Journal of Chromatography B, 1038 (2016) 127–135

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of antipsychotic drugs in hospital and wastewater treatment plant samples by gas chromatography/tandem mass spectrometry☆

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ARTICLE INFO

Article history:
Received 29 May 2016
Received in revised form 12 October 2016
Accepted 23 October 2016
Available online 24 October 2016

Keywords:
Antipsychotics
Solid phase extraction
Gas chromatography–tandem mass spectrometry
Wastewaters samples

ABSTRACT

The development and performance evaluation of a method for the simultaneous determination of six antipsychotic drugs in hospital effluents and wastewater treatment plants (WWTP) samples are herein presented. The method involves an off-line mixed mode (reversed-phase and strong cation exchange) solid phase extraction (SPE) with gas chromatography (GC) coupled to tandem mass spectrometry (MS/MS). The present methodology was validated following internationally accepted criteria, and the studied parameters included selectivity, linearity, limits of detection (LOD) and quantitation (LLOQ), instrumental limits, precision and accuracy, stability and recovery. The procedure was linear for concentrations ranging from 0.1 to 10 μg/L (0.02 to 2 μg/L for haloperidol), with determination coefficients higher than 0.99 for all analytes. Intra- and inter-day precision was lower than 15% for all analytes at the studied concentrations, while accuracy remained between a ±15% interval. Recoveries ranged from 31% to 83%. Low LODs were achieved, between 2 and 10 ng/L, allowing a reliable identification of all analytes at trace levels, using only 50 mL as sample volume. All studied parameters complied with the defined criteria and the method was successfully applied to gather preliminary results of the determination of antipsychotics on hospital effluents and on influent and effluent of WWTPs, opening perspectives for the study of their fate in the aquatic environment.

1. Introduction

Pharmaceuticals are considered a class of new, so called "emerging" contaminants that have raised great concern in the last years [1]. They are continuously being released in the environment mainly due to insufficient removal in wastewater treatment plants (WWTPs) (70–80%), whereas the remaining 20–30% is due to other sources of pollution, such as livestock and industrial wastes, hospital effluents and disposal of unused or expired pharmaceuticals [2].

As a result, the amount of pharmaceuticals and their bioactive metabolites being introduced into the environment is increasingly high, which leads to harmful consequences due to the their recognized (eco)toxicity, as well as unpredictable environmental impact. Nowadays, a large diversity of pharmaceuticals has been found in the environment, including classes such as anti-inflammatory drugs, analgesics, antibiotics, antiepileptics, β-blockers, lipid regulators, antidepressants, anxiolytics, sedatives, contraceptives, etc. [3].

Regarding the psychiatric drugs, anxiolytics, sedatives, hypnotics and antidepressant groups have been determined in wastewaters and aqueous environmental matrices [4]. Surprisingly, the antipsychotics (APs) group has not received practically any research attention. Furthermore, in a recent study of psychiatric drugs use in Portugal, it was verified an increase of the consumption of these drugs in the period 2000–2012, and particularly expres-

☆ Selected paper from the XVI Latin-American Congress on Chromatography (XVICOLACRO) and the 9th National Meeting on Chromatography (9ENC), 5th–9th January 2016, Lisbon, Portugal.

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http://dx.doi.org/10.1016/j.jchromb.2016.10.031
1570-0232/

sive for the group of APs (+171%) [5]. This rising number could be explained by the increase of the prevalence of psychosis disturbs, the lengthening of the duration of treatment, the broadening of licensed therapeutic indications obtained for second-generation APs and the rising proportion of off-label prescriptions of these drugs [6].

Schizophrenia is a debilitating and emotionally devastating illness with long-term impact on patients' lives. Many experts consider schizophrenia to be the most severe expression of psychopathology, encompassing significant disruptions of thinking, perception, emotion, and behavior. Schizophrenia is usually a lifelong psychiatric disability [7]. APs drugs are the elective treatment and they have been broadly classified into two groups. The older agents are referred to as typical or conventional APs or dopamine receptor antagonists, with pharmacologic activity attributed to the blockade of central dopamine receptors, particularly the D_2 receptor subtype. Examples of typical agents include haloperidol (HAL), fluphenazine, thiothixene, chlorpromazine (CPZ), cyamemazine (CYA), levomepromazine (LVMP) and thioridazine [8]. Newer agents, commonly referred to as atypical, serotonin-dopamine antagonists, or second-generation APs, consist of clozapine (CLZ), risperidone, olanzapine, quetiapine (QTP), ziprasidone, aripiprazole, paliperidone, iloperidone, asenapine, and lurasidone, which have demonstrated postsynaptic effects at 5-HT_{2A} and D_2 receptors [9,10]. This new generation of APs largely overcame the extrapyramidal side-effects via decreased activity at dopamine receptors compared with their traditional counterparts [11]. They are the agents of first choice in treatment of schizophrenia and evidence supports that they have superior efficacy for treatment of negative symptoms, cognition, and mood [12].

Nowadays, in our country since 2000 it was verified that the use of first-generation APs remained practically unchanged, and there has been an effective increase in the use of second-generation APs, with QTP being the drug with the highest rate of use [5].

In general, APs are administered at relatively low daily dosages and they are widely metabolized in the body. In consequence, the concentration of these drugs in human specimens is very low, and it exhibits inter-individual variations, which suggests the need for clinical monitoring of patients undergoing therapy, in addition to minimize the side effects [13]. In this regard, AP drugs have been determined in biological matrices by numerous methods, such as gas chromatography (GC) coupled to either nitrogen-phosphorus detector [14,15], mass spectrometric (MS) detection [16] or tandem mass spectrometry (MS/MS) [17], high-performance liquid chromatography (LC) coupled to UV or diode array [18–20], coulometric [21], chemiluminescence [22], MS [23] or MS/MS detectors [24–27], capillary electrophoresis with electrochemiluminescence [28] or UV detection [29], and electrochemical methods [30,31].

Concerning the environment fate, to the best of our knowledge, there are few reports that refer the determination of APs in aqueous matrices in multi-residue analysis, all using LC–MS/MS, namely risperidone in the United States [32], olanzapine in Serbia [33], CPZ, CLZ, olanzapine and risperidone in Greece [34], risperidone and haloperidol in Europe [35], and a total of nine APs (CPZ, olanzapine, CLZ, risperidone, sulpiride, QTP, ziprasidone, aripiprazole and perphenazine) in China [36].

Therefore, the main goal of this work is concerned with the development, optimization and validation of an analytical methodology for the specific and sensitive determination of different antipsychotic drugs (HAL, CPZ, CYA, LVP, CLZ, and QTP). These compounds were selected based on prescription and consumption rates in our country [37]. The methodology used here was based on solid-phase extraction (SPE) using polymeric Strata X-C cartridges, a fast microwave-assisted derivatization and GC–MS/MS. The matrices used in this work are hospital wastewaters and samples and it constitutes a first approach to assess the potential contribution of this group of psychotropic drugs to the pharmaceutical load of WWTPs and therefore it is intended to determine the occurrence and fate of these drugs for a better understanding of the potential environmental implications.

2. Experimental

2.1. Reagents and standards

Standard methanolic solutions of haloperidol (HAL), clozapine (CLZ), chlorpromazine (CPZ) were acquired from LGC Promochem (Barcelona, Spain) at the concentration of 1 mg/mL. Promazine (PRZ) (IS), levomepromazine (LVP) and cyamemazine (CYA) were acquired from Sigma-Aldrich (Lisbon, Portugal). Quetiapine (QTP; 98% purity) was kindly donated by AstraZeneca PLC (London, UK). It should be pointed that PRZ is not commercially available as therapeutic drug in Portugal, and therefore its appearance in an authentic sample, impairing quantitative analysis, is highly unlikely to occur. Furthermore, this compound's chemical structure is similar to that of studied compounds', allowing improving linearity, precision and accuracy, while minimizing analyte losses during the sample preparation process. Methanol (Merck Co, Darmstadt, Germany), hydrochloric acid (HCl) (Panreac, Barcelona, Spain) and ammonium hydroxide (J.T. Baker, Lisbon, Portugal) were of HPLC grade. Potassium dihydrogen phosphate (KH_2PO_4) was acquired from Panreac (Barcelona, Spain), N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and trimethylchlorosilane (TMCS) were purchased from Macherey-Nagel (Düren, Germany). Ultrapure water was obtained by a Milli-Q System (Millipore, Billerica, MA, USA). Phenomenex Strata™-X-C extraction cartridges (200 mg) were obtained from Tecnocroma (Caldas da Rainha, Portugal).

Working standard solutions were prepared by properly diluting the stock solutions with methanol to final concentrations of 0.1, 1 and 10 μg/L of all analytes, except HAL (0.02, 0.2 and 2 μg/L). The internal standard working solution was prepared in methanol to a concentration of 10 μg/mL. All working and stock solutions were stored in the absence of light at 4 °C.

2.2. Sample collection and pre-treatment

The samples used in this work were wastewaters from a hospital and WWTP in the urban region of Porto, Portugal. All samples were collected in amber glass bottles, previously washed with detergent and then rinsed thoroughly with Milli-Q water. Every sample was collected in duplicate (about 0.5 L). The hospital wastewaters were collected at two distinct sampling points: at the effluent of the internment services and at different sites of the final effluent of the hospital WWTP. Influent and effluent wastewater samples were collected from one WWTP network. The samples were then brought to the laboratory in ice-packed containers. Upon arrival, all samples were immediately vacuum-filtered through a 1-μm glass microfibre filter (Type A/E Glass Fiber Filters, Pall Corporation) and then through a 0.45-μm mixed cellulose ester filter (GN-6 Metricel® MCE Membrane Disc Filters, Pall Corporation). Finally, they were stored at 4 °C until analysis.

2.3. Gas chromatography and mass spectrometry conditions

Chromatographic analysis was done using an HP 7890A gas chromatography system equipped with a model 7000 B triple quadrupole mass spectrometer, both from Agilent Technologies (Waldbronn, Germany), a MPS2 auto sampler 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,

Table 1
Retention time and GC–MS/MS parameters (quantitation transitions underlined).

Analyte	Molecular weight (g/mol)	Retention time (minutes)	Transitions (*m*/*z*)	Collision energy (eV)	Dwell time (μs)
PRZ	284.13	10.95	179.2 –> 179.2	5	50
CPZ	318.10	11.73	271.8 –> 257.1	20	19.1
			318.5 –> 233.2	20	10.3
LVP	328.16	11.83	242.0 –> 210.2	20	20.4
			242.0 –> 227.1	20	15.0
CYA	323.14	12.21	323.7 –> 277.2	20	30.8
			323.7 –> 100.3	20	20.4
CLZ	326.13	14.02	255.6 –> 192.1	20	30.6
			255.6 –> 239.0	20	19.9
HAL	375.14	14.69	296.4 –> 296.3	10	10.0
			296.4 –> 103.2	10	50.1
QTP	455.21	18.56	320.7 –> 210.2	30	79.1
			208.8 –> 139.0	30	79.1

CA, USA), was used for the chromatographic resolution of the pharmaceutical substances.

The initial oven temperature was held at 120 °C for 2 min, then raised to 300 °C at 20 °C/min (held for 14 min), giving a total run time of 25 min. The temperatures of the injection port and the ion source were set at 250 °C and 280 °C, respectively. The sample was introduced into the gas chromatograph by splitless injection mode and the flow of helium (carrier gas) was 0.8 mL/min at a constant flow rate.

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 was acquired in the multiple reaction monitoring (MRM) mode using the MassHunter WorkStation Acquisition Software rev. B.02.01 (Agilent Technologies).

Initially, the retention times and mass spectra were obtained by individually injecting derivatized standard solutions at a high concentration (5 μg/mL). Both retention times and mass-to-charge ratio (*m*/*z*) were used for compound identification. Two transitions were chosen for each compound (one quantitative and one qualitative), and the selection was performed in such manner in order to obtain better selectivity and sensitivity for the analytes and fewer matrix interferences. The choice of the ions was based on the higher masses and more abundant mass peaks (especially the most specific masses for each compound) to maximize signal-to-noise ratio in matrix extracts. Tandem mass spectrometry conditions were optimized by injecting a standard solution at different collision energies and dwell times. The transitions were chosen based on the best selectivity and abundance, in order to maximize signal-to-noise ratio in matrix extracts. The most abundant transition was used for quantitation, whereas the second transition was for confirmation. Table 1 resumes the precursor, product ions, collision energies, retention times and dwell time selected for each compound.

2.4. Sample preparation

For APs extraction, the following procedure was used: a 50 mL of water sample was fortified with 25 μL of the IS solution. After stirred-mixed for 15 min, the mixture was loaded onto the SPE cartridges previously conditioned with 2 mL of methanol and 2 mL of acidified ultrapure water (pH 5). Interferences were removed from the sorbent with 2 mL of 0.1 M HCl in water and 2 mL of 0.1 M HCl in methanol. After washing, the cartridges were dried under full vacuum for 15 min to remove all traces of water. Finally, the retained analytes were eluted with 2 mL of 5% ammonia in methanol. The elution solvent was evaporated to dryness under a gentle stream of N_2 and the dry extracts were derivatized using 65 μL of MSTFA/TMCS in a microwave reactor at 800 W during 2 min. After cooling down to room temperature, these extracts were transferred to autosampler vials and a 2 μL aliquot of the resulting solution was then injected in the GC/MS–MS system.

3. Results and discussion

3.1. Derivatization procedure

For the derivatization procedure, a domestic digital microwave oven with a nominal power of 800 W, was used in this study. A comparison was made with a conventional heating block at 85 °C during 45 min. For this purpose, two lots of samples with all the analytes at concentration of 1 and 10 μg/L (0.2 and 2 μg/L from HAL) were prepared. All the samples were extracted by the aforementioned method and derivatization was made using both heating procedures to compare their ability. Similar results were obtained for both sets of experiments (Fig. 1). After application of F-Test and t-Test with a significance level of 0.05 it was verified that both derivatization procedures are comparable. This score represents a major improvement when compared to the classic derivatization process, namely in the reduction of the analysis time.

3.2. Validation procedure

The described method was fully validated according to the guiding principles of the Food and Drug Administration (FDA) [38] and of the International Conference on Harmonization (ICH) [39].The validation was performed following a 5-day validation protocol and included selectivity, linearity and limits, intra- and inter-day precision and accuracy, recovery and stability.

3.2.1. Selectivity

The method's selectivity was evaluated by analyzing blank wastewater samples of ten different origins, to investigate the potential interferences at the retention times and selected transitions of the studied compounds. Samples were pooled and separated in 20 aliquots (ten analyzed as blanks and ten spiked with all the analytes), all spiked with the IS. Quality controls (QC) samples were prepared and analyzed simultaneously. Identification criteria for positivity included an absolute retention time within 2% or ±0.1 min of the retention time of the same analyte in the control sample and the presence of two transitions per compound. To guarantee a suitable confidence in identification, 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 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 [40].

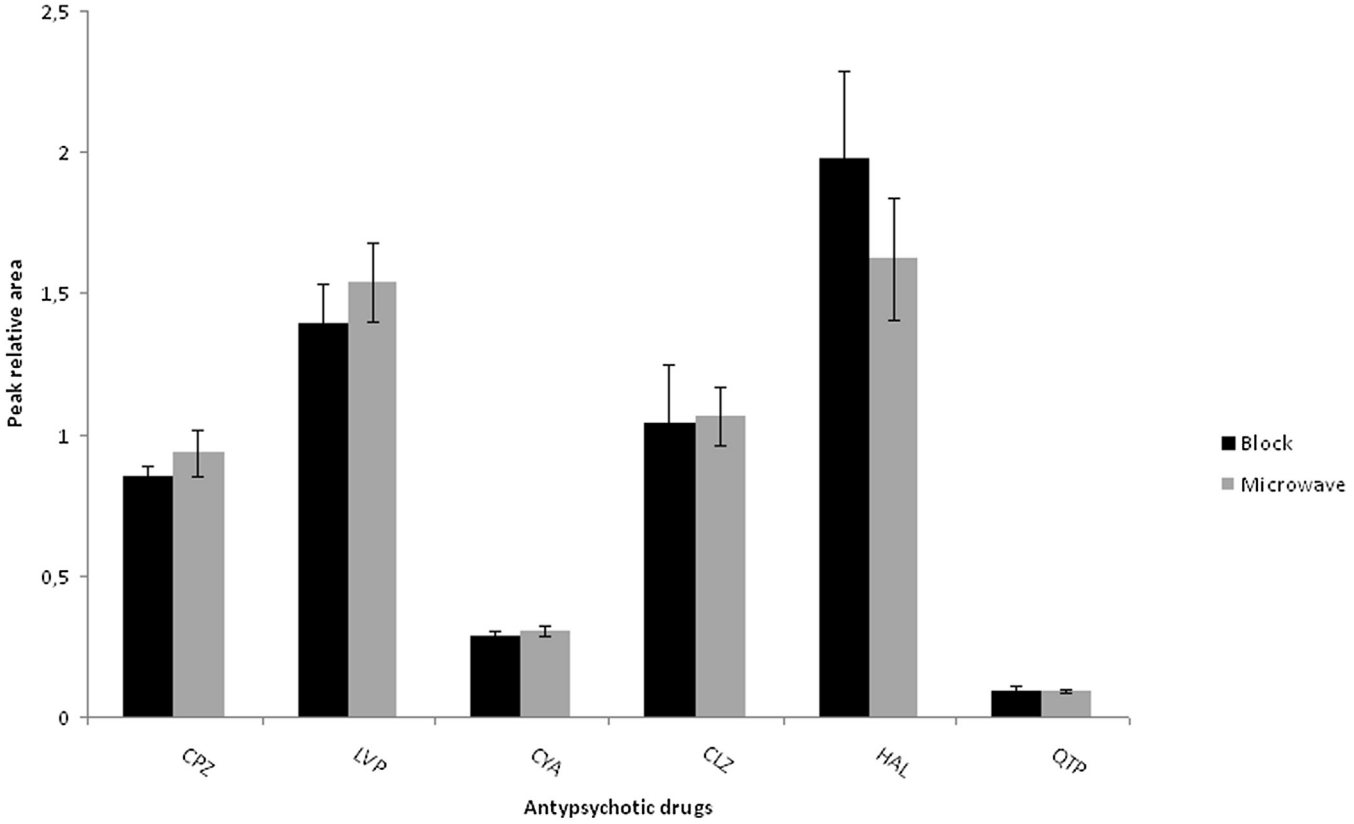

Fig. 1. Comparison between classical derivatization using a thermal block and the use of microwave-assisted derivatization.

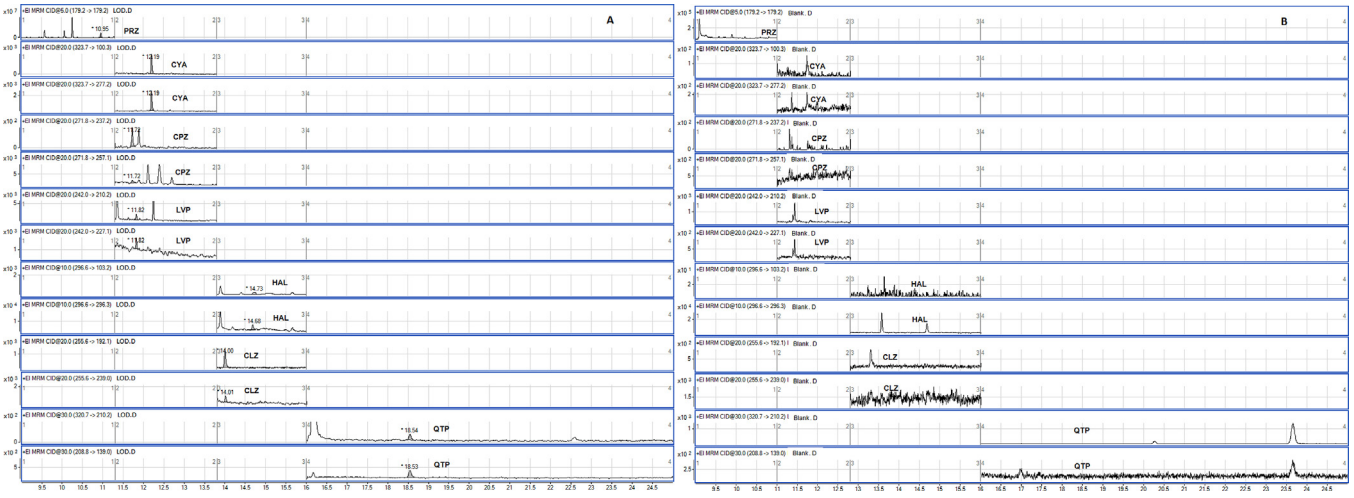

Fig. 2. Ion chromatogram of a spiked sample at the LOD (A) and a blank (B) sample.

Using the above mentioned criteria for positivity, all the analytes were successfully and unequivocally identified in all the spiked samples, whereas in the blank samples no interfering peaks could be detected and/or misidentified as being the analyte. Therefore, the method was considered selective for the selected antipsychotics in wastewater samples. Representative ion chromatograms of a spiked (at the LOD) and a blank sample are shown in Fig. 2.

3.2.2. Calibration model and limits

Linearity of the method was established on spiked samples prepared and analyzed using the described extraction procedure (five replicates) in the range of 0.1–10 μg/L for all compounds except for HAL (0.02–2 μg/L). 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 (R^2) value of at least 0.99 and the calibrators' accuracy within ± 15% (except at the lower limit of quantification (LLOQ), where ± 20% was considered acceptable). The method was linear within the adopted calibration ranges for all analytes; however, since the adopted calibration ranges were wide and in order to compensate for heterocedasticity, weighted least squares regressions had to be adopted. Six weighting factors were evaluated for each analyte ($1/\sqrt{x}$, $1/x$, $1/x2$, $1/\sqrt{y}$, $1/y$, $1/y2$), and the one for which the sum of relative errors (data obtained during the assessment of

Table 2
Linearity data (n = 5).

Analyte	Weight	Linear range (μg/L)	Linearity		R^{2*}	LLOQ (μg/L)	LOD (ng/L)	IQL (μg/L)	IDL (ng/L)
			Slope[a]	Intercept[a]					
CPZ	1/x	0.1–10	0.055 ± 0.016	−0.013 ± 0.0013	0.995 ± 0.001	0.1	10	0.02	2
LVP	1/x	0.1–10	0.066 ± 0.016	0.002 ± 0.0004	0.994 ± 0.003	0.1	10	0.04	4
CYA	1/x	0.1–10	0.029 ± 0.006	−0.001 ± 0.0001	0.993 ± 0.003	0.1	10	0.03	3
CLZ	1/x	0.1–10	0.009 ± 0.003	0.006 ± 0.001	0.993 ± 0.004	0.1	10	0.03	3
HAL	1/x	0.02–2	0.048 ± 0.009	−0.001 ± 0.003	0.994 ± 0.004	0.02	2	0.008	0.8
QTP	1/x	0.1–10	0.003 ± 0.001	0.002 ± 0.001	0.993 ± 0.001	0.1	10	0.03	3

[a] Mean values ± standard deviation.

inter-day precision and accuracy) was the lowest, presenting simultaneously a mean R^2 value of at least 0.99 was chosen (Table 2). This factor was 1/x for all analytes.

By means of these weighted least squares regressions, linear relationships were obtained, and the calibrators' accuracy (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.

Together with each calibration curve, a zero sample (blank sample with IS) and three quality control (QC) samples at low [LQC: 0.8 μg/L; 0.16 μg/L (HAL)], medium [MQC: 3 μg/L; 0.6 μg/L (HAL)] and high [HQC: 6 μg/L; 1.2 μg/L (HAL)] concentrations (n = 3) were also analyzed.

The LLOQ was defined as the lowest concentration that could be measured with adequate precision and accuracy, i.e. with a coefficient of variation (CV, %) less than 20% and a relative error (RE, %) within ± 20% of the nominal concentration. The method's limits of detection (LOD) were determined as the lowest concentrations that showed a discrete peak clearly distinguishable from the blank with a signal-to-noise ratio of at least 3, in which the analytes could be unequivocally identified, and were determined by analyzing six replicates of spiked samples.

The LLOQs were 0.1 μg/L for all antipsychotics except HAL (0.02 μg/L). The LODs were 10 ng/L and 2 ng/L for (HAL). These limits were considered satisfactory, especially when compared to those obtained by other authors. As it can be observed, the LODs obtained by our method were comparable to those reported by Borova et al. [34]. Indeed, these authors report LODs between 2.5–4.9 ng/L for clozapine and chlorpromazine by LC/MS/MS. Furthermore, and to the best of our knowledge, GC–MS/MS has not been used yet for the remaining antipsychotics determination in this kind of sample. Additionally, in this work, even though the derivatization procedure may be a limiting step when comparing liquid chromatography (LC) and GC methods, we consider that the GC technique (coupled to tandem MS) exhibits as principal advantage the fact that it is less susceptible to matrix effects, that requires special attention when it is used a LC technique and even more when dealing with high complexity samples as the wastewater samples.

Instrumental detection limits (IDL) and instrumental quantification limits (IQL) were calculated using signal-to-noise approach in standards prepared in the reagent of derivatization as the concentrations of each compound corresponding to signal-to-noise ratio of 3:1 and 10:1, respectively. These values are resumed in Table 2.

3.2.3. Intra- and inter-day precision and accuracy

Precision was expressed in terms of coefficient of variation (CV, %) and calculated by the one-way analysis of variation (ANOVA).

Intra-day precision was evaluated by analyzing in the same day 5 replicates of blank samples spiked with the studied analytes at 4 concentration levels (0.5, 1, 5 and 10 μg/L) and (0.1, 0.2, 1, and 2 μg/L for HAL). Inter-day precision was evaluated at a minimum of six concentration levels within a 5-day period. The accuracy of the method was characterized in terms of the mean RE between the measured and the spiked concentrations; the accepted limit was ±15% for all concentrations, except at the LLOQ, where ±20% was accepted.

The obtained values for intra-day precision (CV < 12.97%) and bias (±14.34%), as well as to the in inter-day precision (CV < 11.46%) and bias (±12.75%) were acceptable at all concentrations. These results are summarised in Table 3.

Also, intermediate precision (combined intra- and interday) was evaluated using the QC samples prepared and analyzed simultaneously with the calibration curves on 5 different days (15 measurements for each concentration) [38]. The CVs were typically below 13% for all compounds at all concentrations, while accuracy was within ±12% of the nominal concentration (Table 3).

3.2.4. Extraction recovery

For recovery studies, blank samples were spiked with both analytes at three concentrations (0.5, 1 and 10 μg/L) and (0.1, 0.2, and 2 μg/L for HAL) and analyzed by the described method (n = 3), after which the IS was added. At the same time, blank extracts were spiked with the same quantity of the analytes and IS after elution. The obtained peak area ratios were compared between extracted and non-extracted samples (the latter were used as neat standards). Using the aforementioned approach, the absolute recovery values ranged from 31 to 83% for the studied analytes (Table 4).

These recovery values were found adequate, despite of the fact that they were not so high in absolute value. Yet, low limits of detection and quantification were obtained, meaning that the method is sensitive enough to detect low levels of the compounds. Our values are lower than those obtained by other authors, namely concerning CPZ and CLZ [34,36]. However, Borova *et. al* [34] have calculated recoveries using authentic samples, while in our case spiked samples were used, since authentic samples positive for all studied compounds were not available.

3.2.5. Stability

In order to study short-term stability, blank samples were spiked at the above-mentioned LQC, MQC and HQC levels and left at room temperature for 24 h [38]. During the entire stability procedure the analyzed samples were compared with samples freshly prepared and analyzed in the same day (both sets of samples were quantified in the same calibration curve, the obtained concentrations were compared and the respective RE was calculated relatively to the theoretical concentrations; the CVs between the two sets of samples were calculated as well). The analytes were considered stable if both the CV between the two sets of samples and the calculated REs were in accordance with the above-mentioned criteria for precision and accuracy (CVs below 15% and REs within a ±15% interval) [38] (Table 5).

Table 3
Intra- interday and intermediate precision and accuracy.

Analyte	Intraday precision and accuracy (n = 5)				Interday precision and accuracy (n = 5)				Intermediate precision and accuracy (n = 15)			
	Concentration (μg/L)	Measured Concentration (μg/L)[a]	CV (%)	RE (%)	Concentration (μg/L)	Measured Concentration (μg/L)[a]	CV (%)	RE (%)	Concentration (μg/L)	Measured Concentration (μg/L)[a]	CV (%)	RE (%)
CPZ					0.1	0.10 ± 0.00	1.01	−4.49				
	0.5	0.57 ± 0.00	0.33	14.34	0.5	0.55 ± 0.02	4.44	9.48	0.8	0.83 ± 0.03	3.80	4.15
	1	0.96 ± 0.12	12.97	−3.02	1	1.01 ± 0.07	7.07	1.45	3	2.84 ± 0.22	7.92	−5.39
					2	1.75 ± 0.04	2.09	−12.75				
	5	4.46 ± 0.19	4.31	−10.36	5	5.26 ± 0.09	1.70	5.16	6	5.68 ± 0.44	7.71	−5.37
					8	7.75 ± 0.06	0.80	−3.10				
	10	9.58 ± 0.15	1.57	4.52	10	10.12 ± 0.09	0.90	1.23		0.83 ± 0.03	3.80	4.15
LVP					0.1	0.10 ± 0.01	9.59	−2.52				
	0.5	0.55 ± 0.01	2.23	9.74	0.5	0.54 ± 0.01	1.19	7.47	0.8	0.89 ± 0.02	2.53	11.43
	1	1.11 ± 0.11	9.90	11.95	1	1.03 ± 0.08	7.57	3.07	3	3.01 ± 0.18	5.85	1.59
					2	1.82 ± 0.01	0.28	−8.99				
	5	4.42 ± 0.18	4.17	−10.83	5	5.14 ± 0.13	2.55	2.72	6	6.16 ± 0.67	10.89	1.49
					8	7.89 ± 0.56	7.04	−1.32				
	10	9.55 ± 1.18	12.37	2.21	10	10.19 ± 0.66	6.47	1.92				
CYA					0.1	0.10 ± 0.01	11.04	1.13				
	0.5	0.52 ± 0.03	5.66	4.68	0.5	0.50 ± 0.01	2.70	−0.32	0.8	0.79 ± 0.04	5.36	−0.72
	1	0.98 ± 0.08	8.50	1.16	1	1.00 ± 0.02	1.76	−0.46	3	2.79 ± 0.16	5.89	−6.87
					2	1.79 ± 0.11	6.17	−10.40				
	5	4.82 ± 0.37	7.64	−3.60	5	5.23 ± 0.19	3.65	4.64	6	5.90 ± 0.16	2.63	−1.68
					8	7.85 ± 0.35	4.43	−1.81				
	10	9.73 ± 1.02	10.45	−2.70	10	10.03 ± 0.47	4.66	0.31				
CLZ					0.1	0.11 ± 0.00	1.92	6.93				
	0.5	0.50 ± 0.05	9.51	−2.82	0.5	0.50 ± 0.01	1.89	0.87	0.8	0.76 ± 0.04	4.94	−4.85
	1	1.05± 0.03	4.75	5.02	1	1.00 ± 0.08	8.10	−0.36	3	3.21 ± 0.28	8.68	6.93
					2	1.86 ± 0.08	4.51	−6.90				
	5	5.63 ± 0.02	0.35	11.78	5	4.97 ± 0.19	3.91	−0.54	6	6.00 ± 0.35	5.81	0.03
					8	7.89 ± 0.50	6.31	−1.33				
	10	10.71± 0.02	3.70	7.10	10	10.46 ± 0.45	4.28	4.65		0.76 ± 0.04	4.94	−4.85
HAL					0.02	0.02 ± 0.00	0.83	7.54				
	0.1	0.11 ± 0.06	13.2	10.15	0.1	0.10 ± 0.01	6.27	−0.40	0.16	0.16 ± 0.05	6.38	2.80
	0.2	0.22 ± 0.03	11.9	12.40	0.2	0.19 ± 0.01	4.71	−7.22	0.6	0.59 ± 0.38	12.96	−1.45
					0.4	0.37 ± 0.02	4.34	−6.33				
	1	1.69 ± 0.08	1.42	13.46	1	1.03 ± 0.01	1.39	2.67	1.2	1.23 ± 0.46	7.52	2.61
					1.6	1.59 ± 0.03	1.67	−0.59				
	2	2.75 ± 0.90	8.39	7.49	2	2.02 ± 0.00	0.11	0.97				
QTP					0.1	0.10 ± 0.01	5.59	0.07				
	0.5	0.47 ± 0.05	10.29	−5.03	0.5	0.49 ± 0.02	5.02	−2.92	0.8	0.77 ± 0.06	7.51	−3.65
	1	1.01 ± 0.03	3.15	5.11	1	1.03 ± 0.12	11.46	2.81	3	2.67 ± 0.19	7.16	−7.71
					2	1.79 ± 0.06	3.57	−10.66				
	5	5.61 ± 0.16	2.81	12.18	5	4.84 ± 0.33	6.77	−3.26	6	5.36 ± 0.42	7.86	−8.53
					8	8.08 ± 0.12	1.43	0.96				
	10	11.40 ± 0.01	0.08	13.97	10	10.25 ± 0.20	1.94	2.51				

CV—Coefficient of variation; RE—Relative error (measured concentration-spiked concentration/spiked concentration)×100.
[a] Mean values ± standard deviation.

Table 4
Absolute recovery (n = 3).

Analyte	Concentration (μg/L)	Recovery[a] (%)
CPZ	0.5	56.7 ± 3.8
	1	45.3 ± 6.7
	10	43.2 ± 5.4
LVP	0.5	45.0 ± 2.7
	1	34.1 ± 1.4
	10	30.5 ± 4.6
CYA	0.5	62.6 ± 5.1
	1	67.5 ± 3.8
	10	64.6 ± 1.2
CLZ	0.5	66.5 ± 11.7
	1	46.2 ± 1.1
	10	56.3 ± 7.0
HAL	0.1	83.1 ± 1.7
	0.2	75.8 ± 7.5
	2	78.9 ± 6.1
QTP	0.5	83.3 ± 6.7
	1	72.6 ± 6.6
	10	81.3 ± 4.2

[a] Mean values ± standard deviation.

Table 5
Short-term stability (n = 3).

Analyte	Concentration (μg/L)	Measured Concentration (μg/L)[a]	Measured Concentration fresh samples (μg/L)[a]
CPZ	0.8	0.80 ± 0.06	0.85 ± 0.02
	3	2.65 ± 0.06	2.59 ± 0.10
	6	6.18 ± 0.72	5.36 ± 0.07
LVP	0.8	0.82 ± 0.11	0.91 ± 0.01
	3	2.70 ± 0.15	2.58 ± 0.14
	6	5.37 ± 0.08	5.18 ± 0.11
CYA	0.8	0.78 ± 0.04	0.75 ± 0.03
	3	2.56 ± 0.09	2.58 ± 0.47
	6	6.11 ± 0.04	5.48 ± 0.31
CLZ	0.8	0.77 ± 0.12	0.85 ± 0.06
	3	2.68 ± 0.07	2.92 ± 0.12
	6	6.15 ± 0.62	5.73 ± 0.60
HAL	0.16	0.15 ± 0.09	0.16 ± 0.01
	0.6	0.50 ± 0.12	0.68 ± 0.00
	1.2	1.13 ± 0.30	1.22 ± 0.09
QTP	0.8	0.67 ± 0.07	0.70 ± 0.06
	3	2.63 ± 0.18	2.66 ± 0.54
	6	5.51 ± 0.12	5.25 ± 0.01

[a] Mean values ± standard deviation.

3.3. Identification and quantification of antipsychotics in hospital wastewater and WWTP samples

The presented analytical method was developed with the ultimate purpose of accurately measure a representative list of the available antipsychotics on effluents from an urban hospital and on influent and effluent wastewaters from WWTP.

The results obtained are summarised in Table 6, and an elucidative chromatogram of a sample is presented in Fig. 3.

As expected, the higher number of antipsychotics was found at the hospital effluent of the internment services, namely CPZ (<LLOQ) CYA, CLZ, LVP, probably due from their use in the management of emergency episodes. Regarding the final effluent of the hospital WWTP, in different sampling sites, the same compounds were found, except for CPZ, generally at lower levels than those found at the effluent of internment services. In addition, QTP has also been detected.

In fact, the concentrations of APs in these samples were lower than 1 μg/L (excluding QTP in one hospital effluent sample). Yuan et al. [36] reported concentrations of 5.5–12.8 μg/L for CLZ and 2–5 μg/L for QTP, but in psychiatric hospital WWTP samples.

The results obtained for the hospital wastewaters could be explained by the elimination of the emergency services-admitted patients' treatment through the hospital sewage system, and also in the case of day patients, who left the hospital after treatment.

Table 6
Concentration values of the target compounds.

Sample	Origin	Compound[a]	Concentration (μg/L)[a]
#1	Hospital effluent of internment services	CPZ	<LLOQ
		CYA	0.10 ± 0.02
		CLZ	0.50 ± 0.06
		LVP	0.10 ± 0.01
#2	Hospital effluent	LVP	<LLOQ
#3	Hospital effluent	CYA	0.10 ± 0.02
#4	Hospital effluent	CYA	0.85 ± 0.03
		CLZ	<LLOQ
		QTP	2.32 ± 0.09
#5	Hospital effluent	LVP	<LLOQ
		CLZ	<LLOQ
		QTP	< LLOQ
#6	WWTP influent	CLZ	0.15 ± 0.02
		QTP	<LLOQ
#7	WWTP effluent	CLZ	0.10 ± 0.01
#8	WWTP effluent	CLZ	<LLOQ

[a] The other studied compounds are not detected in these samples.

Furthermore, the contribution of this group of psychotropic drugs to the pharmaceutical load of WWTPs was also addressed. At the influent of the urban WWTP network, CLZ and QTP (<LLOQ) were determined, while at the effluent only CLZ (100 ng/L) was detected, probably due to the efficient removal of QTP by the WWTP. These findings confirmed the APs effective elimination in the WWTP and even to its effluent at relevant concentrations.

In a multi-residue method developed by Borova *et al.* [34] for the determination of a higher number of psychoactive pharmaceuticals, illicit drugs, and related human metabolites in five WWTPs, CPZ was not detected in any sample and CLZ was only determined in one WWTP at a concentration lower than 50 ng/L. Yuan et al. [36] determined CPZ (<LLOQ), CLZ (<50 ng/L) and QTP (<10 ng/L) in municipal WWTPs.

Undoubtedly, the use of pharmaceuticals is growing, in agreement with the occurrences of pharmaceutical residues at low μg/L in the WWTP effluents [41], and namely the consumption of APs, and in this sense their input to the aquatic environment is increasing making them of increasing environmental relevance. The number of studies on it is at the moment limited. Moreover, in this study, to really confirm hospital loads, it would be necessary to measure the APs not only as parent compounds, but in their conjugated form as well; therefore the actual occurrence of those compounds in wastewaters may have been underestimated. In fact, these conjugates when passing through the sewage plant could be converted back to the parent compounds, which could lead to higher concentrations in the effluent than in its corresponding influent [42]. Additionally, besides the transformation products, the analysis of the metabolites should be also assessed, to give more information on their occurrence in hospital wastewater and their fate in subsequent WWTPs.

4. Conclusions

An analytical method using solid-phase extraction and gas chromatography/tandem mass spectrometry was developed and optimized for the quantification of six antipsychotics in wastewaters and it was successfully applied to hospital effluents and WWTP residual samples. The validated method was linear in the range of 0.1–10 μg/L (0.02–2 μg/L for HAL), with adequate precision and accuracy and real low limits of detection (≤ 0.01 μg/L) using only 50 mL of sample. Furthermore, the fact that microwave-assisted derivatization resumes the process to 2 min makes the whole procedure quick and less time-consuming.

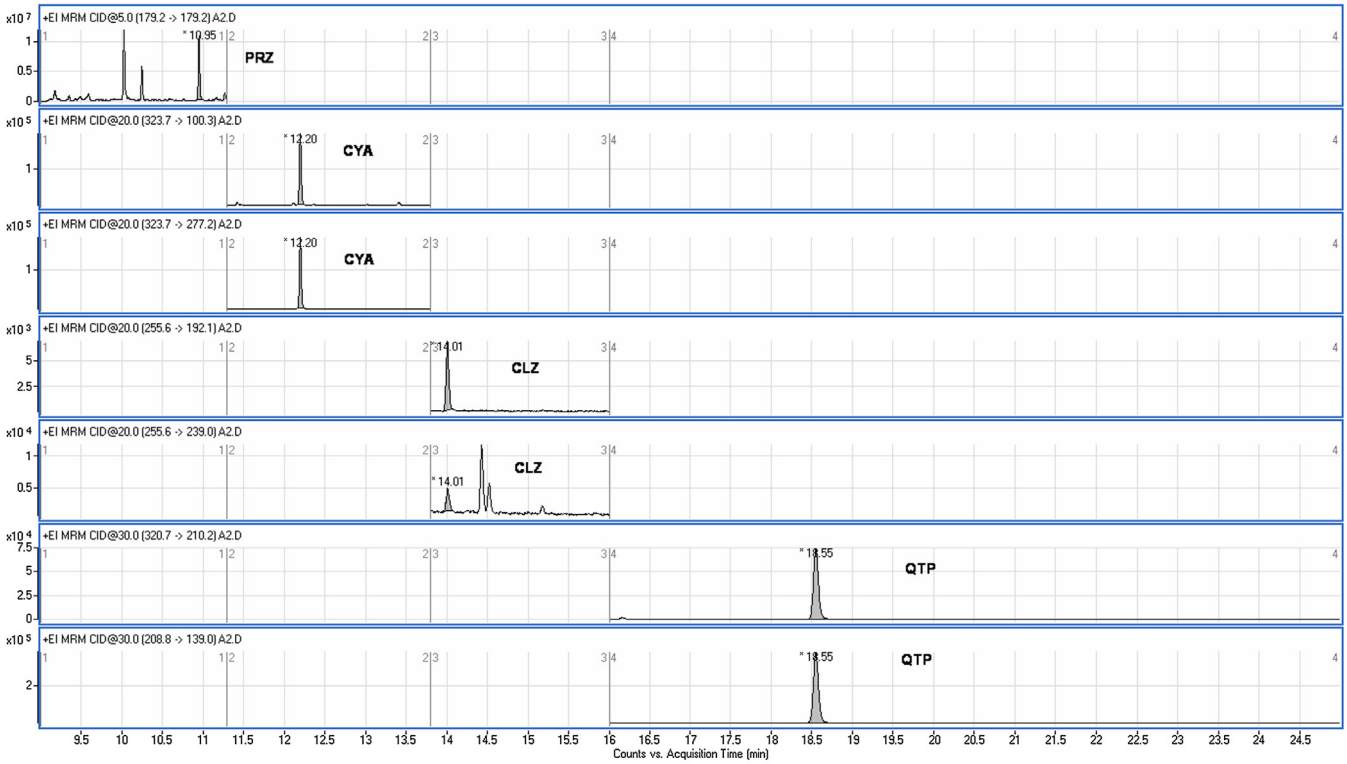

Fig. 3. Ion chromatogram of an authentic sample (CLZ < LLOQ, CYA 0.85 and QTP 2.32 μg/L).

Our work focused in a first instance on the determination of these APs in hospital wastewaters effluents and some preliminary results from field measurements were reported. It was expectable that hospital effluent will contribute to some extent to the pharmaceutical load in the influent entering a WWTP. The analysis of the receiving WWTP confirmed the presence of the drugs and therefore provided more analytical information about the pathway of these emerging pollutants, pointing out the need of the assessment of their ecotoxicology (evaluation of effects on non-target organisms) and also that the removal/degradation processes efficacy of WWTPs should be urgently improved. In conclusion, the present work points out the noteworthy importance of the evaluation of the fate, the occurrence, the persistence and bioaccumulation and the potential toxicity of the APs in the aquatic environment, with possible negative consequences to the aquatic ecosystems and in general to the health public.

Acknowledgments

This work is supported by FEDER funds through the POCI—COMPETE 2020—Operational Programme Competitiveness and Internationalisation in Axis I—Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491) and National Funds by FCT—Foundation for Science and Technology (Project UID/Multi/00709/2013). T. Rosado acknowledges the Programa Operacional Regional do Centro 2007-2013 QREN (Programa "Mais Centro") in the form of a fellowship (CENTRO-07-ST24-FEDER-002012), reference TDM-2-006).

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