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Selective determination of antimycotic drugs in environmental water samples by mixed-mode solid-phase extraction and liquid chromatography quadrupole time-of-flight mass spectrometry

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ABSTRACT

The suitability of mixed-mode (reversed-phase and cationic exchange) solid-phase extraction (SPE) for the selective concentration of basic antimycotic drugs (belonging to triazole, imidazole and allylamine chemical classes) in environmental water samples has been demonstrated for first time. The use of a sequential elution protocol, allowing the removal of neutral and acidic interferences before analytes extraction, led to a significant reduction of matrix effects, during electrospray ionization (ESI), in comparison with results reported for reversed-phase sorbents. In combination with liquid chromatography (LC) quadrupole time-of-flight (QTOF) mass spectrometry (MS) determination, the developed method attained limits of quantification (LOQs) comprised between 2 and 15 ng L^{-1}. After internal surrogate correction, accurate results (in most cases, recoveries ranged between 75 and 117%) were obtained for spiked aliquots of raw and treated wastewater, as well as river water, using quantification against calibration standard solutions in methanol (2% in NH_3). Accurate, scan MS/MS spectra allowed the unambiguous identification of target compounds in environmental samples; furthermore, the information contained in MS spectra was used for the screening of additional antimycotics in the processed samples. Fluconazole, ketoconazole, miconazole and clotrimazole were measured in wastewater samples at concentrations up to 200 ng L^{-1}. The screening capabilities of the LC–QTOF-MS system permitted to identify the systematic presence of climbazole in the processed samples.

1. Introduction

Antimycotics constitute a broad group of drugs designed to treat infections caused by fungi. Most of them are azole (triazoles and imidazoles) or allylamine compounds. In addition to topic applications, some antimycotics are administered orally and, less often, intravenous. Excretion of un-metabolized drugs added to wash off from treated skin areas result in the introduction of these compounds in municipal sewage water [1,2]. Given that antimycotics are designed to act as fungi killers, through inhibition of certain enzymes, they might also disturb the endocrine system of aquatic organisms. In fact, azolic compounds interfere with the production of aromatase enzymes, which are responsible for sexual differentiation of vertebrates during larval stages, and also for the balance between androgenic and estrogenic hormones in mammals [3].

Among the most often prescribed antimycotic drugs, fluconazole (FCZ) has been detected at similar levels in the inlet and outlet streams of sewage treatment plants (STPs), which suggests resistance to biodegradation [4]. Less polar compounds, such as clotrimazole (CTZ), ketoconazole (KTZ), econazole (ECZ) and miconazole (MCZ), have been also found in wastewater entering municipal STPs [4–6]. Although their levels are significantly reduced in treated wastewater, sludge sorption, and not degradation, appears to be the main responsible for such reduction [7,8].

Analytical methods for the determination of antimycotic drugs in environmental water samples usually rely on liquid chromatography (LC) with tandem mass spectrometry (MS/MS), after solid-phase extraction (SPE) of water samples, using reversed-phase type sorbents. With the exception of FCZ, most antimycotics display from moderate to high lipophilic properties; thus, there are prone to sorptive losses on filters and glassware material during SPE. Acidification of water samples results in analytes protonation, increasing their water solubility and minimizing sorption problems [7,9]. On the other hand, reducing the pH of water samples

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increases the retention of acidic compounds on reversed-phase sorbents, resulting in more complex extracts than those obtained at neutral pH. The consequence of these too complex extracts is the existence of significant variations in the yield of electrospray ionization (ESI) for wastewater extracts versus calibration standards. In this sense, several studies have recognized the existence of large ionization suppression effects (up to 75%) after concentration of 100 mL of raw sewage water [5,9]. Although signal suppression can be compensated with isotopic labelled internal surrogates (IS), it results in increased limits of quantification (LOQs).

Mixed-mode SPE sorbents permit increasing the selectivity of reversed-phase materials when applied to the concentration of ionizable compounds. Particularly, the OASIS MCX cartridges have provided cleaner extracts than OASIS HLB ones, when using a suitable elution protocol to fractionate basic analytes (e.g. drugs of abuse) from neutral and acidic interferences [10]. As far as we could trace, with regards to antimycotic drugs, the MCX cartridges have been tested only for the extraction of CTZ from wastewater. In this occasion, authors reported inappropriate results (the attained recoveries are not given); however, details regarding extraction and elution conditions are not provided [7].

The primary aim of this study was to assess the capability of the mixed-mode (reversed-phase and cationic exchanger) OASIS MCX sorbent for the extraction of basic antimycotic drugs from environmental water samples, improving the selectivity of the concentration process by means of a sequential elution strategy. As a secondary aim, we evaluated the use of a hybrid quadrupole time-of-flight (QTOF) MS instrument, instead of a triple quadrupole (QqQ) one, for the determination of target compounds after LC separation. The information contained in accurate, scan MS spectra provided by this system was used to screen the presence of additional antimycotics in the processed samples.

2. Experimental

2.1. Standards, solvents, reagents and samples

ECZ nitrate salt (100%), etaconazole (ETZ, 96.7%), CTZ (100%), FCZ (98%), KTZ (98%), (±)-MCZ nitrate salt (100%) and terbinafine hydrochloride (TRB, 98%) were obtained from Sigma (Milwaukee, WI, USA). CTZ-d5 (98%), used as IS, was acquired from Toronto Research Chemicals (North York, ON, Canada). Relevant properties (pKa and log K_{ow} values) for above compounds are given in Table 1; whereas, their chemical structures are provided as supplementary information, Fig. S1. Individual solutions of each compound and the IS were dissolved in methanol. Further dilutions and mixtures of them were prepared in the same solvent. Calibration standard solutions were dissolved in methanol containing a 2% of NH_3.

Methanol and acetonitrile, HPLC-grade purity; hydrochloric acid (37%), ammonia (25% solution in methanol) and ammonium acetate (99%) were supplied by Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q (Millipore, Billerica, MA, USA) system. SPE cartridges (OASIS HLB 200 mg and OASIS MCX 150 mg) were acquired from Waters (Milford, MA, USA).

Grab samples of raw and treated wastewater were obtained, in different dates, from the same STP, serving a population of 100.000 inhabitants in Galicia (Northwest Spain). The STP was equipped with primary and biological treatment units. Surface water was collected from the river receiving the effluent of this STP. Treated wastewater samples were also obtained from other STPs, in the same geographic area. Samples were taken in glass vessels. Immediately after reception, they were adjusted at pH 3, spiked with the IS and filtered before SPE concentration.

Table 1
Relevant properties and LC–QTOF-MS determination conditions for antimycotic drugs.

Analyte	Abbreviation	pKa	Log K_{ow}	Retention time (min)	Precursor ion (Da)	Collision energy (eV)	Quantification ion (Da)	Other product ions (Da)	Linearity (R^2, 1–200 ng mL^{-1}, 8 levels)	LOQs[a] (ng mL^{-1})
Fluconazole	FCZ	11.01, 2.64	0.4	10.7	307.1113	18	220.0681	238.0783	0.9994	0.5
Etaconazole	ETZ	2.94	3.6	12.7	328.0614	20	158.9763	204.9818	0.9985	0.5
Ketoconazole	KTZ	6.88	4.3	13.0	531.1560	48	82.0530	489.1455	0.9980	0.7
Clotrimazole	CTZ	6.12	4.1	13.5	277.0788	20	165.0699	242.1035	0.9999	0.7
Clotrimazole-d5	CTZ-d5	6.12	4.1	13.6	282.1092	20	170.1018	247.1449		
Econazole Nitrate	ECZ	6.68	5.5	14.1	383.0293	22	125.0153	69.0447	0.9999	0.5
Miconazole Nitrate	MCZ	6.64	6.1	15.1	416.9904	24	158.9763	69.0447	0.9998	1
Terbinafine hydrochloride	TRB	7.1	5.6	15.3	292.2060	14	141.0699	93.0699	0.9996	1

[a] Instrumental LOQs, without considering the SPE step.