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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.

Table 1
Structures, K_{OW} and pK_a values of the statin drugs evaluated.

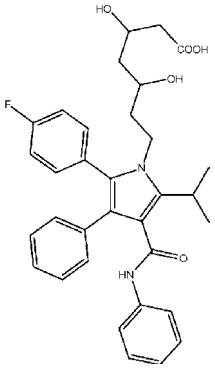
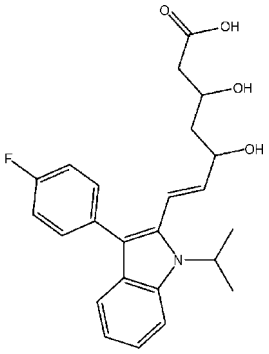
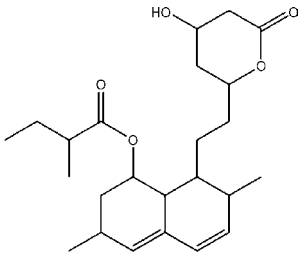
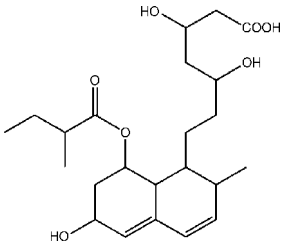
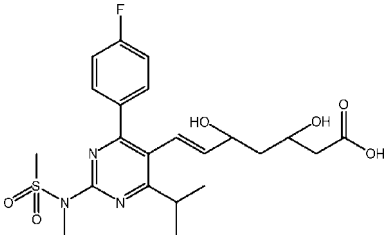
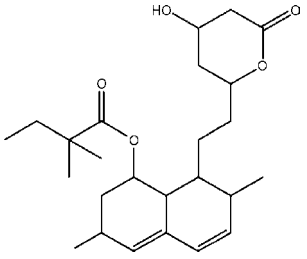
Analyte	Structure	log K_{OW}	pK_a
Atorvastatin		4.46	4.5
Fluvastatin		4.85	4.1
Lovastatin		4.26	13.5
Pravastatin		3.10	4.6
Rosuvastatin		2.05	4.4
Simvastatin		4.68	13.2

Table 2
Optimized parameters of the Q-TOF-MS determination after HPLC separation of statin drugs.

Analyte	Precursor ion (*m*/*z*)	Product ions (*m*/*z*)	Fragmentor (V)	Collision energy (eV)
Atorvastatin	$[M+H]^+$, 559.26	440.23; 466.21	225	20
Lovastatin	$[M+Na]^+$, 427.25	325.18	150	20
Fluvastatin	$[M+H]^+$, 412.19	224.09; 266.13	200	20
Pravastatin	$[M+Na]^+$, 447.24	327.15; 250.81	225	18
Rosuvastatin	$[M+H]^+$, 482.18	258.14; 300.15	225	35
Simvastatin	$[M+Na]^+$, 441.26	325.18	150	20

2.4.2. Dispersive liquid–liquid microextraction

A volume of 9 mL of the filtered sample was put into a conical glass tube, and then 0.5 mL of the dispersive solvent acetone containing 50 μL of the extraction solvent chlorobenzene was rapidly introduced into the aqueous sample. A cloudy state was formed by the fine droplets of the extractant dispersed completely in the aqueous phase. The phase separation was performed by centrifugation at 4000 rpm for 5 min. The sedimented phase was withdrawn with a 100-μL syringe. The extraction procedure was applied one more time. The combined sedimented extracts were evaporated to dryness by a gentle nitrogen stream and reconstituted in 0.1 mL of a methanol:water mixture (2:1, v/v). Finally, 20 μL was injected into the HPLC system.

2.4.3. Stir-bar sorptive extraction

Before use, the coated stir bars were placed into a cleaning solution containing methanol and dichloromethane (50:50, v/v) for 1 h. Afterwards, the stir bars were put into thermodesorption glass tubes, conditioned for 1 h at 300 °C under a nitrogen flow in a Gerstel tube conditioner TC 2, and subsequently immersed into the aqueous sample (250 mL). After a defined stirring time, the stir bar was removed from the aqueous sample, dried with lint-free cloth, and placed into a vial containing 1 mL of methanol:water (2:1, v/v). Finally, the stir bar was removed from the vial and 20 μL of the liquid phase was injected into the HPLC instrument.

3. Results and discussion

3.1. Optimization of SPE, DLLME and SBSE procedures

The extraction procedures were optimized in triplicate using aliquots of 250 mL (for SPE and SBSE) or 9 mL (for DLLME) of tap water spiked with the statin compounds at 0.1, 0.1 and 0.28 μg L^{-1}, for SPE, SBSE and DLLME, respectively. Different spiking levels were used to achieve the same final concentration level after extraction (25 μg L^{-1} for each compound).

3.1.1. Optimization of SPE

A variety of SPE cartridges, including Bond Elut C18-OH, Chromabond tetracycline, Oasis HLB, Supelclean C18 and Supelclean Carbowax were tested in triplicate to find the most efficient extraction cartridge for statins. The best recoveries were obtained with Chromabond tetracycline (mean recovery 82%). Mean recoveries achieved with Bond Elut C18-OH and Supelclean C18 were 80% and 71%, respectively (Fig. 1). Firstly, sample pH 7 was evaluated (Fig. 1a). From the results obtained at pH 7, Carbowax cartridges were discarded and acidification to pH 3 was evaluated. An acidic pH-value of 3 was chosen as it is commonly used in the extraction of pharmaceutical compounds from environmental samples; it is compatible with the solid phases evaluated and provides the almost complete unionization of the statin compounds (pK_a values from 4.1 to 13.5). The analytes evaluated were generally better extracted at neutral pH than under acidic conditions (Fig. 1b), except lovastatin and simvastatin that were similarly extracted under both pH conditions (Fig. 1). The slight increase of extraction recoveries from pH 3 to pH 7 was observed in the statin compounds with a carboxylic group which is ionized at pH 7; pK_a values of such compounds are in the range from 4.1 to 4.6 (Table 1). No significant influence in the extraction recoveries due to log K_{OW} values was observed. Relative standard deviations were similar in all of the experiments (2.9%, 2.4%, 3.4% and 3.6% for Chromabond tetracycline, Bond Elut C18-OH, Supelclean C18 and Oasis HLB, respectively).

Finally, the influence of the elution solvent was evaluated. The best recoveries were obtained with methanol (mean recovery 80.8%). Recoveries with acetone and ethyl acetate were quite similar, 74.5% and 74.4%, respectively (Fig. 1).

3.1.2. Optimization of DLLME

Extraction of analytes by DLLME depends on several factors such as the type and volume of the extraction and disper-

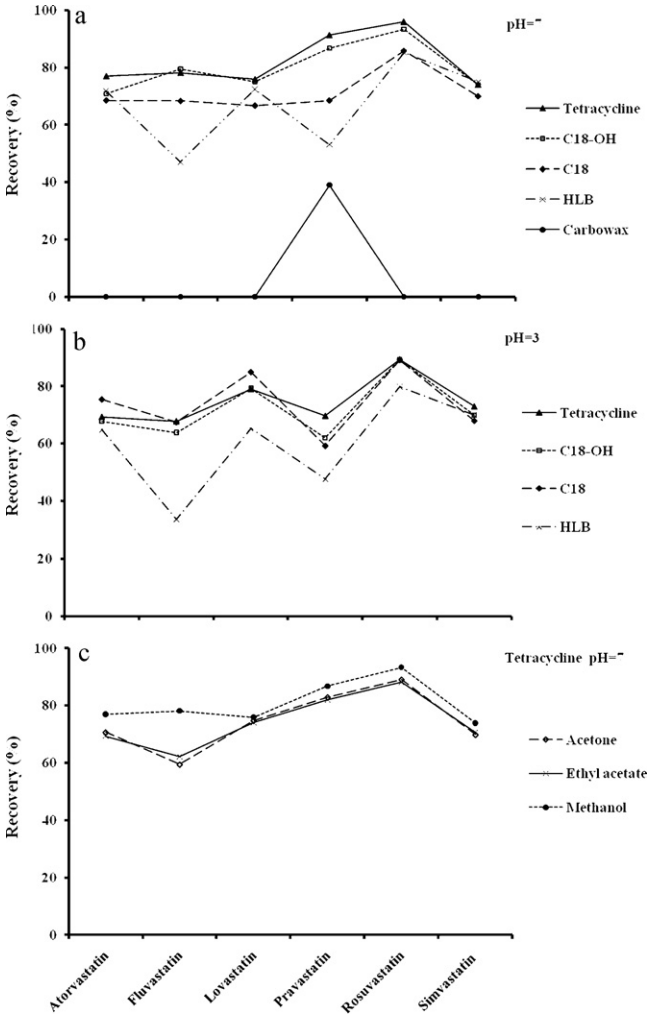


Fig. 1. Influence of (a) solid phase material (using pH 7 and methanol as elution solvent), (b) pH of sample and (c) elution solvent on the extraction recoveries of the SPE procedure (n = 3).

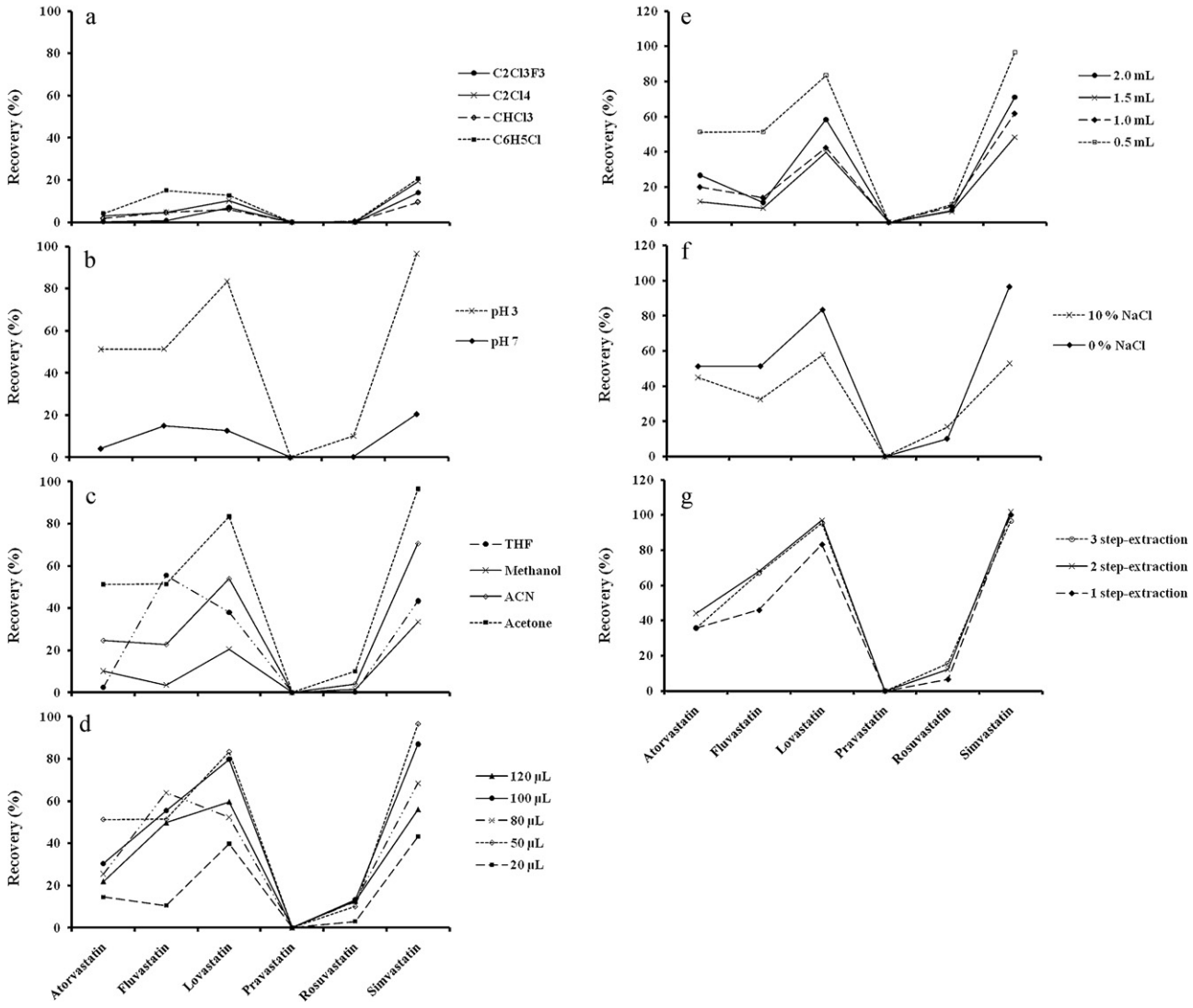


Fig. 2. Influence of (a) extraction solvent (using pH 7, 50 μL of extraction solvent and 0.5 mL of acetone as extraction solvent), (b) pH of sample, (c) dispersive solvent, (d) extraction volume, (e) dispersive volume, (f) salt addition and (g) number of extraction steps on the extraction recoveries of the DLLME procedure ($n = 3$).

sive solvents, sample pH, salt addition and the number of extraction steps.

To optimize the extraction conditions, four extracting solvents (chlorobenzene, chloroform, tetrachloroethylene and trifluortrichloroethylene) and four dispersing solvents (acetone, acetonitrile, methanol, and tetrahydrofuran) were tested in triplicate. The highest recoveries were obtained with chlorobenzene as extraction solvent and acetone as dispersive solvent.

Most of the statins possess acidic moiety and can be present in water in both ionized and unionized forms. The extraction of the analytes in their unionized forms is expected to be easier than when they are present in their ionized form. Thus, the effect of different pH values (3 and 7) was tested. The results confirmed that the extraction efficiencies of all of the analytes increased for samples at low pH. The statins without acidic groups and, consequently, with the highest pK_a values, it is lovastatin (pK_a 13.5) and simvastatin (pK_a 13.5) (Table 1) were the best extracted compounds at acidic pH (Fig. 2b). Extraction recoveries of the statins with a carboxylic group were lower than those without acidic groups even at pH 3 probably due to still being slightly ionized at pH 3. Nevertheless, the statins with the poorest removal rates were those with the lowest log K_{OW} values (pravastatin and rosuvastatin), it means those with the highest affinity for the aqueous phase.

Four extraction volumes of the extraction solvent chlorobenzene were tested (20, 50, 80, 100 and 120 μL). Results obtained showed that the volume of the extraction solvent and the extraction recoveries are related by a non-linear curve with a maximum at 50 μL. The effect of the dispersing solvent volume was studied using four different volumes of acetone: 0.5, 1.0, 1.5 and 2.0 mL. The extraction solvent volume was kept constant at 50 μL. The highest recovery was obtained using 0.5 mL of the dispersing solvent. The decrease of the recoveries when dispersing solvent volumes are increased can be explained by a better solubility of the extraction solvent in the water:acetone mixture. This effect resulted in a reduction of the volumes collected of sedimented phase and also in lower recoveries.

The salt addition (10% of NaCl) was investigated and no influence was observed. Finally, the impact of repeating the extraction step was tested. An additional extraction step increased the recovery of most of the analytes, especially fluvastatin. Another additional step (three step-extraction) did not improve the results significantly, only rosuvastatin recovery increased from 12 to 16%.

All the experiments tested are summarized in Fig. 2.

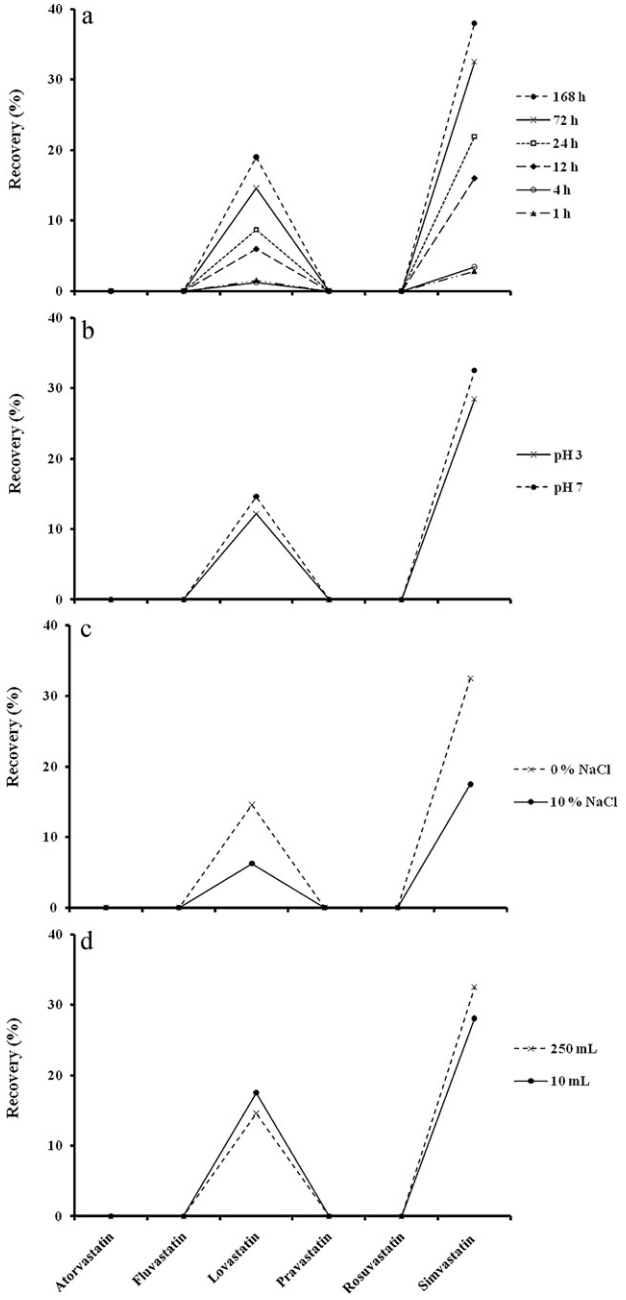


Fig. 3. Influence of (a) stirring time (using pH 7 and 250 mL of sample volume), (b) pH of sample, (c) salt addition and (d) sample volume on the extraction recoveries of the SBSE procedure ($n = 3$).

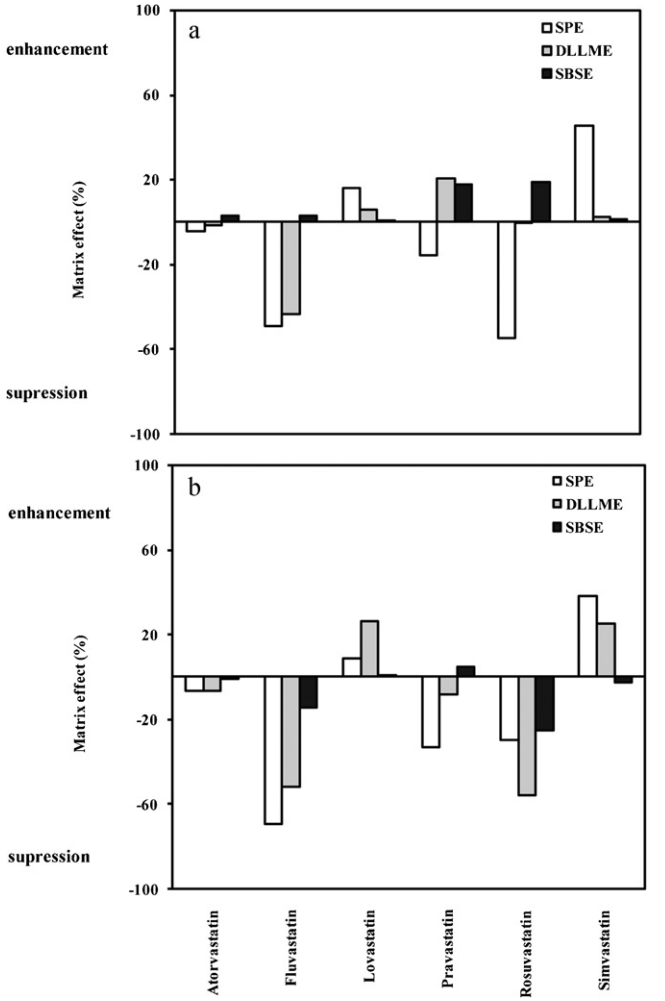


Fig. 4. Matrix effect of (a) river samples and (b) effluent wastewater samples on the determination of statin drugs.

3.1.3. Optimization of SBSE

Important parameters affecting SBSE including time of extraction (1, 4, 11, 24, 72 and 168 h), pH of sample (3 and 7), salt addition and sample volume were optimized. Each parameter was tested in triplicate.

The extraction times to reach the equilibrium are very different and depend on the analyzed sample and, as well, on the analytes. Increasing the stirring time from 1 to 168 h resulted in increasing recoveries from 2 to 19% for lovastatin and from 3 to 38% for simvastatin. As the difference between 72 and 168 h was not very big, 72 h was chosen as extraction time. Even an extraction overnight may lead to sufficient sensitivity depending on the sample concentration.

Once the stirring time had been selected, pH of sample was investigated. The best results, with respect to average signal intensities, were obtained at pH 7. Additionally, with the addition of sodium chloride (10%) no improvement was observed.

Finally, the effect of sample volume (250 and 10 mL) was investigated and no significant difference was detected for the recoveries, so that 250 mL of sample volume was chosen to increase the preconcentration factor. All the results are summarized in Fig. 3.

This procedure was only applicable for lovastatin and simvastatin, the statin compounds without carboxylic groups (Table 1). It seems that the presence of a carboxylic group difficults the sorption onto the polymeric phase. During the SBSE process, the partitioning of the analytes between the sample and the polymeric phase (PDMS) takes place. The knowledge of the distribution coefficients is important to estimate the analyte partitioning and to calculate the recovery rates [25,26]. In Eq. (1), the analyte partition between two non-miscible phases, an aqueous phase (sample) and a polymeric extraction phase, is described:

$$R = \frac{m_s}{m_0} = \frac{K_{SW}/\beta}{1 + (K_{SW}/\beta)} = \frac{1}{(\beta/K_{SW}) + 1} \quad (1)$$

where R is the recovery rate, m_s is the mass of analyte in the stir-bar, m_0 is the initial total mass of analyte, K_{SW} is the partition coefficient between PDMS phase and water, β is the phase ratio (Vw/Vs, where Vs is the volume of PDMS and Vw is the volume of the aqueous phase). This equation shows that the recovery depends on both the values of β and K_{SW}. The K_{SW} value is often approximated by the octanol–water partition coefficient (K_{OW}), which would mean that

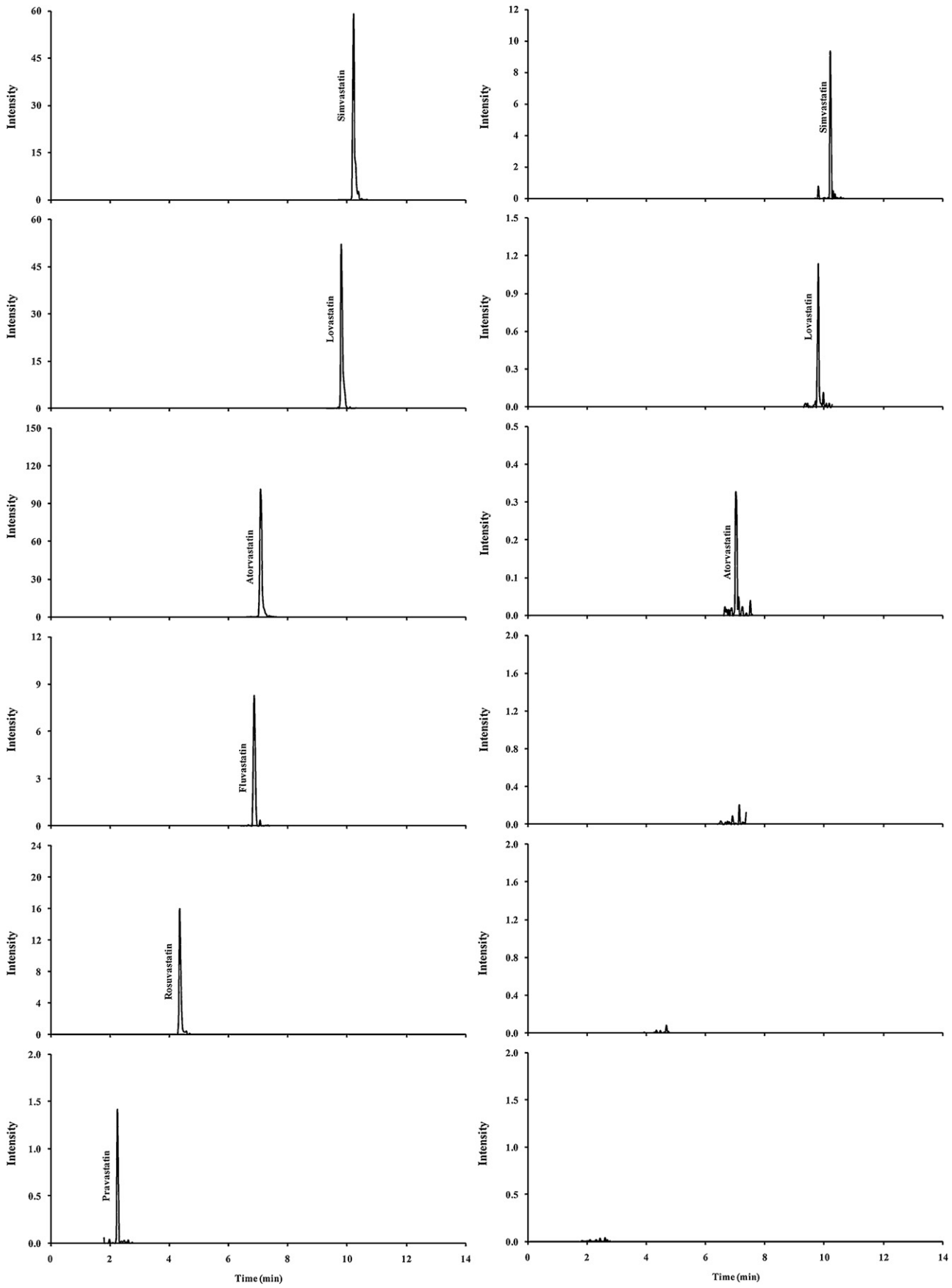


Fig. 5. LC–Q-TOF-MS chromatograms of a standard mixture containing 25 μg L^{-1} of each statin drug (left panel) and an effluent sample of a WWTP (atorvastatin 1.9 ng L^{-1}, lovastatin 5.5 ng L^{-1} and simvastatin 15.7 ng L^{-1}) extracted by DLLME (right panel).

Table 3
Recovery, precision (% RSD) and method detection limits (MDL) of SPE, DLLME and SBSE.

Sample	Technique	Validation parameter	Atorvastatin	Fluvastatin	Lovastatin	Pravastatin	Rosuvastatin	Simvastatin
Pure water	SPE	*R* (%)	77	78	76	87	93	74
		RSD (%)	3.9	6.9	5.8	4.7	1.1	3.8
		MDL ($ng\,L^{-1}$)	0.05	4.09	0.05	11.3	0.31	0.04
	DLLME	*R* (%)	55	68	83	0	17	92
		RSD (%)	3.7	7.3	3.5	–	9.7	3.0
		MDL ($ng\,L^{-1}$)	0.20	13.0	0.13	–	4.90	0.09
	SBSE	*R* (%)	0	0	19	0	0	38
		RSD (%)	–	–	6.0	–	–	3.4
		MDL ($ng\,L^{-1}$)	–	–	0.21	–	–	0.08
River water	SPE	*R* (%)	46	74	80	88	97	78
		RSD (%)	4.6	10.1	6.2	8.1	7.7	6.6
		MDL ($ng\,L^{-1}$)	0.09	4.33	0.05	11.2	0.30	0.04
	DLLME	*R* (%)	39	52	89	0	13	89
		RSD (%)	12.5	13.0	7.6	–	12.1	4.1
		MDL ($ng\,L^{-1}$)	0.28	17.0	0.12	–	6.18	0.10
	SBSE	*R* (%)	0	0	2	0	0	4
		RSD (%)	–	–	6.1	–	–	12.0
		MDL ($ng\,L^{-1}$)	–	–	2.00	–	–	0.70
Effluent waste-water	SPE	*R* (%)	70	86	64	93	93	68
		RSD (%)	8.5	7.1	6.8	15.1	9.9	3.2
		MDL ($ng\,L^{-1}$)	0.06	3.73	0.06	10.5	0.32	0.05
	DLLME	*R* (%)	42	64	87	0	15	82
		RSD (%)	16.4	10.2	5.4	–	10.8	3.0
		MDL ($ng\,L^{-1}$)	0.26	13.8	0.13	–	5.30	0.11
	SBSE	*R* (%)	0	0	5	0	0	6
		RSD (%)	–	–	9.9	–	–	8.1
		MDL ($ng\,L^{-1}$)	–	–	0.80	–	–	0.52

SBSE recovery could be estimated if K_{OW} of the analyte is known. Hydrophobic solutes with a high $\log K_{OW}$ (>3) could be extracted with high recovery rates, while hydrophilic solutes with a low $\log K_{OW}$ (<3) would be poorly extracted [26]. However, the results obtained in this work concerning statin drugs, reveal significant deviations from available K_{OW} coefficients. The statin drugs show high $\log K_{OW}$ values (2.5–4.85). Nevertheless, most of the analytes, except simvastatin and lovastatin, were not extracted by SBSE.

To model the sorption constants of pharmaceutical compounds onto a stir-bar, a new approach considering their properties and the polarity of their functional groups is needed [27]. Because of this fact, K_{SW} was modelled by means of Eq. (2) using the pH dependent octanol–water distribution (D_{OW}), which considers the pK_a value and the ambient pH:

$$\log K_{SW} = 0.74 \times \log D_{OW} + 0.15 \quad (2)$$

For neutral moieties like lovastatin and simvastatin, D_{OW} and K_{OW} are identical:

$$\log D_{OW} = \log K_{OW} \quad (3)$$

In case of acidic moieties like atorvastatin, fluvastatin, pravastatin and rosuvastatin, Eq. (4) is true:

$$\log D_{OW} = \log K_{OW} + \log\left(\frac{1}{1+10^{pH-pK_a}}\right) \quad (4)$$

Taking into account the model described, $\log K_{SW}$ values obtained at pH 7 were 0.48, 1.40, 2.32, 2.33, 3.30 and 3.61 for rosuvastatin, pravastatin, fluvastatin, atorvastatin, lovastatin and simvastatin, respectively. These values could explain the poor recoveries obtained by SBSE for those analytes with $\log K_{SW}$ values lower than 2.5.

3.2. Comparison of SPE, DLLME and SBSE

The accuracy and precision of the optimized procedures were evaluated by analyzing in triplicate spiked pure water, river water and WWTP effluent. Samples were spiked at the concentration levels of 0.10 $\mu g\,L^{-1}$ for SPE and SBSE, and 0.27 $\mu g\,L^{-1}$ for DLLME. Results are shown in Table 3. Quantitative recoveries obtained with SPE were in the range from 74 to 93% in pure water, 46–97% in river water samples and 64–93% in effluent wastewater. Pravastatin and rosuvastatin were poorly extracted by DLLME, recoveries were lower than 17%, so that they could not be determined with the procedure developed. The recoveries obtained by DLLME showed a high dependence on the polarity of the analytes; those more polar were poorly extracted while the less polar, as lovastatin and simvastatin, were efficiently extracted (>82% from pure water, river water and wastewater). SBSE only allowed the extraction of lovastatin and simvastatin but with recovery rates up to 19 and 38%, respectively.

The precision of the method, estimated as relative standard deviation (RSD), varied in the range from 1.1 to 15.1% for SPE, 3.0–16.4% for DLLME and 3.4–12.0% for SBSE.

In general, the linearity of atorvastatin, fluvastatin, pravastatin and rosuvastatin in the three extraction methods was well fitted by a linear expression with coefficients of determination (r^2) up to 0.991 denoting a strong correlation between experimental measurements and fitted expressions. The remaining statins (lovastatin and simvastatin) adhered to non-linear equations, following a polynomic function. In the last cases, coefficients of determination (r^2) exceeded 0.95.

Method detection limits (MDLs) were estimated as the concentrations corresponding to a signal to noise ratio of 3. The method detection limits were in the range of 0.05–10.5 $ng\,L^{-1}$ and 0.04–11.2 $ng\,L^{-1}$ for SPE in wastewater and river water samples, respectively; 0.11–13.8 $ng\,L^{-1}$ and 0.10–17.0 $ng\,L^{-1}$ for DLLME in wastewater and river water samples, respectively; 0.52–0.80 $ng\,L^{-1}$ and 0.70–2.00 $ng\,L^{-1}$ for SBSE in wastewater and river water samples, respectively. In all of the extraction methods, MDLs of atorvastatin, lovastatin and simvastatin in the aqueous samples were lower than those of fluvastatin, rosuvastatin and pravastatin (Table 3).

Despite the limitations of having poorer recoveries than SPE, DLLME and SBSE offered several advantages. Both techniques are simple processes and require low organic solvent volumes. DLLME requires only 30 min for sample treatment, whereas SPE and SBSE require approximately 5 and 72 h, respectively. The total sample volume is lower with DLLME (9 mL) than with SPE (250 mL), facilitating greatly handling and storage. In SBSE, the stir-bar is re-usable whereas SPE cartridges are designed for single use applications.

The matrix in which the analytes are found and the extraction procedure are known to have a powerful effect on electrospray MS by altering the extent to which analytes of interest are ionized [9]. To investigate this phenomenon, we compared the chromatogram of a standard solution containing all statins (25 $\mu g\,L^{-1}$) with the chromatogram of an extract of wastewater and river water spiked after the extraction at the same concentration as the standard solution. The results were highly matrix dependent (Fig. 4). Ion enhancement was observed for lovastatin and simvastatin, while pravastatin, rosuvastatin and fluvastatin were considerably suppressed. Atorvastatin was largely unaffected by the matrix.

The greatest matrix effect was observed for most of the analytes in the SPE procedure. Samples extracted by SPE also coextracted significant amounts of other organic species that may influence in the ion enhancement and suppression. The matrix effect in wastewater samples, when DLLME was employed, was more pronounced for rosuvastatin and lovastatin than for the other statins.

3.3. Application

The optimized procedures were applied to the determination of the concentration levels of the statin drugs in the effluent of a WWTP and in river samples. Fig. 5 illustrates a LC-Q-TOF-MS chromatogram of a standard mixture of statins and an effluent sample of a WWTP. Simvastatin and lovastatin were clearly detected using SPE, DLLME and SBSE as extraction methods; simvastatin was present at mean concentration levels of 1.2 and 11.8 $ng\,L^{-1}$ in river water and effluent wastewater samples, respectively, and lovastatin at 4.3 $ng\,L^{-1}$ in effluent wastewater. However, only atorvastatin was detected by DLLME in effluent samples at 1.9 $ng\,L^{-1}$. The rest of statins were not detected in any of the samples analyzed.

It could be demonstrated that the three procedures yield similar results for the concentration levels of statin drugs in real samples and these levels were in accordance with data reported by several authors [2,4,6,8,21].

4. Conclusions

Three preconcentration techniques including solid phase extraction, dispersive liquid–liquid microextraction and stir-bar sorptive extraction were optimized for the analysis of six hypolipidaemic statin drugs (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin) by LC/Q-TOF-MS in river water and effluent samples of a WWTP. SPE showed the best results for most of analytes (74–93% in pure water, 46–97% in river samples and 64–93% in effluent wastewater). Pravastatin was poorly extracted by DLLME and could not be determined. SBSE only allowed the extraction of lovastatin and simvastatin but with recovery rates up to 19 and 38%, respectively, and an approach was used for modelling the sorption constants onto stir-bar of pharmaceutical compounds.

Despite the limitations of having poorer accuracy than SPE, DLLME and SBSE offer some advantages: both techniques are simple, present low matrix effects and require a low volume of organic solvents. Additionally, DLLME is less time consuming, whereas SBSE employs stir-bars which are re-usable.

To evaluate the applicability of the different procedures, effluent wastewater samples and river samples were analyzed and comparable concentration levels were obtained.

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References

[1] E. Zuccato, D. Calamari, M. Natangelo, R. Fanelli, Lancet 355 (2000) 1789.
[2] H.B. Lee, T.E. Peart, M. Lewina Svoboda, S. Backus, Chemosphere 77 (2009) 1285.
[3] J.B. Quintana, T. Reemtsma, Rapid Commun. Mass Spectrom. 18 (2004) 765.
[4] B. Vanderford, S.A. Snyder, Environ. Sci. Technol. 40 (2006) 7312.
[5] M.J. Benotti, R.A. Trenholm, B.J. Vanderford, J.C. Holady, B.D. Stanford, S.A. Snyder, Environ. Sci. Technol. 43 (2009) 597.
[6] X.S. Miao, C.D. Metcalfe, J. Chromatogr. A 998 (2003) 133.
[7] M. Dawod, M.C. Breadmore, R.M. Guijt, P.R. Haddad, J. Chromatogr. A 1217 (2010) 386.
[8] M.D. Hernando, A. Agüera, A.R. Fernández-Alba, Anal. Bioanal. Chem. 387 (2007) 1269.
[9] V.K. Balakrishnan, K.A. Terry, J. Toito, J. Chromatogr. A 1131 (2006) 1.
[10] R.I. Olariu, D. Vione, N. Grinberg, C. Arsene1, J. Liq. Chromatogr. 33 (2010) 1174.
[11] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcol. Sep. 11 (1999) 737.
[12] W. Buchberger, P. Zaborsky, Acta Chim. Slov. 54 (2007) 1.
[13] D.R. Klein, D.F. Flannelly, M.M. Schultz, J. Chromatogr. A 1217 (2010) 1742.
[14] E. Van Hoeck, F. Canale, C. Cordero, S. Compernolle, C. Bicchi, P. Sandra, Anal. Bioanal. Chem. 393 (2009) 907.
[15] A.R.M. Silva, F.C.M. Portugal, J.M.F. Nogueira, J. Chromatogr. A 1209 (2008) 10.
[16] A.V. Herrera-Herrera, M. Asensio-Ramos, J. Hernández-Borges, M.T. Rodríguez-Delgado, Trends Anal. Chem. 29 (2010) 728.
[17] S. Luo, L. Fang, X. Wang, H. Liu, G. Ouyang, C.T. Luan, J. Chromatogr. A 1217 (2010) 6762.
[18] J. Cheng, J. Xiao, Y. Zhou, Y. Xia, F. Guo, J. Li, Microchim. Acta 172 (2011) 51.
[19] H. Faraji, M. Helalizadeh, Int. J. Environ. Anal. Chem. 90 (2010) 869.
[20] M.A. Farajzadeh, D. Djozan, N. Nouri, M. Bamorowat, M.S. Halamzari, J. Sep. Sci. 33 (2010) 1816.
[21] E. Gracia-Lor, J.V. Sancho, F. Hernández, J. Chromatogr. A 1217 (2009) 622.
[22] M.S. Kostich, J.M. Lazorchak, Sci. Total Environ. 389 (2008) 329.
[23] J.M. Conley, S.J. Symes, M.S. Schorr, S.M. Richards, Chemosphere 73 (2008) 1178.
[24] M. Petrovic, M. Gros, D. Barcelo, J. Chromatogr. A 1124 (2006) 68.
[25] M. Pinxteren, A. Paschkeb, P. Poppa, J. Chromatogr. A 1217 (2010) 2589.
[26] N. Ochiai, K. Sasamoto, H. Kandaa, E. Pfannkochb, J. Chromatogr. A 1200 (2008) 72.
[27] M. Carballa, G. Fink, F. Omil, J.M. Lema, T. Ternes, Water Res. 42 (2008) 287.