Journal of Chromatography B, xxx (2016) xxx–xxx

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

F. Logarinho[a,1], T. Rosado[a,b,1], C. Lourenço[c], M. Barroso[d], A.R.T.S. Araujo[c,e], E. Gallardo[a,b,*]

[a] Centro de Investigação em Ciências da Saúde, Faculdade de Ciências da Saúde da Universidade da Beira Interior (CICS-UBI), Covilhã, Portugal
[b] Laboratório de Fármaco-Toxicologia—UBIMedical, Universidade da Beira Interior, Covilhã, Portugal
[c] Instituto Politécnico da Guarda (IPG), Escola Superior de Saúde, Guarda, Portugal
[d] Serviço de Química e Toxicologia Forenses, Instituto de Medicina Legal e Ciências Forenses-Delegação do Sul, Rua Manuel Bento de Sousa, Lisboa, Portugal
[e] Unidade de Investigação para o Desenvolvimento do Interior do IPG (UDI/IPG), Guarda, Portugal

ARTICLE INFO

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

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-

* Corresponding author at: Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Av. Infante D. Henrique, 6201-556, Covilhã, Portugal.
E-mail address: egallardo@fcsaude.ubi.pt (E. Gallardo).
[1] These authors contributed equally to this paper.

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^{TM}-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,

Please cite this article in press as: F. Logarinho, et al., Determination of antipsychotic drugs in hospital and wastewater treatment plant samples by gas chromatography/tandem mass spectrometry, J. Chromatogr. B (2016), http://dx.doi.org/10.1016/j.jchromb.2016.10.031