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High-performance liquid chromatography quadrupole time-of-flight mass spectrometry method for the analysis of antidiabetic drugs in aqueous environmental samples

Julia Martín[a], Wolfgang Buchberger[b], Juan Luis Santos[a], Esteban Alonso[a], Irene Aparicio[a,*]

[a] Department of Analytical Chemistry, University of Seville, C/Virgen de África 7, E-41011 Seville, Spain
[b] Institute of Analytical Chemistry, Johannes-Kepler-University, Altenbergerstrasse 69, A-4040 Linz, Austria

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ABSTRACT

Antidiabetic compounds are among the most prescribed pharmaceuticals. Nevertheless, their presence in the environment has been scarcely evaluated as there is no method for their determination in environmental samples. This paper reports the development of an analytical method for the determination of traditionally used antidiabetics (metformin and glibenclamide) and novel antidiabetics (vildagliptin, sitagliptin and pioglitazone). The method is based on solid-phase extraction and determination by high-performance liquid chromatography quadrupole time-of-flight mass spectrometry. The method was applied to effluent wastewater, river water and tap water. Mean recoveries of glibenclamide, vildagliptin, sitagliptin and pioglitazone in the matrices evaluated were in the range 78–83%; limits of quantification were in the range 0.4–4.3 ng L^{-1}; and precision values were in the range 2.2–13%. The high hydrophilicity and polarity of metformin complicated its simultaneous extraction. Chromabond Tetracycline cartridges and sample pH 8.5 were applied to the extraction of glibenclamide, vildagliptin, sitagliptin and pioglitazone. Oasis HLB cartridges, neutral sample pH and SDS as ion-pair reagent were used for the extraction of metformin. Validation results of metformin were not as favorable as those of the other antidiabetic drugs but were comparable with others previously reported. The developed method was applied to the first-time determination of the concentrations of the five antidiabetic drugs in wastewater, river water and tap water. Metformin was the antidiabetic drug at the highest concentration in wastewater and surface water (up to 253 ng L^{-1} and 104 ng L^{-1}, respectively). Two of the antidiabetic drugs of recent prescription, sitagliptin and vildagliptin, were found in effluent wastewater at concentrations of 117 ng L^{-1} and 12 ng L^{-1}, respectively, and in river water at concentrations of 35 ng L^{-1} and 6 ng L^{-1}, respectively, whereas the classic antidiabetic drug glibenclamide and the novel drug pioglitazone were not detected.

1. Introduction

Type-2 diabetes mellitus is a long-term metabolic disorder wherein the body becomes resistant to the effects of insulin, a hormone that regulates sugar absorption. According to the American Diabetes Association, this disease affects up to 45% of individuals above 65 year-old, and involves at least 90% of diabetes patients above 20 year-old [1]. The most preferred option to treat this disease is to decrease glucose levels in blood by administration of antidiabetic drugs. Currently, metformin is the most commonly prescribed drug for the treatment of type 2 diabetes [2–5] and, together with acetylsalicylic acid and paracetamol, one of the most prescribed pharmaceutical compounds [6]. However, for many patients, the monotherapy with an oral antidiabetic drug is not sufficient to reach an optimal glycemic control. Sulfonylureas (glibenclamide), thiazolidinedione derivatives (pioglitazone) and gliptins (inhibitors of dipeptidyl peptidase-IV, such as vildagliptin and sitagliptin) are then used. They are novel oral antihyperglycemic drugs which are administered for the treatment of type-2 diabetes in combination with the traditional drugs [7–10]. The drugs can be administered separately or mixed in a single tablet.

The presence of pharmaceutical residues in the aquatic environment has been recognized as one of the most urgent emerging environmental issues. In the last decade, several analytical methods have been developed to investigate the occurrence in the aquatic environment of some of the 3000 pharmaceutical compounds approved for human usage [11]. Analytical methods reported allow

* Corresponding author. Tel.: +34 9 5455 2858; fax: +34 9 5428 2777.
E-mail address: iaparicio@us.es (I. Aparicio).

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the determination of pharmaceutical compounds from several therapeutic categories such as beta-blockers, antibiotics, antiinflammatory drugs, antidepressants and lipid regulators [12–15] but there is a lack of methodologies for the analysis of antidiabetic drugs. Because antidiabetic drugs are used for the treatment of a chronic disease, they are continuously being released to the environment through wastewater discharges from wastewater treatment plants. At present, the few methods reported for the determination of antidiabetic pharmaceuticals in the aquatic environment are limited to the individual determination of the classic antidiabetic drugs metformin [2,16–19] and glibenclamide [20–22] and, recently, to the simultaneous determination of both compounds [12]. Methods reported for the determination of new antidiabetic drugs are restricted to their individual determination in biological matrices [9,10,23,24] or in pharmaceutical preparations [8,25,26]. Due to the physical–chemical properties of metformin, such as its high polarity, conventional methods may result in poor extraction recoveries and poor chromatographic retention. To allow its determination, several of the methods reported for the determination of metformin in plasma propose the use of ion-pair solid phase extraction (IP-SPE) [3,27], hydrophilic interaction liquid chromatography (HILIC) [28] or normal-phase chromatography [29]. Unfortunately, such chromatographic stationary phases are suitable for highly polar compounds but not for the determination of the other antidiabetic drugs which are not so polar as metformin.

The aim of this study was to develop a method for the first-time determination of two classic (metformin and glibenclamide) and three novel antidiabetic drugs (vildagliptin, sitagliptin and pioglitazone) in aqueous environmental samples (wastewater, river water and tap water). The novel antidiabetic drugs selected are the most consumed new-generation antidiabetics [30,31]. The analytical method involves sample pre-treatment by SPE and determination by high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (HPLC/Q-TOF MS). Q-TOF MS, instead of triple quadrupole mass spectrometry (QqQ MS), was employed to minimize the risk of false positive results. Method applicability is tested by the determination of the antidiabetic drugs in effluent wastewater from sewage treatment plants, river water and tap water.

2. Experimental

2.1. Materials and reagents

Antidiabetics were extracted from drug formulations. Glibenclamide was extracted from Glucolón 100 mg (Laboratorio Generfarma, S.L. (Valencia, Spain)); metformin was extracted from Metformin 1000 mg (Sandoz Farmacéutica, S.A. (Madrid, Spain)); pioglitazone was extracted from Actos 15 mg (Takeda Global Research and Development Centre (London, United Kingdom)); rosiglitazone was extracted from Avandia 8 mg (SmithKline Beecham plc (Brentford, United Kingdom)); sitagliptin was extracted from Januvia 100 mg (Merck Sharp & Dohme Ltd. (Hertfordshire, United Kingdom)) and vildagliptin was extracted from Jalra 50 mg (Novartis Europharm Limited (West Sussex, United Kingdom)). Eventual small deviations of the contents from the declared values given for the pharmaceutical formulations were neglected within this work. The chemical structures of each drug are shown in Table 1. Rosiglitazone was used as Internal Standard (I.S.). Film tablets were finely grounded to extract the active agents. An appropriate amount of the ground tablets was weighed and mixed with methanol to obtain a stock solution of 1000 $\mu g\,mL^{-1}$ of each antidiabetic compound. The suspension was treated in an ultrasonic bath for 10 min and filtered through a 0.45 μm pore-size syringe filter. The standard solution, containing a mixture of the antidiabetics at a concentration level of 1 $\mu g\,mL^{-1}$ in each drug, was prepared in methanol. This solution was diluted again with methanol to obtain the final working solutions.

Acetonitrile (ACN), formic acid and methanol (all of chromatographic analysis grade) were purchased from JT Baker (Deventer, The Netherlands). Ammonium formate was purchased from Sigma–Aldrich (Vienna, Austria). Sodium dodecyl sulfate (SDS) and sodium hydroxide were purchased from Fluka (Buchs, Switzerland). Chromabond Tetracycline, Oasis HLB and Supelclean C18 cartridges were purchased from Machery–Nagel (Düren, Germany), Waters (Milford, MA, USA), and Supelco (Bellefonte, PA, USA), respectively. Strata-X and Strata-XCW were purchased from Phenomenex (Aschaffenburg, Germany)

2.2. Sample collection

Effluent wastewater samples, used to test method applicability, were collected in January 2011 from a wastewater treatment plant located in the region of Linz (Austria). River samples were collected in January 2011 from Danube River (Linz, Austria). Tap water samples were collected from Linz. Samples were collected in brown bottles pre-cleaned with acetone and methanol. In order to stabilize samples, ACN was immediately added after sampling to achieve a final concentration of 0.5% (v/v). Stabilized samples were stored at 4 °C. Prior to extraction, samples were filtered through a 0.45 μm glass fiber membrane filter (Whatman, Mainstone, UK). Rosiglitazone was added to filtered samples, as internal standard, to achieve a concentration of 0.2 $\mu g\,L^{-1}$.

2.3. Instrumentation

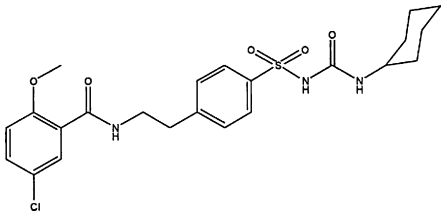
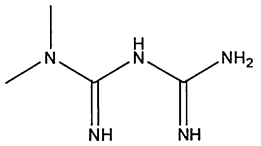
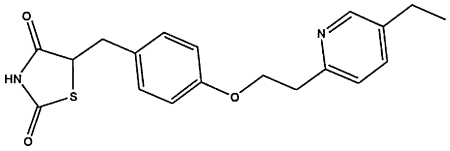
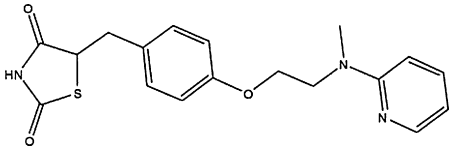
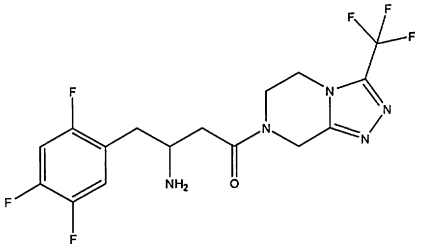
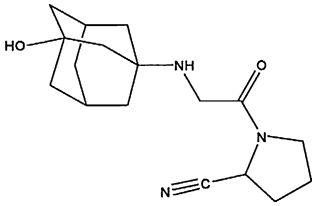
2.3.1. High performance liquid chromatography

Chromatographic analyses were performed on an HPLC 1100 system equipped with a vacuum degasser, a quaternary pump and an autosampler (all from Agilent, Palo Alto, CA, USA). Separations were carried out using a LiChrospher® 100 RP-18 (125 mm × 4 mm i.d.; 5 μm particle size) column (Agilent). The injection volume was 20 μL. Analytes were separated at a flow-rate of 0.7 $mL\,min^{-1}$ by gradient elution with ACN (containing formic acid 0.1%, v/v) (solvent A) and an aqueous 10 mM ammonium formate solution (containing formic acid 0.1%, v/v) (solvent B). The gradient elution program was: 0–2 min: 10% solvent A, 2–3 min: linear increase to 50% A, 3–8 min: linear increase to 90% A, 8–10 min: 90% A, 10–10.1 min: linear decrease to 10% A with a final hold at 10% A until 12.1 min. The column was thermostated at 25 °C.

2.3.2. Mass spectrometry

MS measurements were done in a 6520 quadrupole/time-of-flight (Q-TOF) instrument equipped with an electrospray ionization source (Agilent, Palo Alto, CA, USA). Ionization of analytes was carried out using 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. The instrument was operated in the 4 GHz high-resolution mode. At 4 GHz, these analyzers typically demonstrate a resolution of at least 10,000 at m/z 118 and at least 20,000 at m/z 1522. Mass axis was calibrated over the 70–3200 m/z range using the mixture provided by the manufacturer. A sprayer with a reference solution was used for continuous calibration in the positive ionization mode using the reference masses: 121.05 m/z and 922.01 m/z. Optimization of Q-TOF MS parameters involved the determination of the best ESI mode (positive or negative), cone voltage and the best fragmentation pattern for each compound. For higher sensitivity, product ions were monitored in the range of 5–40 eV. The two most intense product ions were selected for the analysis. Table 2 summarizes the optimized Q-TOF MS

Table 1
Chemical characterization of the antidiabetic drugs.

Name	Structure	Formula	CAS	M.W. (g/mol)	pK_a
Glibenclamide		$C_{23}H_{28}N_3ClO_5S$	10238-21-8	493.1438	6.8
Metformin		$C_4H_{11}N_5$	657-24-9	129.1014	10.3
					12.3
Pioglitazone		$C_{19}H_{20}N_2O_3S$	111025-46-8	356.4387	5.8
					6.4
Rosiglitazone (I.S.)		$C_{18}H_{19}N_3O_3S$	122320-73-4	357.4268	6.8
					6.1
Sitagliptin		$C_{16}H_{15}F_6N_5O$	486460-32-6	407.1181	7.7
Vildagliptin		$C_{17}H_{25}N_3O_2$	274901-16-5	303.3993	Not available

conditions for the analysis of the antidiabetic drugs. The adduct ion $[M+H]^+$ was used as the precursor ion for Q-TOF MS analysis in the positive-ionization mode. The first product ion was used for quantification and the second for confirmation. Q-TOF MS parameters were optimized by direct infusion of individual solutions containing 1 $\mu g\,mL^{-1}$ of each analyte at a flow rate of 0.5 $mL\,min^{-1}$ using an external syringe pump (Model 22; Harvard Apparatus, South Natick, MA, USA).

Table 2
Optimized parameters of the Q-TOF-MS determination.

Antidiabetic drug	Retention time (min)	Precursor ion (m/z)	Product ions (m/z)	Fragmentor (V)	Collision energy (eV)	IDL[a] ($\mu g\,L^{-1}$)
Metformin	2.18	130.1087	71.0619[b]; 60.0541	90	20	0.28
Vildagliptin	5.61	304.2020	154.0993[b]; 97.0738	120	10	0.07
Sitagliptin	6.61	408.1254	235.0798[b]; 193.0685	120	15	0.16
Pioglitazone	6.89	357.1267	134.0914[b]; 119.0746	150	30	0.06
Rosiglitazone (I.S.)	7.37	358.1220	135.0917[b]; 94.0677	130	25	0.10
Glibenclamide	8.56	494.1511	169.0027[b]; 171.0037	100	10	0.20

[a] Instrumental detection limit.
[b] Product ion used for quantification.

2.4. Solid phase extraction

Several solid phase materials (Chromabond Tetracycline, Oasis HLB, Supelclean C18, Strata-X and Strata-XCW cartridges), sample pH (neutral and basic pH) and the use of an ion pair reagent were tested for SPE optimization as it is described below in Section 3. The extraction conditions selected are those described here. Two extraction procedures were used, one for the extraction of glibenclamide, vildagliptin, sitagliptin and pioglitazone and another for the extraction of metformin. Chromabond Tetracycline cartridges were employed for the extraction of glibenclamide, vildagliptin, sitagliptin and pioglitazone. Chromabond Tetracycline cartridges were conditioned using 10 mL of methanol followed by 5 mL of HPLC-grade water. Oasis HLB cartridges were employed for the extraction of metformin. Oasis HLB cartridges were conditioned using 10 mL of methanol, 5 mL of HPLC-grade water and 5 mL of aqueous 2 mM SDS solution (ion pair reagent). Prior to extraction with Chromabond Tetracycline cartridges, the pH of the aqueous samples (wastewater, river water or tap water) was adjusted to 8.5 by addition of 0.1 M sodium hydroxide solution. No pH adjustment was necessary when using Oasis HLB cartridges. Therefore, 250 mL of the sample were percolated 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 washed with another 5 mL of HPLC-grade water and dried for 10 min. The analytes were eluted using four successive aliquots of 1 mL of methanol at a flow-rate of about 1 mL min^{-1}. The eluates were collected in 10-mL collection tubes and evaporated at room temperature to dryness by a gentle nitrogen stream. Then, the extracts were reconstituted in 1 mL of methanol.

2.5. Method validation

Calibration curves were constructed by spiking tap water with the antidiabetic drugs at concentrations ranging from 0.2 to 200 ng L^{-1}. The internal standard method was used for quantification. The quantification of metformin by the internal standard method was corroborated by applying the standard addition method at two spiking levels, 100 and 200 ng L^{-1}. To evaluate the precision and accuracy of the method, effluent wastewater, river water and tap water samples were spiked in triplicate. Limits of detection (LODs) and limits of quantification (LOQs) of the analytes were calculated in effluent wastewater, river water and tap water as the concentrations of analyte corresponding to a signal-to-noise ratio of three and ten, respectively. Product ions used for quantification can be seen in Table 2. Effluent wastewater, river water and tap water samples were analyzed to evaluate the applicability of the method. Peak areas of quantitation ions (Table 2) were used for quantification purposes. Recoveries were calculated by comparison the peak areas of samples spiked before extraction with those of unspiked samples and those of a standard solution at the same concentration level after extraction and concentration (50 μg L^{-1} in each antidiabetic drug). Matrix effect was calculated by comparison the peak areas of spiked sample extracts with those of a standard solution at the same concentration level (50 μg L^{-1} in each antidiabetic drug).

3. Results and discussion

3.1. HPLC-Q-TOF MS

The main chromatographic challenge was the determination of metformin because its high hydrophilicity and polarity. To solve this problem, the use of hydrophilic interaction liquid chromatography (HILIC) [28] or normal-phase chromatography [29] has been proposed. Nevertheless, such conditions are not expected to be suitable for the simultaneous determination of metformin and the other antidiabetic drugs which are not as hydrophilic and polar as metformin. Table 2 summarizes the optimized Q-TOF MS conditions for the analysis of the antidiabetic drugs. The adduct ion $[M+H]^+$ was used as precursor ion for Q-TOF MS analysis in the positive-ion mode. Fig. 1 shows mass spectra of each compound together with the proposed structures of each product ion. Fig. 2 shows HPLC Q-TOF MS chromatograms of a standard solution, a wastewater effluent sample and a river water sample. Peak tailing observed for metformin in effluent wastewater chromatograms did not significantly affect metformin quantification.

3.2. Optimization of SPE procedure

The analytical procedure was optimized using 250 mL aliquots of milli-Q water spiked at 0.2 μg L^{-1} in each antidiabetic compound. The type of solid phase material, sample pH and the use of an ion pair reagent were tested to improve extraction. In each case, the procedure was carried out in triplicate.

3.2.1. Solid phase material

A wide variety of solid phase materials, including Chromabond Tetracycline, Oasis HLB, Strata-X, Strata-XCW and Supelclean C18, were tested to find the most efficient extraction cartridge. Cartridges were previously conditioned with 10 mL of methanol followed by 5 mL of HPLC grade water and then samples at neutral pH were percolated. The elution was performed with four 1-mL aliquots of methanol. Mean recoveries achieved with each cartridge are summarized in Fig. 3. The best extraction recoveries were achieved with Chromabond Tetracycline and Oasis HLB cartridges. No cartridge allowed the optimum extraction of all the antidiabetics. Chromabond Tetracycline cartridges were the most adequate for the extraction of pioglitazone, rosiglitazone and glibenclamide whereas Oasis HLB cartridges were the most adequate for the extraction of metformin, sitagliptin and vildagliptin. Supelclean, Strata-X and Strata-XCW were discarded because the poor recoveries obtained.

3.2.2. Sample pH

The influence of sample pH (pH 7 and pH 8.5) in SPE with Chromabond Tetracycline and Oasis HLB cartridges was evaluated. Extraction recoveries achieved with each SPE cartridge and sample pH are shown in Fig. 4a. The best extraction recoveries, up to 90% except for metformin, were achieved with samples at basic pH using Chromabond Tetracycline cartridges. Nevertheless, metformin and vildagliptin were better extracted using Oasis HLB cartridges and samples at neutral pH than using Chromabond Tetracycline cartridges. Sample pH affected extractions with Chromabond Tetracycline in a lower extent than extractions with OASIS HLB cartridges. In addition, ion suppression of the analytes in ESI-MS in real samples was lower when Chromabond Tetracycline cartridges instead of Oasis HLB cartridges were used. Ion suppression by matrix effect was evaluated by comparison the chromatogram of a standard solution containing all the antidiabetic drugs (50 μg L^{-1}) with the chromatograms of sample extracts spiked at the same concentration as the standard. Oasis HLB cartridges have been designed by Waters (Milford, MA, USA) for the extraction of analytes in a wide range of polarities and pH values. This fact can results in the adsorption of many matrix components resulting in high matrix effects in electrospray ion source. Because of that, Chromabond Tetracycline cartridges and sample pH 8.5 were selected for the extraction of the antidiabetic drugs, except

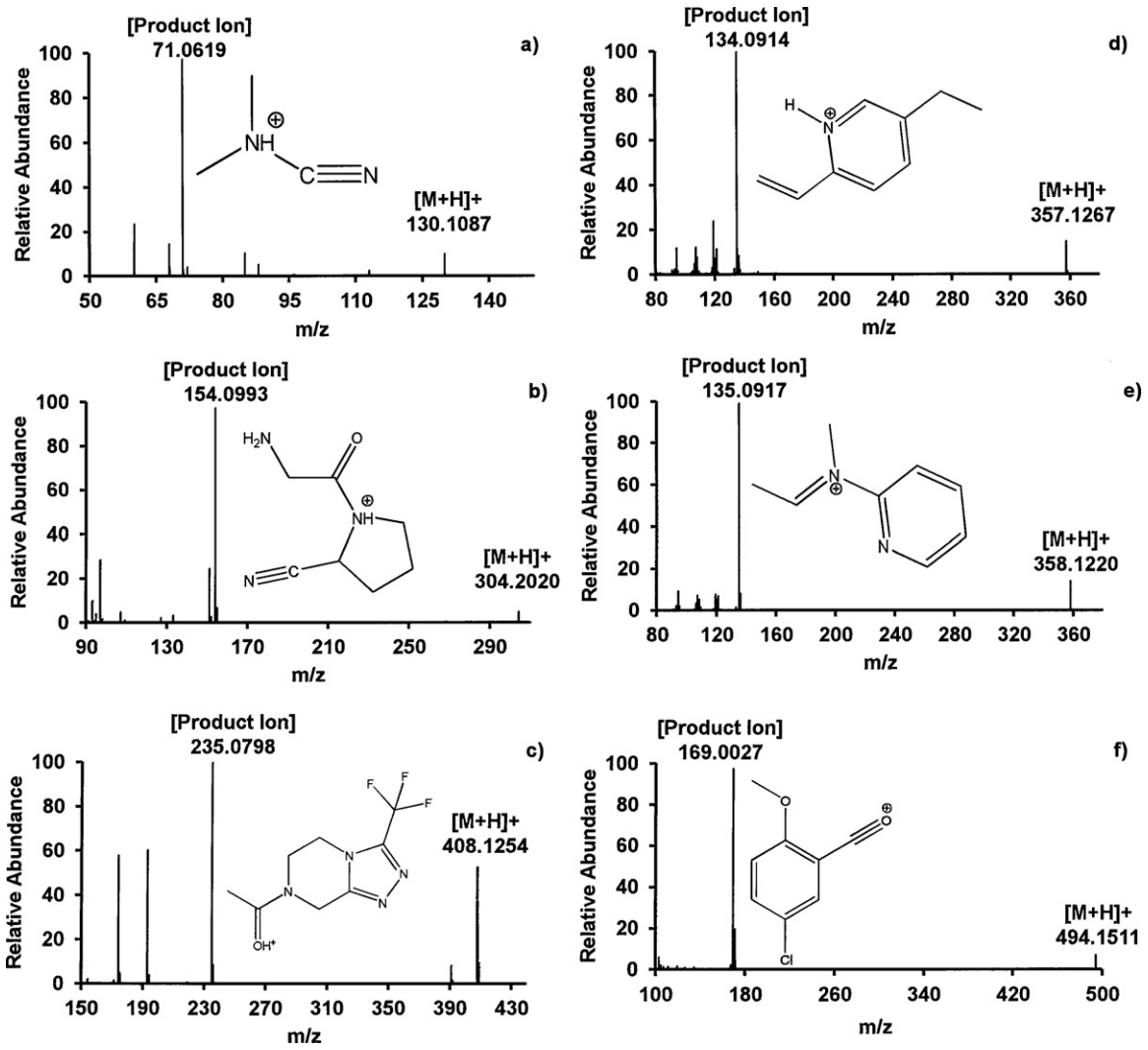


Fig. 1. Product-ion mass spectra of $[M+H]^+$ of (a) metformin, (b) vildagliptin, (c) sitagliptin, (d) pioglitazone, (e) rosiglitazone (I.S.), and (f) glibenclamide.

for metformin. Metformin was extracted from samples at neutral pH using Oasis HLB cartridges.

3.2.3. *Effect of SDS*

Metformin is an aliphatic compound with a low molecular weight, a very high polarity (octanol–water partition coefficient: 0.01) and a high pK_a value. In the environment, metformin is mainly present as a double charged cation. Only at very high pH values, metformin is predominantly in its neutral form. Due to the physical–chemical properties of metformin, conventional analytical methods for trace analysis yield poor recoveries [2–4]. Therefore, in order to improve the problematic extraction of metformin, the use of an ion-pair reagent in the SPE procedure was tested. The cartridges were equilibrated, after conditioning, with SDS as ion-pair (IP) reagent by passing through the cartridge 5 mL of a 2 mM SDS solution. Upon loading the sample, metformin forms a complex with the retained SDS. The complex is less polar than pure metformin and can be easily eluted using methanol. Using SDS as ion-pair reagent and Oasis HLB cartridges, recovery of metformin was increased to 64% (Fig. 4b). However, the use of SDS decreased slightly, from 33 to 27%, the recovery of rosiglitazone and significantly, from 100% to 68%, the recovery of vildagliptin. The use of SDS with Chromabond Tetracycline cartridges did not significantly improve metformin recovery and decreased the recoveries of the other antidiabetics (mean recoveries 64% using SDS and 84% without using SDS). Therefore, the best conditions for the extraction of metformin were extracting samples at neutral pH with Oasis HLB cartridges using SDS as ion pair. The best conditions for the extraction of the other antidiabetics included adjusting sample to pH 8.5 and Chromabond Tetracycline cartridges without the use of an IP reagent (Fig. 4b).

3.3. *Method performance*

Method accuracy (expressed as recovery percentage) and precision (expressed as repeatability in terms of relative standard deviation (% RSD)) were evaluated by recovery experiments of the target compounds in spiked real samples. Samples were spiked with a standard solution at a concentration level of 200 ng L^{-1} in each compound and extracted using Chromabond Tetracycline and Oasis HLB cartridges. Experiments with each type of sample were performed in triplicate. The concentrations obtained from the peak areas and calibration curves of the quantitation ions were compared to the spiked concentrations. Blank samples (unspiked) were also extracted to determine the concentration of analytes present in the sample before spiking. Results are shown in Table 3. Good recoveries were obtained for most of the analytes (60–84% from effluent wastewater, 79–91% from river water and 80–87% from tap water). A small signal suppression was observed in tap water, river water and wastewater for glibenclamide (−9%, −11% and −23%,

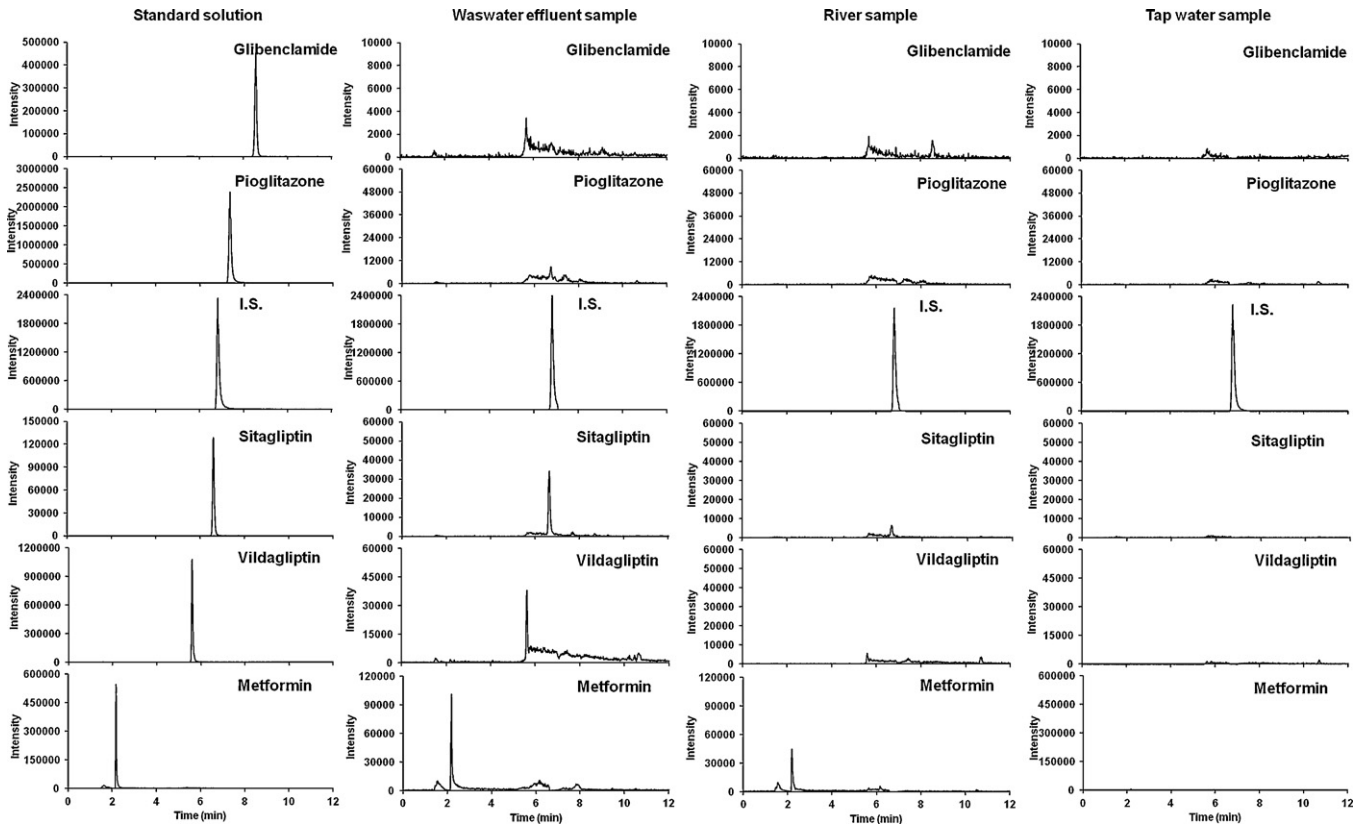


Fig. 2. HPLC Q-TOF MS chromatograms of a standard solution containing 0.1 μg mL^{-1} of each antidiabetic drug, wastewater effluent, river water and tap water (blank sample).

respectively), pioglitazone (−8%, −11% and −16%, respectively), sitagliptin (−10%, −9% and −4%, respectively) and rosiglitazone (−3%, −2% and −1%, respectively). A small signal enhancement was observed for vildagliptin in tap water (10%), river water (14%) and wastewater (16%) and for metformin in tap water (5%). The main matrix effects were observed for metformin in river water (32%) and in effluent wastewater (44%) probably due to its low retention factor. Precision of most of the antidiabetic drugs, in terms of relative standard deviation, was in the ranges from 7.3 to 13% in effluent wastewater, 8.3–13% in river water and 2.2–8.1% in tap water (Table 3). Linearity was determined from peak areas using regression analysis. Data were well fitted by a linear expression with coefficients of correlation (r^2) of 0.993, 0.997, 0.999, 0.999, 0.999 for metformin, glibenclamide, vildagliptin, sitagliptin and pioglitazone, respectively. Good sensitivity was observed with the proposed method with LOQs in the range of 0.4–32.3 ng L^{-1} in real samples (Table 3).

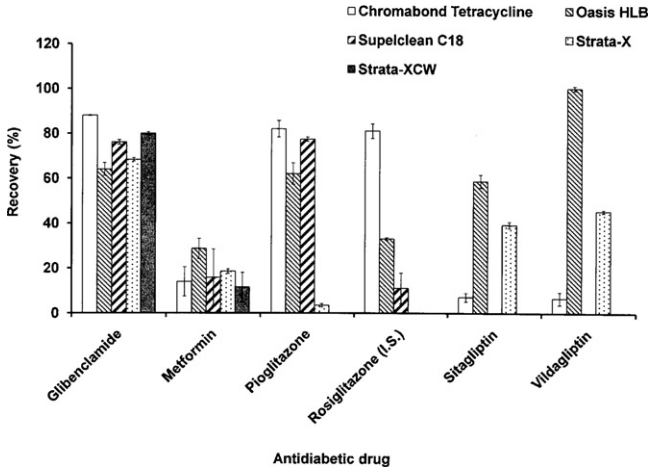


Fig. 3. Influence of solid phase material on SPE recoveries. Samples were percolated at neutral pH and methanol was used as elution solvent (n = 3).

3.4. Analysis of real samples

The optimized method was applied to the first-time determination of the concentrations of the five antidiabetic drugs in wastewater effluent, in river water and in tap water (n = 3). Retention times were used for the identification of the compounds and mass spectra were used for confirmation. No pure standards were used for quantification so concentrations given in this section should be considered just as preliminary information about the presence of antidiabetic drugs in tap water, river water and wastewater. Metformin, sitagliptin and vildagliptin were detected in effluent wastewater at mean concentrations of 253 ng L^{-1}, 117 ng L^{-1} and 12 ng L^{-1}, respectively, and in river water at mean concentrations of 104 ng L^{-1}, 35 ng L^{-1} and 6 ng L^{-1}, respectively. The standard addition method was applied to corroborate metformin determination because its poor between-sample variation due to matrix effects (Table 3). The application of the standard addition method confirmed the quantification of metformin by the internal standard method proposed. Glibenclamide and pioglitazone were not detected in any sample. None of the antidiabetic drugs was detected in tap water. These concentrations are consistent to the extensive prescription of metformin compared to the prescription of the other antidiabetic drugs. The novel antidiabetic drugs, sitagliptin and vildagliptin were found in effluent wastewater and in river water whereas the classical antidiabetic glibenclamide was not detected. This fact shows the significance of the analytical method developed which allows the determination of the new antidiabetic drugs which are present in the aquatic

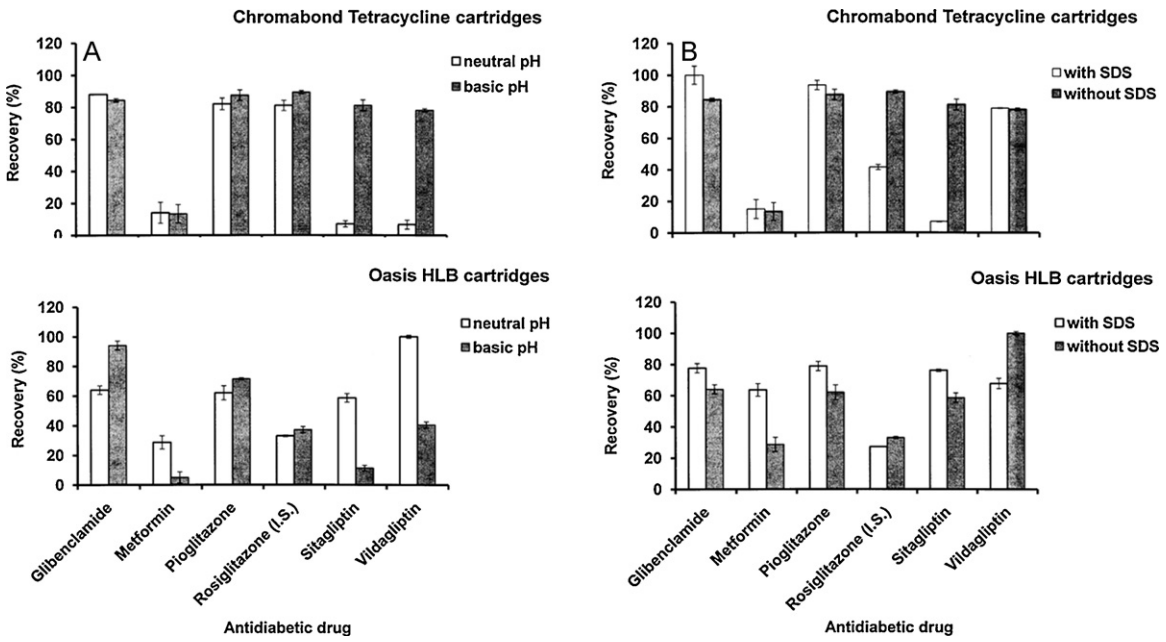


Fig. 4. Influence of sample pH (a) and SDS as ion pair reagent (b) on SPE recoveries using Oasis HLB and Chromabond Tetracycline cartridges ($n = 3$).

Table 3
Recovery, precision (% RSD) and limits of quantification (LOQ) of the optimized method in effluent wastewater, river water and tap water.

Antidiabetic drug	Effluent wastewater ($n = 3$)			River water ($n = 3$)			Tap water ($n = 3$)		
	Recovery (%)	RSD (%)	LOQ (ng L^{-1})	Recovery (%)	RSD (%)	LOQ (ng L^{-1})	Recovery (%)	RSD (%)	LOQ (ng L^{-1})
Glibenclamide	73	7.3	4.3	81	12	3.9	80	6.0	4.0
Metformin	54	19	8.2	31	27	14	16	28	32
Pioglitazone	84	7.6	1.1	82	9.6	1.2	83	2.2	1.1
Sitagliptin	84	13	3.0	79	13	3.2	81	8.1	3.1
Vildagliptin	60	8.2	0.6	91	8.3	0.4	87	5.5	0.4

media at concentrations higher than the classical antidiabetic drug glibenclamide.

4. Conclusions

In this paper, we describe the optimization of an analytical method for the determination of two classic (metformin and glibenclamide) and three novel (vildagliptin, sitagliptin and pioglitazone) antidiabetic drugs in aqueous environmental samples. Extraction of glibenclamide, vildagliptin, sitagliptin and pioglitazone is carried out by solid-phase extraction with Chromabond Tetracycline cartridges and sample pH 8.5 whereas extraction of metformin required the use of Oasis HLB cartridges, neutral sample pH and SDS as ion-pair agent. The method was successfully applied to effluent wastewater, river water and tap water. Good recovery rates (mean values: 75% in effluent wastewater, 83% in river water and 83% in tap water samples) and precision were achieved for most of the analytes, except for metformin. The low retention factor of metformin obtained in reverse-phase chromatography resulted in high matrix effect. Nevertheless, validation parameters of metformin were similar to those reported by other authors. The advantage of using reverse-phase chromatography for the determination of metformin, instead of normal-phase or ion exchange chromatography as several authors propose, is to employ the same chromatographic conditions as for the determination of the other antidiabetic drugs. The applicability of the method was tested by the first time determination of the novel antidiabetic drugs vildagliptin, sitagliptin and pioglitazone in aqueous environmental samples. The concentrations of the novel antidiabetic drugs sitagliptin and vildagliptin obtained in this preliminary study were lower than those of the classic antidiabetic drug metformin (117 ng L^{-1}, 12 ng L^{-1} and 253 ng L^{-1} in wastewater, respectively, and 35 ng L^{-1}, 6 ng L^{-1} and 104 ng L^{-1} in river water, respectively). This fact shows the necessity of analytical methods for the determination of such novel drugs in environmental samples. Pioglitazone and glibenclamide were not detected in the samples.

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