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# Identification of phase I metabolites of cardiovascular and anti-ulcer drugs in surface water samples with liquid-chromatography–mass spectrometry methods

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ABSTRACT

In our study we have identified phase I metabolites of cardiovascular and anti-ulcer agents with the application of predictive multiple reaction monitoring (pMRM) methods with liquid-chromatography–triple-quadrupole mass spectrometry (LC–QQQ-MS) in surface water samples. Targeted accurate mass spectrometry measurements were also carried out for confirmation with liquid-chromatography–time-of-flight mass spectrometry (LC–TOF-MS). pMRM followed by a targeted accurate mass spectrometry measurement can provide a sound basis for the selection of metabolites to be included in analytical methods for the investigation of environmental load of pharmaceuticals. Using LC–QQQ-MS twenty-one metabolites could be identified with two independent transitions at the same retention time and six of them could also be confirmed with LC–TOF-MS.



## 1. Introduction

In the past decades more and more results were published regarding pharmaceutical residues in environmental samples, especially in surface- and wastewater. Initially the methods developed were generally based on gas chromatography–mass spectrometry, but later the analysts of this field turned to liquid chromatography–mass spectrometry. The majority of these methods target at groups of pharmaceuticals such as antibiotics [1–5], endocrine disrupting agents [6–8], X-ray contract media [9–11] or non-steroidal anti-inflammatories [12–15], while others – the so-called "multi-residue methods" – at compounds from many different groups together [16–24]. We have also developed a solid-phase extraction–liquid chromatography–electrospray ionization tandem mass spectrometry method for the identification and quantification of cardiovascular and anti-ulcer agents in surface water samples just recently [25].

It is well known that most of the pharmaceuticals undergo a kind of biotransformation whereby a different molecule excretes from the organism. These metabolites can be even more active than the parent compound in some cases with toxic effects. During pharmaceutical development metabolism pathway is profusely studied therefore metabolites of every pharmaceutical compound are known and well documented. Several papers can be found on determining a substance and its metabolite(s) from human plasma [26–31] or urine [28,32–34]. At the same time there is only limited information on the presence of pharmaceuticals' metabolites in our environment [5,7–9,16–19,22]. Although some studies deal with metabolites as well, their lists of target compounds are rather incomplete and their choice often does not seem to be well based [7–9,16,19].

There are various methodologies for the identification of unknown metabolites in biological samples [35–50]. The most frequently applied technique was first described in 1982 by Perchalski et al. [41], consisting of a product ion scan of the parent drug, followed by a precursor ion and/or neutral loss scan of the biological extract and finally of a product ion scan for the suspected metabolites. Metabolites can most likely be identified based on these experiments and being aware of the typical metabolic pathways [42–46]. Accurate mass measurement by (quadrupole-) time-of-flight mass spectrometers is another usually applied technique [37,43,47]. Beyond these there are further specialties like isotope cluster analysis [48], $MS^n$ experiments for structural characterization [43], application of correlation analysis [44] and mass defect filter [37] or applications of low resolution quadrupole mass spectrometer for accurate mass measurement [49,50].

The above mentioned procedures can adequately apply for few-component analyses. But environmental analysis methods usually target minimum a dozen compounds, which makes most of these

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approaches rather tedious and uneconomic. It seems more reasonable to choose the metabolites to be included in the target compound list based on pharmaceutical studies. But with more than ten target compounds, each having two to three major metabolites the number of metabolite standards to be purchased increases extremely. On the other hand it is also possible that a supposed metabolite cannot be found in environmental samples at all. So in these cases a trial method should be applied to select metabolites worth to be targeted and then purchased for method development. Such a trial method can be based on the MS/MS fragmentation of the parent compound completed with metabolite prediction. Due to short cycle times the multiple reaction monitoring (MRM) approach allows a wide range of potential transitions to be targeted with sufficiently low detection limits [38,40].

The types of investigated compounds in our method are as follows: beta-blockers, HMG-CoA reductase inhibitors, angiotensin-converting enzyme (ACE) inhibitors, protonpump inhibitors and $H_2$-receptor antagonists [25]. They are presented in Table 1 together with their MRM transitions, collision energies and fragmentor voltages. Since an increasing number of publications describe the negative effects of drug metabolites on inferior animals in surface water [51–53] we have come to the conclusion that monitoring the parent compound by itself is not enough. Therefore we have searched in bioanalytical studies for the already identified metabolites of all the investigated compounds and have found almost a hundred of them. During phase I biotransformation processes hydroxylation is the main reaction. This occurs in respect of beta-blockers and of HMG-CoA reductase inhibitors as well [32,54–57]. Functional group losses are also frequent as observed in the case of the beta-blocker carvedilol [55,56]. Besides, there are so-called prodrugs among ACE-inhibitors becoming active after a biotransformation reaction such as hydrolyzation in the case of enalapril (to enalaprilate [58]) or ramipril (to ramiprilate [31]). For anti-ulcer agents the most common biotransformation reactions are hydroxylation and oxidation of their sulphur atom leading to sulphone metabolites [27–29,59]. Some publications mention the activity range of the metabolites as well. For instance the hydroxyl-metabolites of carvedilol, of fluvastatin and lovastatin have increased activity and diacetolol, desmethyl-carvedilol and exomethylene-lovastatin are also active metabolites of the parent compound.

Under our actual research we have developed a trial method for the identification of cardiovascular and anti-ulcer agents' phase I metabolites from surface water after solid phase extraction enrichment of the samples. The method uses predictive MRM methods and targeted accurate mass measurements. Predictive-MRM methods were generated based on knowing the drugs' metabolic pathway, the expected mass difference and on the known fragmentation pattern of the parent compound. In the case of the targeted accurate mass measurements the accurate mass of a suspected metabolite is calculated on the basis of the known biotransformation reactions and the expected mass difference.

Our procedure can provide a sound basis for choosing the compounds to be involved in an analytical method for investigating the environmental load of pharmaceuticals including metabolites. Under this procedure one can reduce the number of standards to be purchased; therefore metabolite identification in environmental samples could be significantly more cost effective. This can induce more extensive studying of environmental loads including monitoring pharmaceutical metabolites as well.

## 2. Materials and methods

### 2.1. Chemicals and materials

Acetonitrile, methanol of HPLC gradient grade quality as well as acetone and n-hexane for gas chromatography were from Merck (Darmstadt, Germany). Water was deionized in our laboratory using a Millipore (Billerica, MA, USA) Milli-Q water purification system. Ammonium acetate (cryst. Extra pure, Ph Eur), acetic acid

**Table 1**
Compound specific LC–MS/MS parameters for the parent compounds.

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

$R_t$: retention time (min); FragV: fragmentor voltage (V); MRM1: quantifier transition; CE1: collision energy for MRM1 (V); MRM2: qualifier transition; CE2: collision energy for MRM2 (V).

(Extra pure, Ph Eur) and 25% aqueous $NH_4OH$ (analytical grade) were also from Merck (Darmstadt, Germany). Glass filters (with 16–40 μm porosity) were purchased from Spektrum-3D (Hungary). Oasis HLB (500 mg, 12 mL; PN: 186000116) cartridges were from Waters Co. (Ireland).

### 2.2. Sample preparation

Five liters of Danube river water was filtered through glass filter and its pH was adjusted to 10 with the addition of 25% aqueous $NH_4OH$. The sample was extracted in five 1000 mL parts with Oasis HLB (500 mg, 12 mL) solid phase extraction cartridges which were pre-conditioned with 5 mL of n-hexane, 5 mL of acetone, 10 mL of methanol, and then equilibrated with 10 mL Millipore water, pH adjusted to 10 with the addition of 25% aqueous $NH_4OH$. Samples were loaded at a flow rate of 3–4 mL/min through PTFE tubes with a Supelco Visiprep vacuum manifold. After sample loading, the solid phase was washed with 2 mL of 5% methanol in 2% aqueous $NH_4OH$ and the cartridges were then dried for at least 10 min with air flow. Subsequently, all five cartridges were eluted with 2 mL × 2.5 mL of methanol separately but the eluates were collected in the same vial and then evaporated to dryness by a gentle stream of nitrogen. Finally the sample was reconstituted in 500 μL of 10% methanol in Millipore water reaching a 10,000-fold enrichment. This considerable enrichment was carried out to facilitate the full-scan time-of-flight measurements.

### 2.3. Liquid chromatography

All mass spectrometric measurements were carried out after liquid chromatographic separation. An Agilent 1200 system (Agilent Technologies, Germany) consisting of a binary pump-SL (G1312B), a vacuum degasser (G1379A), an autosampler-SL (G1329B) with thermostat (G1330B) and a thermostated column compartment (G1316A) was used. Zorbax Eclipse Plus-C18 (2.1 mm × 100 mm, 1.8 μm; PN: 959764-902) column equipped with an in-line filter containing replacement frits (2 mm, 0.2 μm) (PN: 5067-1555, Germany) was used at a column temperature of 50 °C. For gradient elution 10% ammonium acetate, pH adjusted to 5 with acetic acid (A) and acetonitrile with 0.15% acetic acid (B) was used at a flow rate of 250 μL/min. Elution was started at 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 composition was held for 7 min.

For triple quadrupole measurements (LC–QQQ-MS) sample aliquots of 5 μL, while for time-of-flight measurements (LC–TOF-MS) sample aliquots of 10 μL was injected onto the chromatographic column.

### 2.4. Triple-quadrupole mass spectrometry

An Agilent 6460 Triple Quadrupole (G6460A) mass spectrometer (Agilent Technologies, Germany) equipped with electrospray ionization source was used in multiple reactions monitoring (MRM) mode. Instrument was check-tuned just before the experiments. Instrument parameters were the following: sheath gas temperature 200 °C, sheath gas flow rate 4 L/min, desolvation gas ($N_2$) temperature 350 °C and its flow rate 10 L/min, nebulizer gas ($N_2$) pressure 35 psi, capillary voltage 3500 V and nozzle voltage 500 V. The collision gas was also nitrogen and all the experiments were carried out in positive ionization mode.

The acquisition method was based on the parent drug and its metabolic pathway. In the first step the fragmentor voltage, the two most intensive fragments of the parent compound and their collision energies were determined. In the next step, according to the metabolic pathway, the mass difference of a supposed metabolite was added to the parent drug's mass and to the fragments' mass leading to four different transitions for every metabolite. A detailed scheme can be seen in Section 3. The applied fragmentor voltage and collision energies were the same as for the parent compound. Dwell times were 30 ms. The optimized parameters are presented in Table 2.

MassHunter Data Acquisition for Triple Quad B.02.01. software was used for data acquisition and MassHunter Qualitative Analysis B.03.01. software was used for data evaluation.

### 2.5. Time-of-flight mass spectrometry

An Agilent 6210 Time-of-Flight (G1969A) mass spectrometer (Agilent Technologies, Germany) equipped with electrospray ionization source was used. Instrument was low-mass calibrated at seven points in positive ionization mode with fluorinated phosphazines, triazines and betaine (G1969/85000 ES/TOF Calibration Ions) just before the experiments. Source conditions were the following: positive ion electrospray, capillary voltage 3500 V, source temperature 350 °C and flow 10 L/min, nebulizer gas ($N_2$) pressure 35 psi, octopole RF Peak 250 V and skimmer1 65 V. Fragmentor voltage was set to 50, 100 and 150 V. Data files were acquired in continuum (profile) mode, and spectra were stored from $m/z$ 50 to 1650. Five scans were averaged. Reference masses were 121.050873 and 922.009798.

MassHunter Data Acquisition for 6200 Series TOF & 6500 Series Q-TOF B.02.00. software was used for data acquisition and MassHunter Qualitative Analysis B.03.01. software for data evaluation.

## 3. Results and discussion

Bioanalytical studies [26–30,32,33,52,54,55,58–67] specify almost a hundred major metabolites for the twenty-six cardiovascular and anti-ulcer agents (see Table 1) targeted in our previous method [25]. Instead of purchasing reference material for all of these metabolites, we decided to identify the most important ones in surface waters by pMRM and accurate mass measurements. pMRM methods for the identification of the most important metabolites were based on the acquisition method of the parent compounds. The two most intensive product ions of the parent compounds were determined (Eq. (1)):

$$M^+ \rightarrow F_1 \quad \text{and} \quad F_2 \quad \text{at the same retention time} \tag{1}$$

where $M^+$ is the protonated parent compound (precursor ion) and $F_1$ and $F_2$ are the main product ions of the parent compound.

After a biotransformation reaction the mass of the parent molecule changes by a mass of "$x$", and the fragments will be as presented by Eq. (2), because theoretically the mass difference appears on either the first fragment ($F_1$) or on the second fragment ($F_2$), while the other one remains unchanged:

$$[M^+ + x] \rightarrow [F_1 + x] \quad \text{and} \quad F_2 \quad \text{or} \quad F_1 \quad \text{and} \quad [F_2 + x] \quad \text{at the same retention time} \tag{2}$$

By this procedure four transitions were built up for all the metabolites except for hydroxyl-sulphone-lansoprazole and dihydroxy-atorvastatin in case of which six transitions could be generated. Fragmentor voltages and collision energies determined formerly for the parent drugs were used for all of these transitions.

By evaluating the data identification of a metabolite was based on two independent transitions at the same retention time. Twenty-one metabolites were identified by this method six of which could be confirmed with accurate mass measurement. The two independent transitions of these confirmed compounds can be seen in Figs. 1–6. Table 2 presents all the compounds identified by predictive MRM method as well as their biological activity. The

**Table 2**
Identified metabolites with their optimized parameters and detected fragment ions (*in italics*) with their retention time.

| Parent drug | Metabolite [Ref] | Activity[d] | Mass diff. | Precursor | FragV | Prod1 (CE) | Prod2 (CE) | Prod3 (CE) | Prod4 (CE) | Prod5 (CE) | Prod6 (CE) | $R_t$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Atenolol | Hydroxy- [52,66] | 10% | +16 Da | 283 | 100 | *178 (20)* | 162 (20) | 123 (40) | *107 (40)* | – | – | 7.65 |
| Betaxolol | Acide- [32] | ?[a] | −31 Da | 277.1 | 70 | *116.1 (20)* | 85.1 (20) | 161 (20) | *130 (20)* | – | – | 7.34 |
| Lovastatin | 6′-/3″-Hydroxy- [54] | 60%/15% | +16 Da | 421.3 | 50 | *199.1 (10)* | 215.1 (10) | 285.1 (5) | *301.1 (5)* | – | – | 6.62 |
| Metoprolol | α-Hydroxy- [52,57] | ? | +16 Da | 284.2 | 140 | 116.1 (15) | *132.1 (15)* | *74.1 (20)* | 90.1 (20) | – | – | 8.93 |
| Oxprenolol | Desisopropyl- [34,67] | ? | −42 Da | 224.1 | 110 | *72.2 (15)* | 30.2 (15) | 116.2 (15) | *74.2* (15) | – | – | 9.04 |
| Simvastatin | 6′-Carboxy- [54] | 27% | +30 Da | 449 | 100 | *155 (10)* | 125 (10) | 203 (10) | *173 (10)* | – | – | 7.09 |
| Acebutolol | Diacetolol [26] | Active | −28 Da | 309.1 | 70 | 88 (25) | *116 (25)* | 218 (25) | *190 (25)* | – | – | 3.99 |
| Atorvastatin | Dihydroxy-[c] | ? | +32 Da | 591.4 | 120 | *440.3 (20)* | 456.3 (20) | 472.3 (20) | 466.2 (15) | 482.2 (15) | *498.2 (15)* | 6.81 |
| Betaxolol | Hydroxy- [32] | ? | +16 Da | 324.1 | 70 | 116.1 (20) | *132.1 (20)* | *161 (20)* | 177 (20) | – | – | 7.85 |
| Carvedilol | Desmethyl- [55,56] | Active | −14 Da | 393 | 150 | *224 (25)* | 210 (25) | 283 (20) | *269 (20)* | – | – | 9.22 |
| | Hydroxy- [55,56] | 1300% | +16 Da | 423 | 150 | 224 (25) | *240 (25)* | *283 (20)* | 299 (20) | – | – | 6.95 |
| Cimetidine | Hydroxymethyl-/sulphoxide-[b] [27,28] | ? | +16 Da | 269.1 | 90 | *95 (30)* | 111 (30) | 159 (10) | *175 (10)* | – | – | 5.82 |
| | Amide- [27,28] | ? | +18 Da | 271.1 | 90 | *95* (30) | 113 (30) | 159 (10) | *177 (10)* | – | – | 10.45 |
| Enalapril | Enalaprilate [58] | 100% | −28 Da | 349.2 | 140 | 234.2 (15) | *206.2 (15)* | *303.2 (15)* | 275.2 (15) | – | – | 7.95 |
| Fluvastatin | 5-/6-Hydroxy- [54] | 88%/45% | +16 Da | 428.2 | 130 | *224 (30)* | 240 (30) | 266.1 (15) | *282.1 (15)* | – | – | 2.47 |
| | Desisopropil-propionic acid- [54] | Inactive | −74 Da | 336.2 | 130 | 224 (30) | *150 (30)* | 266.1 (15) | *192.1 (15)* | – | – | 2.26 |
| Lansoprazole | Hydroxy-sulphone- [29,59] | ? | +32 Da | 402.1 | 80 | 252.1 (10) | *284.1 (10)* | 268.1 (10) | 119.1 (15) | 151.1 (15) | *135.1 (15)* | 5.84 |
| Lovastatin | Exomethylene- [54] | 50% | −2 Da | 403.3 | 50 | 199.1 (10) | *197.1 (10)* | *285.1 (5)* | 283.1 (5) | – | – | 5.84 |
| Omeprazole | Hydroxysulphone- [59] | ? | +32 Da | 378.1 | 100 | 198 (10) | *230 (10)* | 136.1 (30) | *168.1 (30)* | – | – | 7.91 |
| Oxprenolol | Desallyl- [34,67] | ? | −40 Da | 226.1 | 110 | *72.2 (15)* | 32.2 (15) | 116.2 (15) | *76.2 (15)* | – | – | 6.99 |
| Pantoprazole | Sulphide- [59] | ? | −16 Da | 368.2 | 110 | 200.1 (10) | *184.1 (10)* | *138.1 (30)* | 122.1 (30) | – | – | 8.42 |

Mass diff.: calculated mass difference of the metabolite from the parent compound; precursor: calculated protonated metabolite precursor ion; FragV: fragmentor voltage (V); Prod1–Prod6 (CE): calculated fragment ions of the metabolites (their collision energy in V); $R_t$: retention time (min).

[a] No data regarding the activity was found.

[b] Two different biotransformation reactions induce the same mass difference.

[c] Only hydroxyl-atorvastatin was published [55] dihydroxy-atorvastatin was just an assumption.

[d] Activity of the metabolite if the parent compound's activity is 100%.

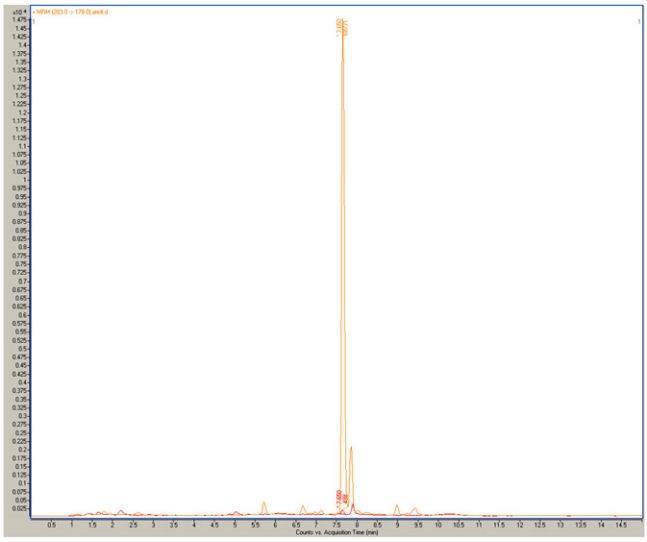

**Fig. 1.** MRM transitions of hydroxy-atenolol (283 > 178 and 283 > 107) at $R_t$ = 7.65 min.

transitions indicating the presence of a compound in the sample are given in italics. Compounds the presence of which was confirmed by accurate mass measurement are seen in the upper part of the table.

In case of accurate mass measurements the enriched sample was introduced to the time-of-flight LC–MS three times with the application of three different fragmentor voltages (50, 100, 150 V). In course of data evaluation ion chromatograms of the calculated suspected metabolite's mass were extracted and formulas were generated based on this extracted mass spectrum. A formula was accepted if the difference between the calculated and the measured masses was less than 10 ppm and if their isotope pattern was similar. The results are summarized in Table 3 with the appropriate fragmentor voltage. As seen six metabolites could be identified using LC–TOF-MS out of the twenty-one predicted with the help of LC–QQQ-MS. The difference can be explained by the reduced sensitivity of the full scan mode applied with LC–TOF-MS compared to the high sensitivity of the LC–QQQ-MS.

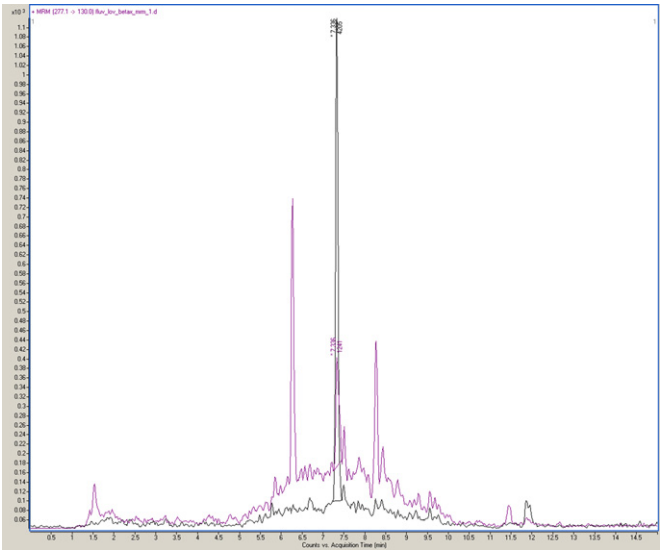

**Fig. 2.** MRM transitions of betaxolol-acide (277.1 > 116.1 and 277.1 > 130) at $R_t$ = 7.34 min.

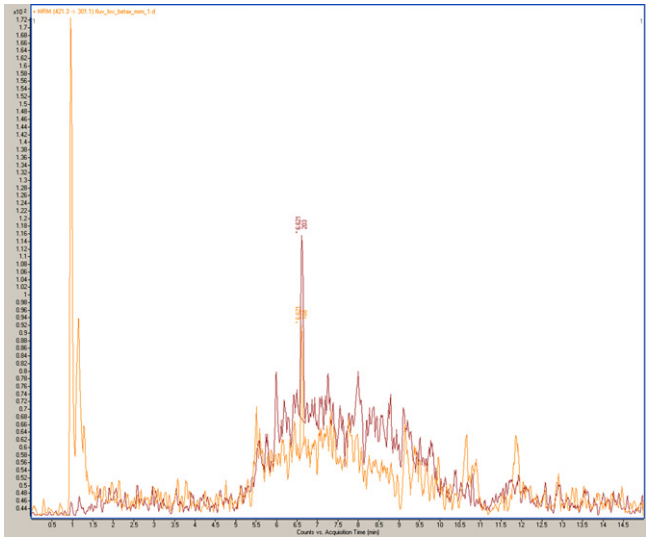

**Fig. 3.** MRM transitions of 6′-/3″-hydroxy-lovastatin (421.3 > 199.1 and 421.3 > 301.1) at $R_t$ = 6.62 min.

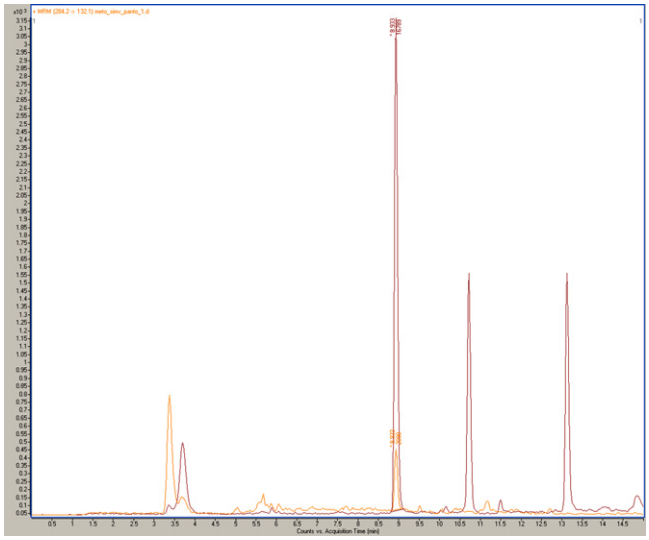

**Fig. 4.** MRM transitions of α-hydroxy-metoprolol (284.2 > 132.1 and 284.2 > 74.1) at $R_t$ = 8.93 min.

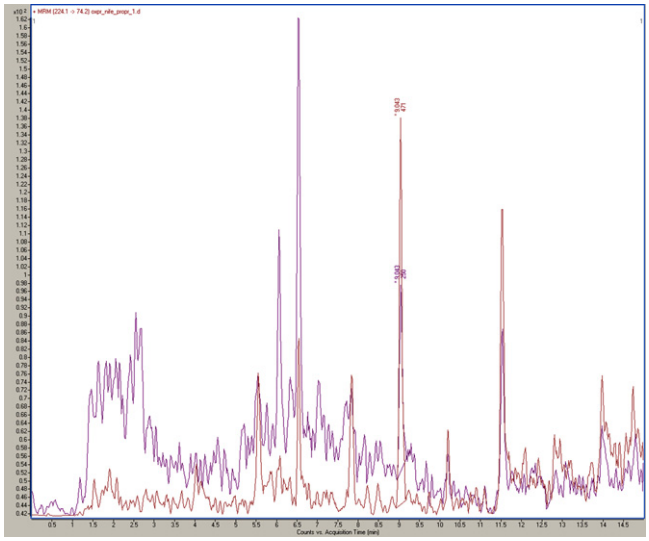

**Fig. 5.** MRM transitions of desisopropyl-oxprenolol (224.1 > 72.2 and 224.1 > 74.2) at $R_t$ = 9.04 min.

**Table 3**
Exact mass measurements.

| Metabolite | FragV | Calculated *m/z* | Measured *m/z* | Difference (ppm) |
|---|---|---|---|---|
| Hydroxy-atenolol | 100 | 283.1652 | 283.1658 | −2.12 |
| Betaxolol-acide | 50 | 268.1543 | 268.1562 | −6.97 |
| Hydroxy-lovastatin | 100 | 421.2585 | 421.2591 | −2.26 |
| Hydroxy-metoprolol | 100 | 284.1856 | 284.1841 | +5.30 |
| Desisopropyl-oxprenolol | 150 | 224.1281 | 224.1291 | −4.55 |
| Carboxy-simvastatin | 100 | 449.2534 | 449.2524 | +2.09 |

FragV: fragmentor voltage (V).

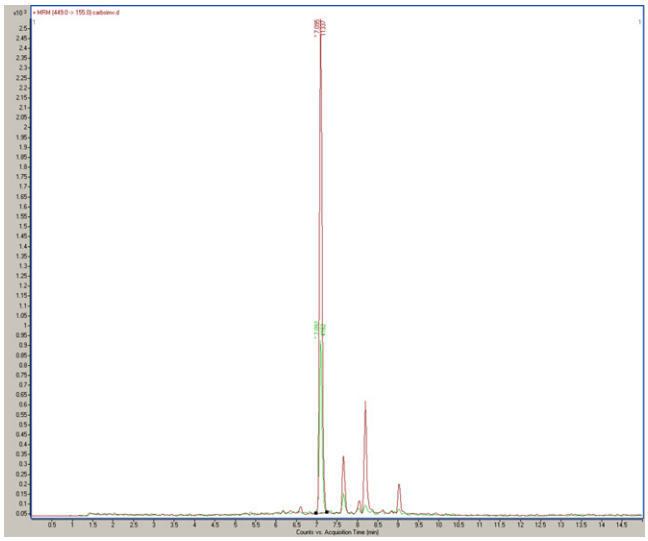

**Fig. 6.** MRM transitions of 6′-carboxy-simvastatin (449 > 155 and 449 > 173) at $R_t$ = 7.09 min.

Even though only 10% of the absorbed atenolol is metabolized in humans [66] the sensitivity of LC–TOF-MS was still good enough to confirm the presence of hydroxyl metabolite in surface water. The two major metabolites of betaxolol are α-hydroxy-betaxolol and betaxolol-acide [32]. Both were detected with LC–QQQ-MS, but only the later could be confirmed by LC–TOF-MS. Lovastatin and simvastatin undergo extensive metabolism in human bile and in both cases the 6′-position was identified as the major metabolism site. The main human metabolites are therefore 6′-β-hydroxy-, 6′-exomethylene-lovastatin and 6′-β-carboxy- and 6′-β-hydroxy-simvastatin, while in case of lovastatin 3″-hydroxy metabolite is also frequent [54]. The confirmed hydroxyl-lovastatin carries on 15–60%, carboxy-simvastatin carries on 27% activity of the parent compound. Metoprolol is less extensively metabolized and the major metabolite is not the confirmed α-hydroxy-metoprolol but 4-(2-hydroxy-3-isopropylamino-propoxy)-phenylacetic acid which could not be detected [52]. Finally oxprenolol is extensively metabolized generating many metabolites [34,67], out of which desallyl-oxprenolol and desisopropyl-oxprenolol were detected by LC–QQQ-MS, but only the later could be confirmed by LC–TOF-MS.

## 4. Conclusions

Twenty-one phase I metabolites of cardiovascular and anti-ulcer agents from surface water samples have been identified with predictive MRM methods, out of which six have been confirmed with accurate mass measurements. Identification was based on knowing the metabolic pathways and the fragmentation of the parent drug. MRM methods were built up based on assumptions by adding the metabolic mass differences to the parent compound and the fragments as well. In all cases a metabolite was regarded as identified if two independent transitions at the same retention time indicated so. The evaluation of the accurate mass measurements was based on the calculated metabolite masses. The presence of metabolites was confirmed by extracted ion chromatograms, allowing <10 ppm acceptable mass difference between measured and calculated masses, and checking the similarity of the isotope patterns.

By applying this trial method the metabolite target list of an environmental survey on pharmaceuticals can be rationalized. In our case it has been narrowed from almost a hundred to only six compounds. As a result by avoiding the needless targeting at any suspected metabolites the cost of the environmental survey covering also the most prevalent metabolites can be held under control.

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