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Comparison of different extraction methods for the determination of statin drugs in wastewater and river water by HPLC/Q-TOF-MS

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ABSTRACT

Three preconcentration techniques including solid phase extraction (SPE), dispersive liquid–liquid microextraction (DLLME) and stir-bar sorptive extraction (SBSE) have been optimized and compared for the analysis of six hypolipidaemic statin drugs (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin) in wastewater and river water samples by high performance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (HPLC/Q-TOF-MS). Parameters that affect the efficiency of the different extraction methods such as solid phase material, sample pH and elution solvent in the case of SPE; the type and volume of the extracting and dispersive solvent, pH of sample, salt addition and number of extraction steps in the case of DLLME; and the stirring time, pH of sample, sample volume and salt addition for SBSE were evaluated. SPE allowed the best recoveries for most of the analytes. Pravastatin was poorly extracted by DLLME and could not be determined. SBSE was only applicable for lovastatin and simvastatin. However, despite the limitations of having poorer recovery than SPE, DLLME and SBSE offered some advantages because they are simple, require low organic solvent volumes and present low matrix effects. DLLME required less time of analysis, and for SBSE the stir-bar was re-usable. SPE, DLLME and SBSE provided method detection limits in the range of 0.04–11.2 ng L^{-1}, 0.10–17.0 ng L^{-1} for 0.52–2.00 ng L^{-1}, respectively, in real samples. To investigate and compare their applicability, SPE, DLLME and SBSE procedures were applied to the detection of statin drugs in effluent wastewater and river samples.

1. Introduction

The interest in the presence of pharmaceutically active compounds in the environment has grown during the last years because it has become evident that sewage treatment plant effluents are a significant source for releasing pharmaceuticals after human use into the environment. Due to the high persistence and widespread occurrence of lipid-regulating agents in aquatic environments, their presence in drinking water has also been reported [1–5]. Lipid regulating agents can be divided into two main groups namely "the fibrate" and "the statin" class. Both classes are among the most frequently prescribed drugs. In contrast to the extensive information related to the fibrate class in the environment, only a few papers have been published about the presence of pharmaceuticals belonging to the statin class (cholesterol-reducing agents) [6]. Only a few of these methods were designed for the separation of a mixture of statin drugs [6,7].

The determination of statin drugs in environmental water samples involves solid phase extraction (SPE) and high performance liquid chromatography–mass spectrometry (HPLC/MS–MS) determination. However, in addition to the target analytes, a significant amount of matrix components may be coextracted by SPE and signal suppression may be observed in mass spectrometric analyses [8–10].

Matrix interferences might be different if stir bar sorptive extraction (SBSE) (introduced by Baltussen et al. in 1999 [11]) is used instead of SPE. SBSE is based on sorption of the analytes into a film of polydimethylsiloxane (PDMS) (same principle as solid phase microextraction) by means of the partition equilibrium established between the aqueous matrix and the PDMS phase. PDMS coated onto a glass-coated magnetic stir bar is commercialized as Twister and provided by Gerstel. Usually, SBSE is combined with thermal desorption but solvent desorption is possible as well giving the possibility for replicate analysis and LC combination [12]. Among the observed benefits of this technique are its relative speed and its minimal solvent requirement. However, few papers have reported the extraction of pharmaceutically active compounds from environmental samples by SBSE [13–15].

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Recently, dispersive liquid–liquid microextraction (DLLME) has also been applied, as an alternative to SPE, with the aim to decrease signal suppression during liquid chromatography–mass spectrometric analyses. DLLME is an innovative liquid phase microextraction mode that is based on a three component solvent system. Solvents employed in DLLME are a mixture of a high-density solvent (extractant) and a water-miscible polar solvent (disperser) which is rapidly introduced into the aqueous sample to form a cloudy solution [16]. This technique has demonstrated a very good performance for pesticides or polychlorinated biphenyls in tap, lake and river water, so that it seems to be interesting to extend the applications to other analytes and more complex matrices such as wastewater [17–20].

Analyses of the statin pharmaceuticals have been performed with HPLC/MS–MS, using mainly single quadrupole and triple quadrupole (QqQ) MS instruments [21–23]. The use of HPLC and time-of-flight (TOF) MS, and a combination of quadrupole and TOF (Q-TOF) has proved to be a powerful tool for the identification of trace constituents of complex mixtures and/or for confirming their presence [24].

In the current work, three preconcentration techniques (SPE, DLLME and SBSE) were investigated and compared for the extraction of six of the most used statin drugs (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin) prior to their analysis by HPLC/Q-TOF-MS. The method has been developed for their simultaneous determination in rivers and effluent wastewater.

2. Experimental

2.1. Materials and reagents

Statins were not available as pure standards but were extracted from the following commercially available drug formulations: Sortis 10 mg (atorvastatin), Pfizer Corporation Austria GmbH (Vienna, Austria); Actavis 80 mg (fluvastatin), Actavis Group PTC (Hafnarfjödur, Island); Mevacor 20 mg (lovastatin), Merck Sharp and Dohme GmbH (Vienna, Austria); Pravastatin Pharma 20 mg (pravastatin), Pharma Arzneimittel GmbH (Graz, Austria); Crestor 10 mg (rosuvastatin), AstraZeneca Österreich GmbH (Vienna, Austria); Simvastatin Tablet 40 mg (simvastatin), Pharma Arzneimittel GmbH (Graz, Austria). Eventual small deviations of the contents from the declared values given for the pharmaceutical formulations were neglected within this work. The chemical structures are shown in Table 1. The internal standards Irganox 3114 and Irganox 1035 were obtained from Ciba-Geigy (Basle, Switzerland). For extraction of the active agents from the film tablets they were finely ground and an appropriate amount was weighed and mixed with methanol to give a stock solution of 1000 $\mu g\,mL^{-1}$ of each statin. The suspension was treated in an ultrasonic bath for 10 min and filtered through a syringe filter of 0.45 μm pore size. A standard solution, containing a mixture of the statins at a concentration of 1 $\mu g\,mL^{-1}$ of each drug was prepared in methanol. This solution was diluted again using methanol:water (2:1, v/v) to obtain the final working solutions.

Acetonitrile (ACN), acetone, dichloromethane, formic acid, methanol and tetrahydrofuran (THF) (all of chromatographic analysis grade) were purchased from JT Baker (Deventer, The Netherlands). Chlorobenzene (C_6H_5Cl), chloroform ($CHCl_3$), tetrachloroethylene (C_2Cl_4), trifluorotrichloroethylene ($C_2Cl_3F_3$) were obtained from Merck (Darmstadt, Germany). Ammonium formate was purchased from Sigma-Aldrich (Vienna, Austria). SPE cartridges, Bond Elut C18-OH, Chromabond tetracycline, Oasis HLB, Supelclean C18 and Supelclean Carbowax were purchased from Varian (Darmsdtadt, Germany), Machery-Nagel (Düuren, Germany), Waters (Milford, MA, USA) and Supelco (Bellefonte, PA, USA), respectively.

Twister stir bars of 2 cm length coated with a 0.5 mm layer of polydimethylsiloxane were obtained from Gerstel (Mühlheim, Germany).

2.2. Sample collection

Effluent wastewater samples, used to test method applicability, were collected during September 2010 from a waste water treatment plant (WWTP) in the region of Linz (Austria). River samples were collected during September 2010 from the River Danube.

Water samples were collected in brown bottles pre-cleaned with acetone and methanol. Immediately after sampling acetonitrile was added to achieve a concentration of 0.5% (v/v) in order to stabilize the samples. Stabilized samples were stored at 4 °C in a refrigerator. Prior to extraction, water samples were filtrated through a 0.45 μm membrane filter. Irganox 3114 (in the case of SPE and DLLME) and Irganox 1035 (in the case SBSE) were added to filtered samples as surrogate standards to achieve a final concentration of 25 $\mu g\,L^{-1}$.

2.3. Instrumentation

2.3.1. High performance liquid chromatography

Chromatographic analyses were performed on an 1100 HPLC system equipped with a vacuum degasser, a quaternary pump, an autosampler and a UV–Vis diode array detector (all from Agilent, Palo Alto, CA, USA).

Separations were carried out using a Zorbax Eclipse XDB-C18 (5 mm × 4.6 mm i.d.; 1.8 μm particle size) column (Agilent). Analytes were separated by gradient elution with ACN (containing 0.1%, v/v formic acid) (A) and an aqueous 5 mM ammonium formate solution (containing 0.1%, v/v formic acid) (B) at a flow-rate of 1 $mL\,min^{-1}$. The linear gradient elution program was: 0 min, 30% A; 5 min, 50% A; 8 min, 60% A; 9 min, 100% A; 12 min, 100%; 12.1 min, 30% A; 14 min, 30% A. The column was thermostated at 25 °C.

2.3.2. Mass spectrometry

MS measurements were done with a 6510 quadrupole/time-of-flight (Q-TOF) instrument equipped with an electrospray ionization source (Agilent). Results were obtained with the following settings: MS capillary voltage 3800 V, drying-gas flow rate 12 $L\,min^{-1}$, drying-gas temperature 350 °C, and nebulizer pressure 60 psi.

Various adduct ions, such as $[M+H]^+$ and $[M+Na]^+$, have been used as precursor ions for Q-TOF-MS analysis in the positive-ion mode. The optimization of MS parameters was performed by flow injection analysis of each compound. Table 2 summarizes the optimized Q-TOF-MS conditions for the analysis of statin drugs.

2.4. Extraction procedures

2.4.1. Solid phase extraction

Chromabond tetracycline cartridges were conditioned by passing two times 5 mL methanol followed by 5 mL HPLC grade water through them. Thereafter, the aqueous samples (250 mL) were passed through the cartridges at a flow-rate of approximately 10 $mL\,min^{-1}$. Then, each sample bottle was rinsed with 10 mL of HPLC grade water, and the rinse was added to the cartridge. The cartridges were eluted using four successive 1 mL aliquots of methanol at a flow-rate of about 1 $mL\,min^{-1}$. The eluates were collected in a 10-mL collection tube and concentrated to almost dryness by a gentle nitrogen stream. Then, samples were reconstituted in 1 mL of a methanol:water mixture (2:1, v/v). Finally, 20 μL was injected into the HPLC system.