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Determination of antihypertensive and anti-ulcer agents from surface water with solid-phase extraction–liquid chromatography–electrospray ionization tandem mass spectrometry

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ABSTRACT

Pharmaceuticals are emerging contaminants in surface water and they must be measured to follow their effects on the aquatic environment. We developed a solid-phase extraction and liquid chromatography–electrospray ionization tandem mass spectrometry (SPE-LC–ESI-MS/MS) method for the determination of twenty-six pharmaceutical compounds – which belong to antihypertensive and anti-ulcer agents – from surface water samples. The selection of pharmaceuticals was based on usage frequency in Hungary. During method development Oasis HLB, SampliQ Polymer SCX and Si-SCX SPE cartridges were tested. As LC eluent ammonium formate, ammonium acetate buffers at pH 3 and 5 were investigated and for quantitation both matrix-matched and internal standard calibration was used. For matrix effect assessment post-extraction spike method was applied which can separate the extraction efficiency from ion suppression for better determination of recovery. Method detection limits (MDLs) varied between 0.2 and 10 ng/L. Precision of the method, calculated as relative standard deviation (RSD), ranged from 0.2 to 14.6% and from 1.2 to 22.4% for intra- and inter-day analysis, respectively. The method was applied to analyze Danube water samples. Measured average concentrations varied between 2 and 39 ng/L for eleven compounds and another one could be detected under LOQ.

1. Introduction

Due to growing consumption, improper disposal of unused or expired drugs and disability of waste water treatment plants to remove them entirely, pharmaceuticals are emerging contaminants in the environment [1,2]. Huge amount of drugs are used in medical and veterinary treatment. After excretion they can be found in the environment either in their parent forms or as metabolites or as transformation products, generated during the waste water treatment [3].

Acute toxicity is not the biggest concern, but over long periods of time the continuous inflow of pharmaceuticals in surface water even at low levels could cause changes in organisms. Moreover, mixtures of pharmaceuticals could also have even stronger negative impact on aquatic fauna and flora [4,5].

Consequently there is a need for reliable analytical methods, which enable the sensitive and selective determination of these substances, even at trace levels. Several methodologies are already available for the determination of different kinds of pharmaceuticals in surface and waste waters. Among them there are a few groups which are fairly well investigated such as antibiotics [6–10], endocrine disruptors [11–13], non-steroidal anti-inflammatories [14–17], psychiatric drugs [18,19] and X-ray contrast media [20–22]. Nevertheless, there are other increasingly applied types of pharmaceuticals, like antihypertensive drugs, which should also be studied.

Measuring polar compounds, such as polar pharmaceuticals and their even more polar metabolites, is a kind of challenge: applying GC–MS they have to be derivatized, which is very time-consuming and generally compromises the reliability of the method, while applying LC–MS derivatization can be avoided but one has to deal with matrix effects during the atmospheric pressure ionization of the molecules [23–28]. The already existing methods for pharmaceutical residue analysis are either based on gas chromatography–mass spectrometry [29–31] or liquid chromatography–tandem mass spectrometry [18,19,32–38], but there is a growing tendency of applying LC–MS/MS, due to its unique selectivity and sensitivity.

There are two common methods to assess matrix effects [39]: the post-column infusion method, defined by Bonfiglio et al. [40], and the post-extraction spike method, proposed by Matuszewski et al. [41,42]. The post-column infusion method provides a qualitative assessment of matrix effects, identifying chromatographic regions most likely to experience matrix effects [43]. In contrast,

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the post-extraction spike method quantitatively assesses matrix effects by comparing the response of an analyte in neat solution to the response of the analyte spiked into a blank matrix sample that has been carried through the sample preparation process. In this manner, quantitative effects on ion suppression or enhancement experienced by all analytes in the sample can be measured. So far, there are mostly bioanalytical methods applying the latter procedure to assess matrix effects [41,42,44] but environmental samples are also matrix loaded therefore it is worth to extend this approach for handling large volumes of surface water samples.

We developed a solid-phase extraction–liquid chromatography–electrospray ionization tandem mass spectrometry method for the determination of twenty-six basic pharmaceutical compounds. Since in Hungary the most often prescribed drugs are antihypertensive and anti-ulcer agents the following types of pharmaceuticals were chosen: four H_2-receptor antagonists, three proton pump inhibitors, nine β-blockers, three selective calcium-channel blockers, three angiotensin-converting enzyme inhibitors and four HMG-CoA reductase inhibitors (also called statins). During method development we evaluated four different solid-phase extraction methods with the application of three different kinds of sorbents (one silica-based and two polymeric types) and compared the effects of different modifiers and pH of the mobile phase. For matrix effect assessment post-extraction spike method was applied. Quantitation was done with internal standard calibration, with the application of three deuterium-labeled internal standard combined with matrix-matched calibration. The method performance parameters such as linearity, accuracy, precision and limit of detection and quantitation were determined before the method was applied to river water samples.

2. Material and methods

2.1. Chemicals and materials

All pharmaceutical standards were of high purity grade (>90%). Acebutolol, atenolol, betaxolol, carvedilol, cimetidine, esmolol, metoprolol, nifedipine, nizatidine, oxprenolol, propranolol and sotalol were purchased from Sigma-Aldrich (Hungary). Atorvastatin–calcium, famotidine, lisinopril·$2H_2O$, lovastatin, pantoprazole–sodium, ranitidine·HCl, ramipril and simvastatin were from Wessling NCo. by courtesy. Atenolol-d_7, enalapril-d_5 and lansoprazole-d_4 were purchased from CDN Isotopes (Quebec, Canada). Nimodipine and omeprazole were from Calbiochem (Darmstadt, Germany). Fluvastatin–sodium was from USP (Rockville, MD). Amlodipine besylate and enalapril maleate were from Richter Gedeon Co. by courtesy. Lansoprazole was from LGC Standards (Wesel, Germany).

Acetonitrile, methanol of HPLC gradient grade quality; acetone, n-hexane and dichloromethane for gas chromatography; diethyl-ether and ethyl-acetate for chromatography were from Merck (Darmstadt, Germany). Water was deionized in our laboratory using a Millipore (Billerica, MA, USA) Milli-Q water purification system. Ammonium formate (cryst. Extra pure, Ph Eur), ammonium acetate (cryst. Extra pure, Ph Eur), formic acid (Extra pure, Ph Eur) and acetic acid (Extra pure, Ph Eur) were form Merck (Darmstadt, Germany). 25% aqueous NH_4OH (analytical grade) was also from Merck (Darmstadt, Germany). Paper filters (3hw type) were purchased from Spektrum-3D (Hungary).

Standard and internal standard stock solutions of 1 mg/mL were prepared in methanol, with the exception of statin compounds (atorvastatin, fluvastatin, lovastatin and simvastatin), which were prepared in acetonitrile because they proved to be degradable in methanol [45,46]. All stock solutions were stored at −18 °C in a refrigerator for a maximum time of two months. Working and calibration solutions were prepared in 10% methanol in Millipore water and stored in the dark below 4 °C.

2.2. Solid-phase extraction

Pharmaceuticals were extracted from 500 mL of tap or surface water with the application of four different extraction methods using Waters Oasis HLB (500 mg, 6 mL), Agilent SampliQ Polymer SCX (150 mg, 6 mL) and Agilent SampliQ Si-SCX (500 mg, 6 mL) solid phase extraction cartridges. Particles in the water were removed by filtering through paper filter. Before extraction 50 ng of internal standards were added and in the case of spiked samples 500 μL solution containing all the compounds at a concentration of 25, 50, 100 or 300 ng/mL was also added.

2.2.1. Extraction Method I

pH of the water samples was adjusted to 10 with 25% aqueous NH_4OH. SampliQ Polymer SCX cartridges were conditioned with 5 mL of methanol and equilibrated with 5 mL of Millipore water pH adjusted to 10 with 25% aqueous NH_4OH. Samples were introduced onto the cartridges through PTFE tubes at a flow rate of 3–4 mL/min. After sample loading the cartridges were washed with 5 mL of Millipore water pH adjusted to 10 with 25% aqueous NH_4OH. Cartridges were then dried for at least 10 min with vacuum, and subsequently, the pharmaceuticals were eluted with 2.5 mL of methanol and 2.5 mL of methanol–25% aqueous NH_4OH (1:1, v/v) in the same collection vial.

2.2.2. Extraction Method II

pH of the water samples was adjusted to 10 with 25% aqueous NH_4OH. SampliQ Si-SCX cartridges were conditioned with 5 mL of methanol and equilibrated with 5 mL of Millipore water pH adjusted to 10 with 25% aqueous NH_4OH. Samples were introduced onto the cartridges through PTFE tubes at a flow rate of 3–4 mL/min. After sample loading the cartridges were washed with 5 mL of Millipore water pH adjusted to 10 with 25% aqueous NH_4OH. Cartridges were then dried for at least 10 min with vacuum, and subsequently, the pharmaceuticals were eluted with 2.5 mL of methanol and 2.5 mL of methanol–25% aqueous NH_4OH (1:1, v/v) in the same collection vial.

2.2.3. Extraction Method III

Oasis HLB cartridges were conditioned with 5 mL of methanol and equilibrated with 5 mL of Millipore water. Samples were introduced to the cartridges through PTFE tubes at a flow rate of 3–4 mL/min. After sample loading, the solid phase was washed with 5 mL of Millipore water. Cartridges were then dried for at least 10 min with vacuum, and subsequently, the pharmaceuticals were eluted with 5 mL of methanol.

2.2.4. Extraction Method IV

pH of the water samples was adjusted to 10 with 25% aqueous NH_4OH. Oasis HLB cartridges were conditioned with 5 mL of n-hexane, 5 mL of acetone, 10 mL of methanol, and then equilibrated with 10 mL of Millipore water, pH adjusted to 10, with 25% aqueous NH_4OH. Samples were introduced to the cartridges through PTFE tubes at a flow rate of 3–4 mL/min. After sample loading, the solid phase was washed with 5 mL of 5% methanol in 2% aqueous NH_4OH. Cartridges were then dried for at least 10 min with vacuum, and subsequently, the pharmaceuticals were eluted with 5 mL of methanol.

In all four cases extracts were evaporated to dryness by a gentle stream of nitrogen and reconstituted in 500 μL of 10% methanol in Millipore water, with the exception of the post-extraction spiked

Table 1
Compound specific LC–MS/MS parameters.

Compounds	R_t	TS	FragV	MRM1	CE1	MRM2	CE2	MRM ratio
Acebutolol	6.037	2	70	337.1 > 116	25	337.1 > 218	25	16.0
Amlodipine	6.946	2	100	409.1 > 238	10	409.1 > 294.1	10	55.4
Atenolol	1.629	1	130	267.1 > 144.9	25	267.1 > 190	20	45.6
Atenolol-d_7	1.612	1	120	274.2 > 145	25	274.2 > 79.1	20	109.3
Atorvastatin	8.082	2	120	559.4 > 440.3	20	559.4 > 466.2	15	14.8
Betaxolol	6.711	2	70	308.1 > 116.1	20	308.1 > 161	20	23.9
Carvedilol	6.880	2	150	407.1 > 224.1	25	407.1 > 283	20	81.8
Cimetidine	1.597	1	90	253.1 > 95	30	253.1 > 159	10	90.7
Enalapril	6.650	2	140	377.2 > 234.2	15	377.2 > 303.2	15	28.4
Enalapril-d_5	6.637	2	120	382.2 > 239.1	15	382.2 > 308.2	15	39.4
Esmolol	6.265	2	100	296.1 > 219	15	296.1 > 254.1	15	26.8
Famotidine	1.551	1	60	338.1 > 189	15	338.1 > 155	30	52.9
Fluvastatin	8.100	2	130	412.2 > 224	30	412.2 > 266.1	15	85.3
Lansoprazole	7.118	2	80	370.1 > 252.1	10	370.1 > 119.1	15	30.0
Lansoprazole-d_4	7.107	2	90	374.1 > 252	5	374.1 > 123	20	36.7
Lisinopril	3.051	1	110	406.3 > 84.1	30	406.3 > 246.2	20	24.8
Lovastatin	9.729	2	50	405.3 > 199.1	10	405.3 > 285.1	5	90.7
Metoprolol	6.119	2	140	268.2 > 116.1	15	268.2 > 74.1	20	93.8
Nifedipine	7.712	2	70	347.1 > 315.1	0	347.1 > 254.1	15	56.3
Nimodipine	8.466	2	70	419.2 > 343.1	5	419.2 > 301.1	25	56.6
Nizatidine	1.896	1	100	332.1 > 58.1	30	332.1 > 155	15	40.9
Omeprazole	6.680	2	100	346.1 > 198	10	346.1 > 136.1	30	46.7
Oxprenolol	6.391	2	110	266.1 > 72.2	15	266.1 > 116.2	15	31.5
Pantoprazole	6.899	2	110	384.2 > 200.1	10	384.2 > 138.1	30	91.6
Propranolol	6.588	2	90	260.1 > 116.2	15	260.1 > 183.2	15	71.6
Ramipril	7.139	2	120	417.3 > 234.2	25	417.3 > 130.1	25	22.3
Ranitidine	2.374	1	90	315.2 > 176.1	15	315.2 > 130.1	25	60.9
Simvastatin	9.746	2	80	419.3 > 199.2	5	419.3 > 285.2	5	89.3
Sotalol	1.748	1	100	273.1 > 255	5	273.1 > 133	30	60.8

R_t – retention time (min); TS – time segment; FragV – fragmentor voltage (V); MRM1 – quantifier transition; CE1 – collision energy for MRM1 (V); MRM2 – qualifier transition; CE2 – collision energy for MRM2 (V); MRM ratio – ratio of the two MRMs (%).

samples, which were reconstituted in 500 μL solution containing all the compounds at a concentration of 25, 50, 100 or 300 ng/mL.

For all solid-phase extraction procedures a Supelco Visiprep vacuum manifold was used.

2.3. Liquid chromatography

LC separations were performed on an Agilent 1200 system (Agilent Technologies, Germany) consisting of a binary pump with internal solvent selection valve (G1312A), a vacuum degasser (G1379B), an autosampler (G1367B) with thermostat (G1330B) and a thermostated column compartment (G1316A). Sample aliquots of 5 μL were injected with needle wash (from flushport, acetonitrile–methanol 1:1 (v/v), 5 s) onto a Zorbax Eclipse Plus-C18 column (2.1 mm × 100 mm, 1.8 μm) equipped with an in-line filter containing replacement frits (2 mm, 0.2 μm) (PN: 5067-1555, Germany). The column was kept at 50 °C. Three elution systems were tested: (1) 10 mM ammonium formate, pH adjusted to 3 with formic acid (A1) and acetonitrile with 0.1% formic acid (B1); (2) 10 mM ammonium acetate, pH adjusted to 3.5 with acetic acid (A2) and acetonitrile with 0.5% acetic acid (B2); (3) 10 mM ammonium acetate, pH adjusted to 5 with acetic acid (A3) and acetonitrile with 0.15% acetic acid (B3). Gradient elution was carried out at a flow rate of 250 μL/min. The elution started with 10% eluent B and then the amount of it was linearly increased to 80% within 4 min, and within another 4 min to 100%. This eluent composition was held for 4 min, and then the percent of eluent B was immediately lowered back to 10%. Before the next injection, the system was allowed to equilibrate for 8 min.

2.4. Mass spectrometry

The flow from the LC column was transferred to an Agilent 6460 Triple Quadrupole mass spectrometer (Agilent Technologies, Germany) equipped with an electrospray ionization source, supported by the new Agilent Jet Stream Technology for thermal gradient focusing, which uses heated nitrogen to improve ion generation and desolvation. The temperature of the sheath gas was 350 °C, while the flow rate was 11 L/min. Nitrogen was used as desolvation and nebulizing gas at a temperature of 350 °C, a flow rate of 10 L/min and a pressure of 35 psi. The capillary voltage was 3500 V, while the nozzle voltage was 1000 V. The collision gas was also nitrogen.

Positive ions were acquired in multiple reaction monitoring (MRM) mode. For all compounds two transitions were monitored. Optimized parameters are summarized in Table 1. The compounds were grouped into two time segments. In the first one there were seven compounds and atenolol-d_7 internal standard, while in the second one was the other nineteen compounds with enalapril-d_5 and lansoprazole-d_4 internal standards. Dwell time of the first time segment was 50 ms, while that of the second one was 25 ms.

MassHunter Data Acquisition for Triple Quad B.02.01. software was used for data acquisition and MassHunter Qualitative Analysis B.03.01. and MassHunter Quantitative Analysis B.03.02. softwares were used for data analysis.

2.5. Matrix effect assessment: post-extraction spike method

Three sets of samples were constructed. The first set of samples consisted of neat calibration standards prepared in 10% methanol in Millipore water, at a concentration level of 25, 50, 100 and 300 ng/mL. The second set of samples were first extracted and spiked after extraction with the analytes in the same solvent and at the same concentration level as in set 1. In set 3, the analytes were spiked before extraction into the samples with the addition of 500 μL solution containing all the compounds at a concentration level of 25, 50, 100 and 300 ng/mL.

In each case 500 mL Danube river water samples were used.

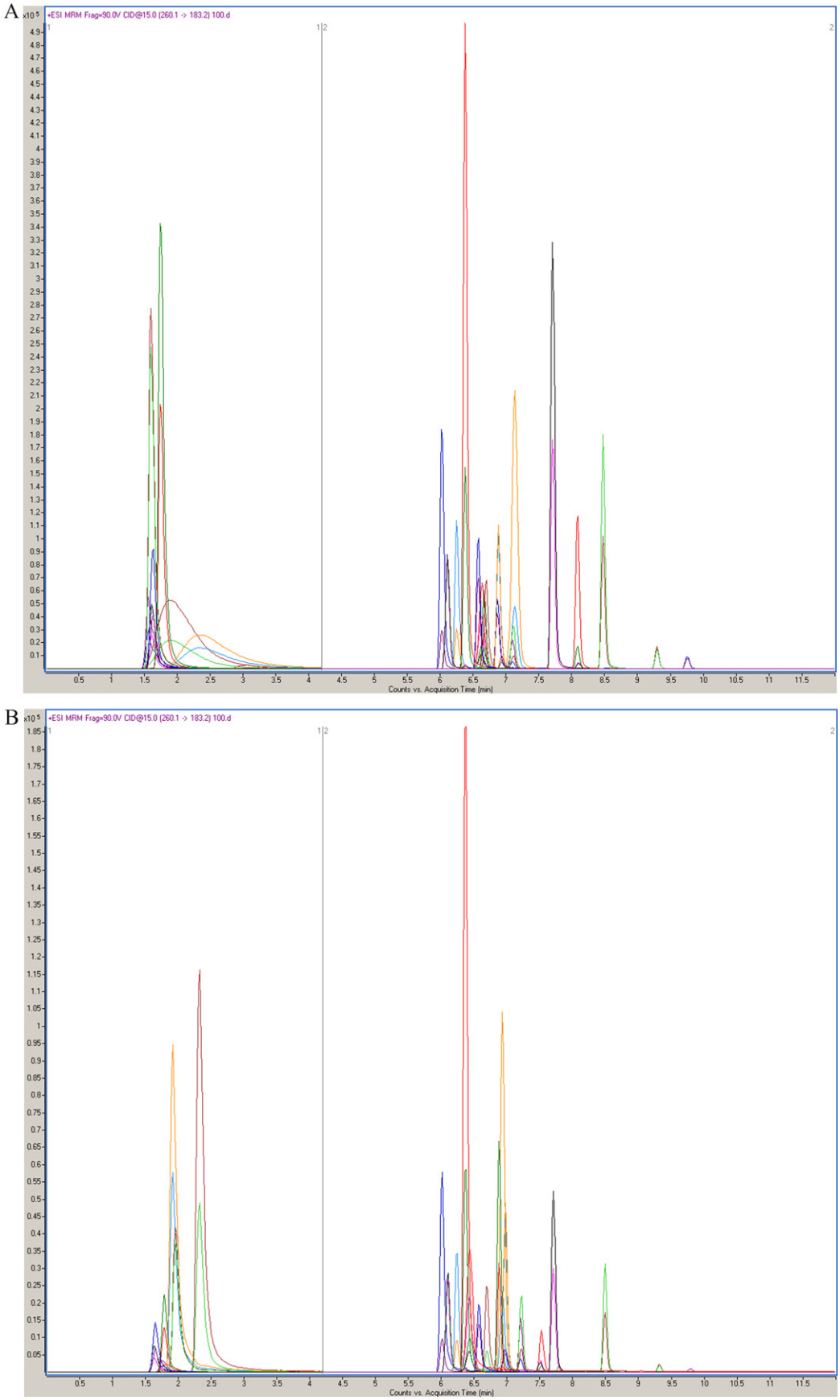

Fig. 1. Comparison of different eluent modifiers and buffer pH with MRM chromatograms. (a) MRM chromatogram of a standard solution at a concentration of 100 ng mL^{-1}, with eluent composition of 10 mM ammonium formate, pH 3.0 and 0.1% formic acid in acetonitrile. (b) MRM chromatogram of a standard solution at a concentration of 100 ng mL^{-1}, with eluent composition of 10 mM ammonium acetate, pH 5.0 and 0.15% acetic acid in acetonitrile.

Table 2
Results of post-extraction spike method (in case of matrix effect [ME (%)] a value of >100% indicates ionization enhancement, while a value of <100% indicates ionization suppression).

Compounds	Extraction methods											
	I			II			III			IV		
	ME (%)	RE (%)	PE (%)	ME (%)	RE (%)	PE (%)	ME (%)	RE (%)	PE (%)	ME (%)	RE (%)	PE (%)
Acebutolol	113	94	106	106	99	105	112	102	115	106	94	100
Amlodipine	118	49	58	105	111	117	59	49	28	145	50	72
Atenolol	171	90	153	108	100	108	96	45	43	43	99	43
Atorvastatin	101	96	97	99	8	8	97	51	49	105	73	77
Betaxolol	109	48	53	135	104	140	108	102	110	115	91	105
Carvedilol	106	27	29	116	73	85	120	135	163	148	47	69
Cimetidine	127	3	4	98	52	51	8	5	0	75	48	36
Enalapril	114	59	68	96	2	2	115	100	115	110	99	109
Esmolol	105	68	71	111	98	109	107	97	104	107	90	97
Famotidine	114	7	8	88	30	27	3	15	0	77	53	41
Fluvastatin	99	95	94	96	9	9	61	2	1	107	71	76
Lansoprazole	117	129	150	107	35	38	62	66	41	112	89	100
Lisinopril	94	11	11	113	1	2	97	25	24	118	3	3
Lovastatin	118	87	105	102	7	16	75	69	51	84	77	65
Metoprolol	104	80	83	114	102	117	101	101	101	104	94	98
Nifedipine	104	93	97	105	85	89	87	7	6	106	77	81
Nimodipine	110	95	104	100	112	112	85	5	4	128	84	107
Nizatidine	109	19	20	90	54	49	33	1	0	84	78	66
Omeprazole	118	111	131	107	59	63	70	65	46	111	88	97
Oxprenolol	107	81	87	117	102	119	100	101	101	106	92	97
Pantoprazole	111	93	103	102	15	16	93	89	82	108	92	99
Propranolol	105	23	24	157	104	162	100	113	113	103	95	98
Ramipril	111	81	89	96	3	3	109	93	102	111	93	103
Ranitidine	106	20	21	140	18	25	29	1	0	89	64	57
Simvastatin	107	30	30	101	3	11	70	55	38	78	36	28
Sotalol	104	95	98	101	98	98	132	72	94	87	81	70

2.6. Quantitation

For quantitation the combination of matrix-matched and internal standard calibration was used. Matrix-matched calibration solutions were prepared by extraction of 100 mL Danube water samples applying Oasis HLB cartridges with *Extraction Method IV*. After evaporation the samples were reconstituted in 1 mL solution containing all the compounds at a concentration of 5, 10, 25, 50, 75, 100 and 300 ng/mL and all three internal standards at a concentration of 50 ng/mL. Calibration curves were generated using linear regression analysis.

For all compounds two MRM transitions were monitored: the more intensive was used for quantitation and the less intensive for confirmation. Other confirmation parameters were the ratio of the two MRM transitions and the retention time of the compounds (see Table 1).

Table 3
Method validation results.

Compounds	Linearity (R^2)	IDL (pg inj.)	MDL (ng L^{-1})	MQL (ng L^{-1})	Accuracy (%)		
					10 ng/mL	50 ng/mL	300 ng/mL
Acebutolol	0.9971	5	0.2	1	88	111	98
Amlodipine	0.9998	5	5	10	71	92	97
Atenolol	0.9997	5	0.2	1	91	105	98
Atorvastatin	0.9992	14	4	5	90	103	99
Betaxolol	0.9979	5	1	2	88	103	100
Carvedilol	0.9985	5	5	10	103	108	99
Cimetidine	0.9924	5	0.2	1	88	112	97
Enalapril	0.9999	5	0.2	1	108	103	100
Esmolol	0.9974	10	5	10	77	104	99
Famotidine	0.9949	5	1	2	76	105	99
Fluvastatin	0.9979	5	1	2	89	108	100
Lansoprazole	0.9996	15	4	10	93	109	98
Lisinopril	0.9985	7	–	–	–	–	–
Lovastatin	0.9996	12	3	10	79	83	93
Metoprolol	0.9966	3	0.2	1	89	103	100
Nifedipine	0.9989	15	5	15	86	91	97
Nimodipine	0.9991	12	5	10	87	94	98
Nizatidine	0.9824	5	1	2	86	104	98
Omeprazole	0.9993	5	1	2	91	99	98
Oxprenolol	0.9978	5	3	8	85	94	97
Pantoprazole	0.9952	8	2	4	93	98	98
Propranolol	0.9975	5	1	2	95	99	100
Ramipril	0.9997	3	0.5	2	94	98	103
Ranitidine	0.9841	3	0.5	2	69	76	96
Simvastatin	0.9989	12	3	10	66	71	79
Sotalol	0.9985	5	0.2	1	86	88	92

Table 4
Results of precision experiments.

Compounds	Precision (%RSD)											
	Instrumental (n = 5)						Method (n = 3)					
	Intra-day			Inter-day			Intra-day			Inter-day		
	10 ng/mL	50 ng/mL	300 ng/mL	10 ng/mL	50 ng/mL	300 ng/mL	10 ng/mL	50 ng/mL	300 ng/mL	10 ng/mL	50 ng/mL	300 ng/mL
Acebutolol	2.4	0.8	0.5	6.8	5.9	2.7	0.7	4.6	2.1	7.8	8.9	9.4
Amlodipine	4.5	1.5	1.3	22.9	10.8	9.7	0.2	0.9	1.1	10.2	11.3	10.9
Atenolol	0.9	3.0	0.7	8.2	9.6	2.9	1.4	0.7	4.6	1.8	1.7	5.8
Atorvastatin	2.8	1.1	1.7	0.9	4.2	1.8	2.2	4.1	8.2	9.5	13.2	15.3
Betaxolol	2.3	0.9	0.4	2.6	2.5	3.1	1.4	2.9	3.1	5.9	9.5	3.5
Carvedilol	3.0	1.2	2.7	11.8	4.2	3.7	2.3	4.9	5.8	14.0	22.3	18.9
Cimetidine	1.0	0.9	0.8	19.2	17.9	12.6	0.2	1.1	0.4	7.0	8.2	9.7
Enalapril	1.1	1.1	0.4	3.6	2.2	1.7	3.6	0.6	0.4	6.9	4.4	1.2
Esmolol	1.1	0.9	0.5	1.6	1.5	2.6	4.9	9.1	12.1	22.3	16.3	21.3
Famotidine	2.0	2.3	1.6	3.8	8.0	4.3	3.9	5.7	6.7	15.0	5.0	11.8
Fluvastatin	4.0	3.5	2.0	5.2	14.5	3.3	5.7	9.0	4.6	9.8	13.0	11.1
Lansoprazole	2.8	3.1	2.1	24.1	20.2	5.2	2.3	2.6	0.6	6.9	3.0	2.0
Lisinopril	2.3	2.1	1.6	5.5	8.2	7.7	–	–	–	–	–	–
Lovastatin	2.2	1.3	1.6	11.4	6.1	13.6	10.2	4.7	6.4	11.8	8.8	8.4
Metoprolol	1.5	0.9	0.8	4.0	3.2	3.5	4.5	0.8	5.3	13.2	11.1	10.3
Nifedipine	1.4	1.1	0.3	3.2	2.8	2.8	1.6	4.6	5.8	10.2	9.7	8.8
Nimodipine	1.6	1.5	0.6	3.5	12.3	2.8	4.3	2.6	3.2	9.1	8.5	8.0
Nizatidine	0.7	0.9	0.6	15.0	17.5	13.6	5.8	4.5	4.3	15.3	13.4	12.5
Omeprazole	1.2	1.1	0.7	20.3	19.9	2.4	2.2	1.7	0.9	5.1	6.5	4.9
Oxprenolol	0.8	0.9	0.7	4.3	3.1	3.1	4.8	4.5	2.3	9.4	8.1	7.5
Pantoprazole	1.6	1.5	0.6	5.3	9.9	1.4	5.7	1.4	2.0	7.8	6.5	5.9
Propranolol	1.8	1.4	0.5	3.1	2.6	3.7	2.8	1.2	0.8	10.2	15.1	9.9
Ramipril	1.1	1.0	0.7	4.2	2.7	2.2	1.2	0.4	0.4	5.9	1.9	2.2
Ranitidine	2.6	1.4	1.8	15.2	15.4	12.5	5.7	4.3	3.9	11.3	9.6	6.6
Simvastatin	2.0	2.9	1.5	21.0	11.2	12.4	14.6	10.7	9.8	22.4	19.9	21.0
Sotalol	1.4	1.7	0.8	5.0	4.8	2.3	3.4	2.7	3.1	13.2	11.1	8.4

2.7. Method validation

During method validation 500 mL of Danube river water samples were extracted with Oasis HLB (500 mg, 6 mL) cartridges applying *Extraction Method IV*. For separation eluent combination of 10 mM ammonium acetate, pH adjusted to 5 (A3) and acetonitrile with 0.15% acetic acid (B3) was applied.

Accuracy of the method was evaluated as the percentage of deviation from the known added amount of analyte in the sample at three concentration levels, namely 10, 50 and 300 ng/mL.

Precision was evaluated as the relative standard deviation (RSD) of replicate measurements. Both intra- and inter-day precision of the instrument and of the analytical method were assessed. Instrumental repeatability and reproducibility was determined by five successive injections of standard solutions at a concentration level of 10, 50 and 300 ng/mL in one day and in five successive days, respectively. Intra-day and inter-day precision of the analytical method was determined by three replicates of 500 mL Danube water sample analysis spiked before extraction at a concentration level of 10, 50 and 300 ng/L for all compounds in one day and in three different days, respectively.

Method detection limits (MDLs) and method quantification limits (MQLs) were determined from spiked surface water samples, as the minimum detectable amount of analyte with a signal-to-noise ratio of 3 and 10, respectively.

The instrumental detection limits (IDLs) were estimated from the injection of a standard solution successively diluted until reaching a concentration level corresponding to a signal-to-noise ratio of 3.

2.8. Applying the method for river water samples

Danube river water samples were collected in South Budapest (at 1644 km) seven times during two months in winter. Samples were collected in amber glass bottles and filtered immediately after arriving in the laboratory. All samples were analyzed on the same day as collected.

500 mL samples were spiked with 500 μL of a solution containing the three internal standards at a concentration of 50 ng/mL. Samples' pH was adjusted to 10 with 25% aqueous NH_4OH and then extracted with Oasis HLB applying *Extraction Method IV*. After evaporation the sample residues were reconstituted in 500 μL 10% methanol in Millipore water thus a 1000-fold enrichment could be achieved.

3. Results and discussion

3.1. Investigation of the composition of the eluent system

For LC separation different eluent pH and modifiers have been tested. Because of the basic functionality of the measured components the Zorbax Eclipse Plus C18 type column was chosen which is especially designed for separation of basic compounds. This column's maximum working temperature is 60 °C and it is stable up to pH 8.0. Therefore regarding the column lifetime alkaline eluent pH has not been tested. Only pH 3.0 with 10 mM ammonium formate, pH 3.5 and 5.0 with 10 mM ammonium acetate were tested with the addition of the same amount of acid (formic acid or acetic acid) to acetonitrile as for buffer solutions during pH adjustment.

Although, a little higher peak intensities were obtained with the application of formate buffer (pH 3.0), much better peak shapes were achieved in the early eluting region (for lisinopril, nizatidine and ranitidine) with acetate buffer (Fig. 1). In case of acetate buffer pH 3.5 and 5.0 were compared: both of them gave good peak shapes but at pH 5.0 signal intensities were a bit higher. Therefore the eluent system of 10 mM ammonium acetate, pH 5.0 (A3) and acetonitrile with 0.15% acetic acid (B3) was chosen for further investigations.

Table 5
Concentrations measured in Danube river water samples.

Therapeutic group	Compounds	Measured concentrations (ng/L)		
		Average	Minimum	Maximum
β-Blockers	Acebutolol	2	1	3
	Atenolol	14	11	19
	Metoprolol	29	18	55
	Sotalol	22	9	36
H_2-receptor antagonists	Cimetidine	34	17	46
	Famotidine	19	5	34
	Nizatidine	29	29	29
	Ranitidine	39	9	60
ACE-inhibitors	Enalapril	3	2	4
	Ramipril	3	2	5
Proton pump inhibitors	Lansoprazole	<LOQ	<LOQ	<LOQ
	Pantoprazole	6	4	7

<LOQ – could be detected but not quantified.

3.2. Matrix effect assessment

The usually proposed method to compensate for matrix effects is the use of stable isotopically labeled internal standard which elutes at the same time as the compound, but it is also advised to use one for every single compound. In case of a multi-compound method, as for environmental analysis, it is not affordable for lots of laboratories.

In our method there are twenty-six compounds to be measured while we have only three deuterated internal standards from three of the six different therapeutical classes. The other difficulty of their use is the application of time segments, which is useful for better MS detection, but separates the compounds into two groups. For instance atenolol-d_7 is in the first time segment, because atenolol is an early eluting compound, while the other β-blockers – with the exception of sotalol – elute in the second time segment at a very different eluent composition. Thus it cannot be effectively used for compensation in the case of the other seven β-blocker compounds.

Consequently, the use of stable isotopically labeled internal standard is not fully appropriate in this case, therefore, we also used matrix-matched calibration. Due to this combination accuracy and precision results (summarized in Tables 3 and 4) are more convenient.

3.2.1. Post-extraction spike method

In bioanalytical measurements there is a routinely applied method for matrix effect assessment: the post-extraction spike method [41]. It can quantitatively determine the effect of matrix ions, namely ion suppression or enhancement. It makes a difference between matrix effect and the "extraction effectiveness", which is usually expressed as recovery. It introduces the expression "process efficiency" which includes both matrix effect and recovery.

In environmental analysis where one has to determine low levels of concentrations from huge sample volumes it can be useful to monitor both of these two parameters and it also makes easier to choose between extraction procedures.

Applying this method three sets of samples were prepared. The first set of samples consisted of neat calibration standards. The second set of samples were first extracted and spiked after extraction with the analytes in the same solvent at the same concentration level as in set 1. Any difference of the peak areas of the analytes in comparison with those observed in set 1 would be indicative of an effect of sample matrix. In set 3, analytes were spiked before extraction into the samples. The differences here would reflect a combined effect of a sample matrix and potential differences in recovery of analytes.

By comparing the absolute peak areas obtained in sets 1–3 we can determine the matrix effect (ME), the recovery (RE) of the extraction procedure and overall "process efficiency" (PE). If the peak areas obtained in set 1 are depicted as "A", that obtained in set 2 as "B" and that obtained in set 3 as "C", the ME, RE and PE values can be calculated as follows:

$$\mathrm{ME}\,(\%) = \frac{B}{A} \times 100 \quad (1)$$

$$\mathrm{ME}\,(\%) = \frac{C}{B} \times 100 \quad (2)$$

$$\mathrm{PE}\,(\%) = \frac{C}{A} \times 100 = \frac{(\mathrm{ME} \times \mathrm{RE})}{100} \quad (3)$$

The obtained results for 500 mL Danube water samples with all four extraction methods are summarized in Table 2. Comparing the process efficiencies [PE (%)] it can be seen that applying *Extraction Method IV* only six compounds have lower than 60% while with the other methods there are more compounds with a lower value of that. Among this six lisinopril was the worst (3%), while only two other compounds have a value lower than 40% (simvastatin and cimetidine). With the exception of atenolol in all cases the lower process efficiency was due to the inconvenient recovery [RE (%)].

In some cases notable ion enhancement can be observed increasing the process efficiency, for example in case of amlodipine, carvedilol and nimodipine. Nevertheless significant ion suppression can cause lower process efficiency in spite of a high recovery, as can be seen for atenolol with *Extraction Method IV*.

3.3. Method validation

All validation data are summarized in Tables 3 and 4.

Matrix-matched calibration curves were generated using linear regression analysis and fitted well ($R^2 > 0.99$, except for nizatidine and ranitidine, where $R^2 > 0.98$), over the established concentration range (5–300 ng/mL).

Accuracy ranged from 66 to 108% at 10 ng/mL, from 71 to 112% at 50 ng/mL and from 79 to 103% at 300 ng/mL. Only ranitidine (69%) and simvastatin (66%) gave lower than 70% accuracy at 10 ng/mL concentration.

Instrumental intra- and inter-day precision was evaluated at a concentration level of 10, 50 and 300 ng/mL with five successive injections in one day and in five successive days. Repeatability, expressed as relative standard deviation (RSD), varied between 0.7 and 4.5% at 10 ng/mL, between 0.8 and 3.5% at 50 ng/mL and between 0.3 and 2.7% at 300 ng/mL, while reproducibility, expressed as relative standard deviation (RSD), ranged from 0.9 to 22.9% at 10 ng/mL, from 1.5 to 20.2% at 50 ng/mL and from 1.7 to 13.6% at 300 ng/mL concentration.

Intra- and inter-day precision of the analytical method was evaluated at a concentration level of 10, 50 and 300 ng/L with three replicates of Danube water sample analysis in one day and in

three different days, respectively. Repeatability, expressed as relative standard deviation (RSD), varied between 0.2 and 14.6% at 10 ng/L, between 0.4 and 10.7% at 50 ng/L and between 0.4 and 12.1% at 300 ng/L, while reproducibility, expressed as relative standard deviation (RSD), ranged from 1.8 to 22.4% at 10 ng/L, from 1.7 to 22.3% at 50 ng/L and from 1.2 to 21.3% at 300 ng/L concentration.

Instrumental detection limits (IDLs) varied between 3 and 15 pg per injection. Method detection limits (MDLs) ranged from 0.2 to 5 ng/L and method quantification limits (MQLs) were from 1 to 15 ng/L which are comparable with or even better than the results of other reported methods [18,31,47].

3.4. Application for river water samples

To demonstrate the applicability of the developed method Danube water samples were analyzed which were collected in Budapest (Hungary), seven times during two months in winter.

As can be seen in Table 5 twelve compounds could be detected and with the exception of lansoprazole all of them could be quantified as well. These compounds belong to the group of β-blockers (four), H_2-receptor antagonists (four), angiotensin-converting enzyme inhibitors (two) and proton pump inhibitors (two). The measured average concentrations varied between 2 ng/L (acebutolol) and 39 ng/L (ranitidine). The minimum concentrations varied between 1 ng/L (acebutolol) and 29 ng/L (nizatidine), while the maximum concentrations ranged from 3 ng/L (acebutolol) to 60 ng/L (ranitidine). Five compounds have an average concentration higher than 20 ng/L (cimetidine, metoprolol, nizatidine, ranitidine and sotalol) and other two have an average concentration higher than 10 ng/L (atenolol and famotidine).

Comparing these measured concentrations to other compounds' already determined in Danube river [48–50] we can conclude that these compounds are also considerable pollutants in the aquatic environment. Only caffeine, paracetamol and X-ray contrast media could be determined at higher concentrations while the others are present in the same concentration range as our compounds.

4. Conclusions

We have developed a solid-phase extraction followed by liquid chromatography–tandem mass spectrometry method for the determination of twenty-six anti-ulcer and antihypertensive agents. During method development four different extraction methods were tested with the application of Oasis HLB, SampliQ Polymer SCX and Si-SCX SPE cartridges and three different eluent combinations were also investigated. Finally Oasis HLB was chosen for sample preparation with a procedure different from the usually applied one. The sample pH was adjusted to 10 and an eluent combination of 10 mM ammonium acetate, pH 5.0 and acetonitrile with 0.15% acetic acid was applied. The post-extraction spike method proposed by Matuszewski et al. [41] was applied for matrix effect assessment and it proved to be a good choice also for environmental samples. The method was validated and gave good linearity and precision data. Detection limits were low enough that the method could be applied to river water samples successfully. Danube water samples were analyzed and twelve compounds could be detected while eleven of them could also be quantified with an average concentration between 2 and 39 ng/L.

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