	−6.19
	60	60.49±5.31	8.79	2.81
	200	200.39±3.76	1.88	1.18
	400	402.30±8.55	2.13	−0.84
	600	599.06±6.73	1.12	−0.60
	800	801.16±8.63	1.08	0.21
	1,000	996.84±9.85	0.99	0.19
QTP	4	4.19±0.21	5.11	4.94
	30	29.60±1.57	5.32	−1.34
	60	58.19±4.70	8.09	−3.02
	200	197.98±3.55	1.80	−1.00
	400	392.66±5.12	1.30	−2.48
	600	604.61±8.48	1.40	2.67
	800	813 33±8.87	1.09	2.19
	1,000	986.43±6.96	0.71	−1.95
HAL	6	6.01±0.20	3.45	0.22
	12	12.30±0.68	5.57	2.21
	40	38.04±1.65	4.36	−5.79
	80	82.13±1.27	1.55	2.59
	120	122.11±2.78	2.28	1.69
	160	157.00±2.16	1.38	−1.91
	200	200.67±2.21	1.10	0.33
OLZ	0.8	0.87±0.03	3.74	9.93
	6	5.66±0.31	5.52	−5.68
	12	11.56±0.36	3.08	−3.67
	40	38.57±0.60	1.56	−3.56
	80	81.44±0.73	0.90	1.80
	120	121.89±0.82	0.67	1.57
	160	161.73±4.05	2.51	1.08
	200	197.06±2.70	1.37	−1.47

Table 4 (continued)

Compound	Spiked	Measured	CV%	RE%
CYA	4	4.02±0.31	7.85	0.75
	30	28.69±1.62	5.66	−4.74
	60	104.23±3.32	5.17	6.48
	200	189.24±3.26	1.73	−5.63
	400	397.84±7.63	1.92	−1.51
	600	609.00±7.05	1.16	3.95
	800	798.21±9.16	1.15	−0.48
	1,000	997.81±8.22	0.82	−0.51

All concentrations in nanograms per milliliter

CPZ chlorpromazine, *CLOZ* clozapine, *LVMP* levomepromazine, *QTP* quetiapine, *HAL* haloperidol, *OLZ* olanzapine, *CYA* cyamemazine, *CV* coefficient of variation, *RE* relative error ((measured concentration-spiked concentration/spiked concentration)×100)

from the target concentration. These data are presented in Table 5.

Extraction efficiency

Using the aforementioned approach, the obtained values for efficiency ranged from 62 to 92 % for all analytes, and are presented in Table 6. Vardakou et al. [11] report recovery values of 86 % for CLOZ, while Mercolini et al. [6] have obtained 96 % for LVMP and CLOZ. For CPZ, HAL, CLOZ, and QTP, Mercolini et al. [5] obtained values ranging from 96 to 98 %. Our lower efficiencies may be justified

Table 5 Intermediate precision and trueness ($n=15$)

Compound	Spiked	Measured	CV%	RE%
CPZ	50	51.50±1.13	4.74	3.00
	500	510.37±5.94	3.66	2.07
CLOZ	50	50.71±1.17	6.82	1.42
	500	496.80±4.00	3.61	−0.64
LVMP	50	51.06±0.154	4.92	2.13
	500	506.43±3.08	2.52	1.29
QTP	50	51.31±0.69	3.95	2.64
	500	494.51±2.30	2.29	−1.10
HAL	10	10.34±0.08	2.52	3.40
	100	101.93±1.49	4.15	1.93
OLZ	10	10.22±0.08	4.45	2.18
	100	104.63±0.74	3.58	4.63
CYA	50	50.53±0.83	3.55	1.07
	500	503.28±5.50	2.30	0.66

All concentrations in nanograms per milliliter

CPZ chlorpromazine, *CLOZ* clozapine, *LVMP* levomepromazine, *QTP* quetiapine, *HAL* haloperidol, *OLZ* olanzapine, *CYA* cyamemazine, *CV* coefficient of variation, *RE* relative error ((measured concentration-spiked concentration/spiked concentration)×100)

Table 6 Absolute recovery (*n*=3)

Concentration (ng/mL) analyte	10	50	40	200	120	600
CPZ		81.94±5.50		72.80±0.37		78.56±4.57
CLOZ		84.75±4.72		83.87±5.78		70.34±2.99
LVMP		77.33±5.50		69.42±5.52		84.22±5.34
QTP		80.43±3.93		92.19±1.32		83.85±2.14
CYA		72.67±2.54		84.92±3.76		88.14±6.56
HAL	62.22±3.22		64.92±2.88		68.70±3.03	
OLZ	81.83±2.15		73.02±1.04		84.33±5.09	

Mean values±standard deviation

CPZ chlorpromazine, *CLOZ* clozapine, *LVMP* levomepromazine, *QTP* quetiapine, *HAL* haloperidol, *OLZ* olanzapine, *CYA* cyamemazine

by the fact that a multi-analyte method was desired, and as such the extraction conditions had to be the same for all analytes. Still, the determination of APs in plasma samples by means of MEPS has not been published in the literature yet, and therefore our results cannot be compared with similar methods.

Stability

Concerning processed samples stability, the obtained CVs were lower than 5 % for HAL, CPZ, CLOZ, LVMP, QTP, and CYA, meaning that this analytes are stable in the extracts for at least 24 h at room temperature; the exception was OLZ, which was found to be unstable under the studied conditions (analyte losses of at least 50 % were observed for this particular compound). This is in accordance with previously published reports [2, 36], which have shown that the compound is unstable in blood. This fact was also confirmed in our short-term stability experiments. The obtained CVs concerning short-term stability were less than 5 % for HAL, CPZ, CLOZ, LVMP, QTP, and CYA, meaning that this analytes are stable in the samples for at least 24 h at room temperature, again with the exception of OLZ. In what concerns freeze and thaw samples stability, after comparison of the analyzed samples with freshly prepared ones, we found that HAL, CPZ, CLOZ, LVMP, QTP, and CYA were
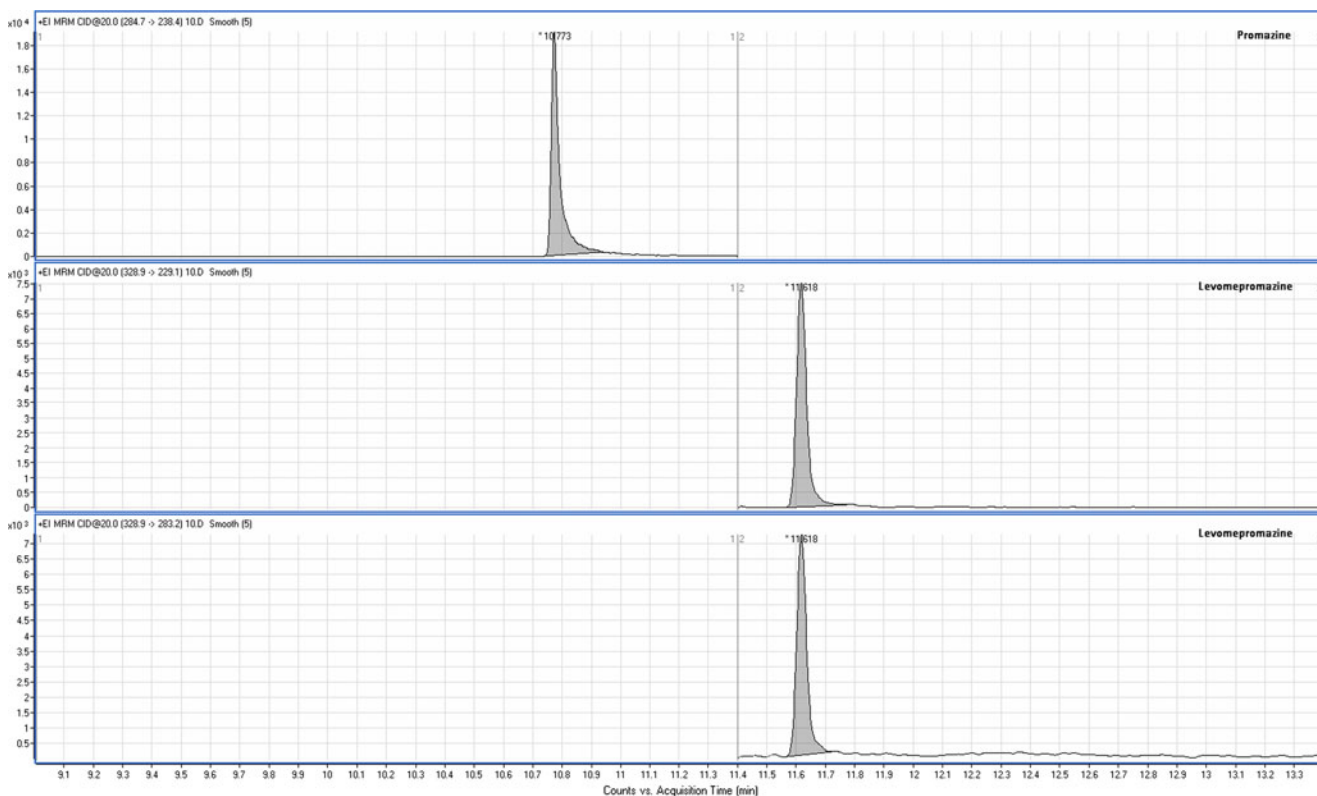

Fig. 3 Chromatogram obtained by analysis of an authentic sample positive for LVMP (7.8±0.02 ng/mL)

stable for at least three freeze/thaw cycles, with the exception of OLZ, which was found to be unstable under those conditions.

Method applicability

The herein described procedure is being routinely applied for AP monitoring in psychiatric patients (from the Psychiatry Department of the local Hospital Centro Hospitalar Cova da Beira, E.P.E., Covilhã, Portugal). Figure 3 shows the obtained chromatogram from one of these samples (positive for LVMP, with a concentration of 7.8±0.02 ng/mL (n=3)).

Conclusions

A simple and sensitive procedure employing MEPS-GC-MS/MS was developed and fully validated for the qualitative and quantitative determination of AP drugs in human plasma samples. The used sample volume was as low as 0.25 mL; this provides a significant advantage, particularly when there is little sample availability, enabling further exams to be performed on the same sample.

Method selectivity, linearity, intra- and interday precision and trueness, limits, and extraction efficiency were considered adequate. The fact that low detection and quantitation limits were obtained in reduced sample volumes enables the detection of small amounts of the compounds, which makes this procedure useful for those laboratories performing routine plasma analysis. The presented method assay has been found to be applicable for clinical toxicological casework; this method may also find some applicability in forensic scenarios, mainly in overdose cases since the obtained sensitivity allow for adequate sample dilution prior to MEPS extraction.

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