Journal of Chromatography A, 1248 (2012) 104–121

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry

Meritxell Gros[a,*], Sara Rodríguez-Mozaz[a], Damià Barceló[a,b]

[a] Catalan Institute for Water Research (ICRA), C/Emili Grahit 101, 17003 Girona, Spain
[b] Department of Environmental Chemistry, IDAEA-CSIC, C/Jordi Girona 18-26, 08034 Barcelona, Spain

ARTICLE INFO

Article history:
Received 9 January 2012
Received in revised form 18 May 2012
Accepted 22 May 2012
Available online 29 May 2012

Keywords:
Pharmaceuticals
Ultra-high-performance liquid chromatography
Quadrupole-linear ion trap
Information dependent acquisition
Multi-residue analytical method

ABSTRACT

The present work describes the development of an analytical method, based on automated off-line solid phase extraction (SPE) followed by ultra-high-performance liquid chromatography coupled to quadrupole linear ion trap tandem mass spectrometry (UPLC-QqLIT) for the determination of 81 pharmaceutical residues, covering various therapeutic groups, and some of their main metabolites, in surface and treated waters (influent and effluent wastewaters, river, reservoir, sea and drinking water). For unequivocal identification and confirmation, two selected reaction monitoring (SRM) transitions per compound are monitored. Quantification is performed by the internal standard approach, indispensable to correct matrix effects. Moreover, to obtain an extra tool for confirmation of positive findings, an information dependent acquisition (IDA) experiment was performed, with SRM as survey scan and an enhanced product ion (EPI) scan as dependent scan. Compound identification was carried out by library search, matching the EPI spectra achieved at one fixed collision energy with those present in a library. The main advantages of the method are automation and speed-up of sample preparation by the reduction of extraction volumes for some matrices, the fast separation of a big number of pharmaceuticals, its high sensitivity (limits of detection in the low ng/L range), selectivity, due to the use of tandem mass spectrometry, reliability since a significant number of isotopically labeled compounds are used as internal standards for quantification and finally, the analysis of tap, reservoir and sea waters, since information about occurrence of pharmaceuticals in these matrices is still sparse. As part of the validation procedure, the method developed was applied to the analysis of pharmaceutical residues in waste and surface waters from different sites in Catalonia (North East of Spain).

1. Introduction

Among the vast array of contaminants of anthropogenic origin reaching water supplies, pharmaceutically active compounds (PhACs) have one of the largest inputs into the environment. Several studies have proven that some pharmaceuticals are not completely degraded during conventional wastewater treatment [1–5] being therefore, discharged into receiving water bodies (rivers, lakes and seas), which may be used as sources for the abstraction of drinking water. It is difficult to predict which environmental and public health implications may arise from the occurrence of pharmaceuticals in freshwater ecosystems. Some of the most notorious adverse effects that they might have in the environment, besides acute or chronic toxicity, are resistant development of pathogenic bacteria due to the occurrence of antibiotics [6,7], genotoxicity [8,9], and endocrine disruption [10,11]. Furthermore, some PhACs such as antidepressants and antibiotics can be prone to bioconcentration/bioaccumulation in aquatic organisms, particularly in fish [12–14]. Therefore, the presence of pharmaceuticals in environmental waters, especially in drinking water and raw waters used for its production must be considered an important issue in terms of human health safety.

In this context, it is important to set up fast, sensitive and reliable analytical methods that enable the determination of a wide range of pharmaceuticals in environmental waters at the low concentration levels found. Nowadays, a large number of analytical methods are already available for their determination in both surface and wastewaters [15–21]. Nevertheless, a more limited number of methods for their analysis in drinking and sea waters are found in the literature. This is probably due to the analytical difficulties

* Corresponding author. Tel.: +34 972183380; fax: +34 972183248.
E-mail address: mgros@icra.cat (M. Gros).

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2012.05.084

encountered to quantify them at the ultra-trace levels that they might actually be present.

Current research regarding the development of analytical methods for the determination of pharmaceuticals in environmental matrices is oriented toward improvements of these methodologies, with the aim of increasing sample throughput, minimizing sample manipulation, decreasing solvent consumption and increasing overall method efficiency, in terms of selectivity and sensitivity. Advances on hyphenated LC–tandem MS, the technique per excellence for pharmaceutical determination, have driven to improved sensitivity, allowing the detection of sub-ppt concentrations, as well as to the identification of a big number of compounds simultaneously without losing sensitivity. On the other hand, high complexity of environmental samples often requires application of high-resolving power techniques, such as Orbitrap and TOF mass spectrometers, or hybrid mass spectrometers, like QqTOF and QqLIT, in order to provide additional structure information needed for unequivocal identification of contaminants and confirmation of positive findings. While TOF, QqTOF and Orbitrap mass spectrometers provide exact mass measurements and evidence on isotopic patterns, useful to propose chemical structures to confirm the identity of target compounds [22–25], QqLIT allows the application of the information dependent acquisition (IDA) function, where a targeted screening, monitoring one SRM transition per compound, is performed in combination with an enhanced product ion scan (EPI). In this way, MS/MS spectra are achieved, which are afterwards matched with MS/MS spectra recorded in a library [15,17,26,27].

With respect to liquid chromatography, the overall trend has moved towards the use of ultra high performance liquid chromatography (UHPLC), using short, narrow bore columns, packed with lower particle sizes (sub-2-μm), high mobile phase flow rates and ultrahigh pressures. In this way, a much faster chromatographic separation of a large number of compounds can be achieved, in comparison with conventional HPLC. Shortening the analysis time is important for attaining the high sample throughput required in laboratories conducting monitoring studies, since long runs are not tolerable for truly high-throughput analysis. On the other hand, narrower peaks are obtained using UHPLC, which facilitates peak resolution and reduces co-elution of interferences. As a result, matrix effects during detection are diminished.

Regarding sample preparation, new approaches are characterized by the application of green chemistry, which allowed the automation of the sample pre-treatment processes and also by on-site, on-line and even direct analysis [27,28]. Nevertheless, solid phase extraction (SPE) still remains as the most widely used mean of extraction and pre-concentration of organic contaminants in aqueous matrices.

The present work describes the development of an analytical method based on automated off-line solid phase extraction (SPE) followed by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry, for the fast and simultaneous determination of 81 multiple-class human and veterinary pharmaceutical residues as well as some of their metabolites and transformation products, in several surface and treated waters, including waste, river, reservoir, drinking and sea water. Target compounds were selected because of their high human consumption in Spain as well as their high occurrence and ubiquity in the aquatic environment, according to the information found in the scientific literature. Moreover, some human metabolites have been added to the list of compounds to be monitored. The vast majority of analytical methods published in the literature focus their attention on parent compounds and rarely include metabolites and/or transformation products (TPs). Some of the metabolites are still bioactive and may have high stability and mobility in the environment. Therefore, it is very important to assess their fate in order to assess the environmental risks associated with the release of pharmaceuticals). Quantitative analysis was performed by selected reaction monitoring (SRM) mode, monitoring two SRM transitions. For extra confirmation, an information dependent acquisition (IDA) experiment was performed, combining SRM with an EPI scan. Compound identification was carried out by library search with a library developed based on the EPI spectra at the registered collision energy.

The work presented in this manuscript offers several advantages and improvements in comparison with previous analytical methods developed by the authors [17,29]. These advantages are: (i) the minimization of sample manipulation for some matrices and automation of sample preparation (SPE is performed automatically and low samples volumes are used for some matrices: i.e. 25 mL for influent wastewaters, 50 mL for effluent wastewaters and 100 mL for river waters), (ii) the list of target pharmaceuticals to be monitored has been updated (adding some pharmaceuticals widely consumed in Spain and whose information about their occurrence in Spanish aquatic environment is sparse) and some human metabolites have been included, (iii) its high sensitivity (limits of detection are in the low ng/L range, even though using less samples volumes for pre-concentration of wastewaters), (iv) its selectivity (due to the inclusion of the IDA approach for confirmation). In the present study, some improvements and changes have been made to the IDA experiment, in comparison with previous works reported by the authors [17], (v) the use of a larger number of isotopically labeled compounds for internal standard calibration (almost one for each therapeutic group) and (vi) the analysis of reservoir and sea waters, since information about occurrence of pharmaceuticals in these type of waters is still sparse. Finally, the developed method was successfully applied to the analysis of pharmaceutical residues in several waste and surface waters from different sites in Catalonia (North East of Spain). Results indicate that pharmaceuticals are widespread pollutants in these types of matrices.

2. Materials and methods

2.1. Chemicals and reagents

All pharmaceutical standards were of high purity grade (>90%). Compounds with number (see Table 1) 1–17, 20, 23, 25, 28–30, 32–34, 36–42, 44–47, 49, 51, 54–57, 59–79 and 81 were purchased from Sigma–Aldrich. Compounds with numbers 9, 18, 19, 27, 43, 52, 53 and 80 were purchased from the US Pharmacopeia (USP), whereas substances with numbers 24, 26, 31, 35, 50, 48 and 79, were acquired from the European Pharmacopeia (EP). Substances with number 24, 26, 28–31, 37, 41, 42, 55, 61, 69, 79 and 81 were purchased as hydrochloride salts, 12, 17 and 18 as sodium salts, 19 as calcium salt, 25 as hydrobromide salt, 43 as tartrate, 50 as besylate, 51 as potassium salt, 54 as hydrogen sulfate and 56 as hemisulfate. Finally substances with number 21, 22 and 58 were acquired in Toronto Research Chemicals (TRC). Isotopically labeled compounds, used as internal standards, were erithromycin-N,N-dimethyl-^{13}C, xylazine-d_6, azaperone-d_4, ibuprofen-d_3, diazepam-d_5, meloxicam-d_3, ronidazole-d_3, ofloxacin-d_3, and fluoxetine-d_5 (as hydrochloride salts) from Sigma–Aldrich. Dexamethasone-d_4, indomethacine-d_4, antipyrine-d_3, cimetidine-d_3, bezafibrate-d_6, gemfibrozil-d_6, carbamazepine-d_{10}, citalopram-d_4 (as hydrobromide), atenolol-d_7, warfarin-d_5, hydrochlorothiazide-d_2, valsartan-d_8 and glyburide-d_3 were purchased from CDN isotopes (Quebec, Canada) and azithromycin-d_3, sulfamethoxazole-d_4, acetaminophen-d_4, venlafaxine-d_6, amlodipine-d_4 (as maleic acid salt), verapamil-d_6 (as hydrochloride salt) and furosemide-d_5 were from Toronto Research Chemicals (Ontario, Canada). On the other