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Determination of β-blockers and β_2-agonists in sewage by solid-phase extraction and liquid chromatography–tandem mass spectrometry

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Abstract

A method using solid-phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been developed for the determination of 12 β-blockers and β_2-agonists in wastewater samples. Extraction of the drugs was effected by an Oasis MCX cartridge with a strong cation resin adsorbent. Matrix coextractives were removed from the SPE cartridge by methanol prior to the elution of the drugs with a mixture of dichloromethane, 2-propranol, and ammonium hydroxide. The extract was analyzed by LC–MS/MS with electrospray ionization operating in the positive mode. Recovery of the 12 compounds was in most cases better than 85% at the fortification levels of 500 and 50 ng/L, with standard deviations between 3 and 7%. Based on a concentration factor of 250, the method detection limits ranged from 6 to 11 ng/L for the target compounds. No degradation of these drugs in spiked sewage effluent samples was observed over a storage period of 7 days at 4 °C in the dark. Many β-blockers were detected in the 14 wastewater samples collected from seven Canadian sewage treatment plants; those occurring at the highest concentrations were atenolol, acebutolol, sotalol, and metroprolol, with overall median concentrations of 1370, 339, 282, and 257 ng/L, respectively. However, there was only a small decrease in the median concentrations of these β-blockers between the primary and final effluent, suggesting that these drugs are not easily removed by the sewage treatment processes. As a result of selective extraction and effective removal of coextractives, no matrix effect was observed for the samples during LC–MS/MS analyses.
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Keywords: β-Blockers; β_2-Agonists; Sewage; Solid-phase extraction; LC–MS/MS

1. Introduction

β-Adrenergic blocking agents, or β-blockers, are prescription drugs used to treat hypertension, congestive heart failure, and abnormal heart rhythms, relieve angina, and prevent cardiac infarctions (heart attacks) in humans. They work by blocking the effects of adrenaline on our body's β-receptors, thereby slowing the nerve impulses to the heart and reducing its workload. Some β-blockers, such as timolol, are mainly used as ophthalmic solutions for the treatment of glaucoma. The most commonly prescribed β-blockers in Canada include acebutolol, atenolol, metoprolol, nadolol, propranolol, labetalol, and sotalol [1]. β_2-Agonists such as clenbuterol and salbutamol, in contrast, are used in much lower quantities as human and veterinary medicine to treat pulmonary disorders and asthma. However, because of their stimulatory activity on respiration and the central nervous system, these β_2-agonists are sometimes misused as performance enhancement drugs in horse racing and human sports [2,3]. Structurally, all of the above-mentioned compounds have a common –CH(OH)–CH_2–NH– side chain (Fig. 1). In many cases, this chain is either bonded directly to an aromatic ring (β-blockers with a suffix alol and the β_2-agonists) or via a CH_2–O– bond (β-blockers with a suffix olol). All of these compounds have at least one chiral center, with labetalol having two and nadolol having three (Fig. 1). Except for timolol, which is marketed as the pure, active *S*-enantiomer, the other drugs are sold as 50:50 racemic mixtures in the *S*- and *R*-forms, which could possess markedly different pharmacodynamic and pharmacokinetic behaviors [4].

The elimination of β-blockers after human consumption occurs, in most cases, via the hepatic metabolism in the liver and/or renal excretion of the unchanged drug [5]. While the lipophilic β-blockers (e.g. propranolol, alprenolol) are extensively metabolized and eliminated predominantly after

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glucuronidation, the more hydrophilic β-blockers (e.g. atenolol, nadolol, and sotalol) are almost exclusively excreted unchanged in urine [5]. Due to their prevalence of use, β-blockers have been observed in many environmental samples. Ternes [6] reported the occurrence of metoprolol, propranolol, nadolol, and bisoprolol, with median concentrations between 25 and 730 ng/L, in sewage treatment plant (STP) effluents. Sotalol was detected in 3 out of 105 groundwater samples with a maximum concentration of 590 ng/L [7]. Metoprolol, nadolol, and propranolol were found in over 71% of the 34 effluent samples collected in the United States, with median concentrations from 18 to 51 ng/L [8]. Concentrations of a few selected β-blockers in surface water and sewage samples collected in eastern [9] and western [10] Canada have also been reported.

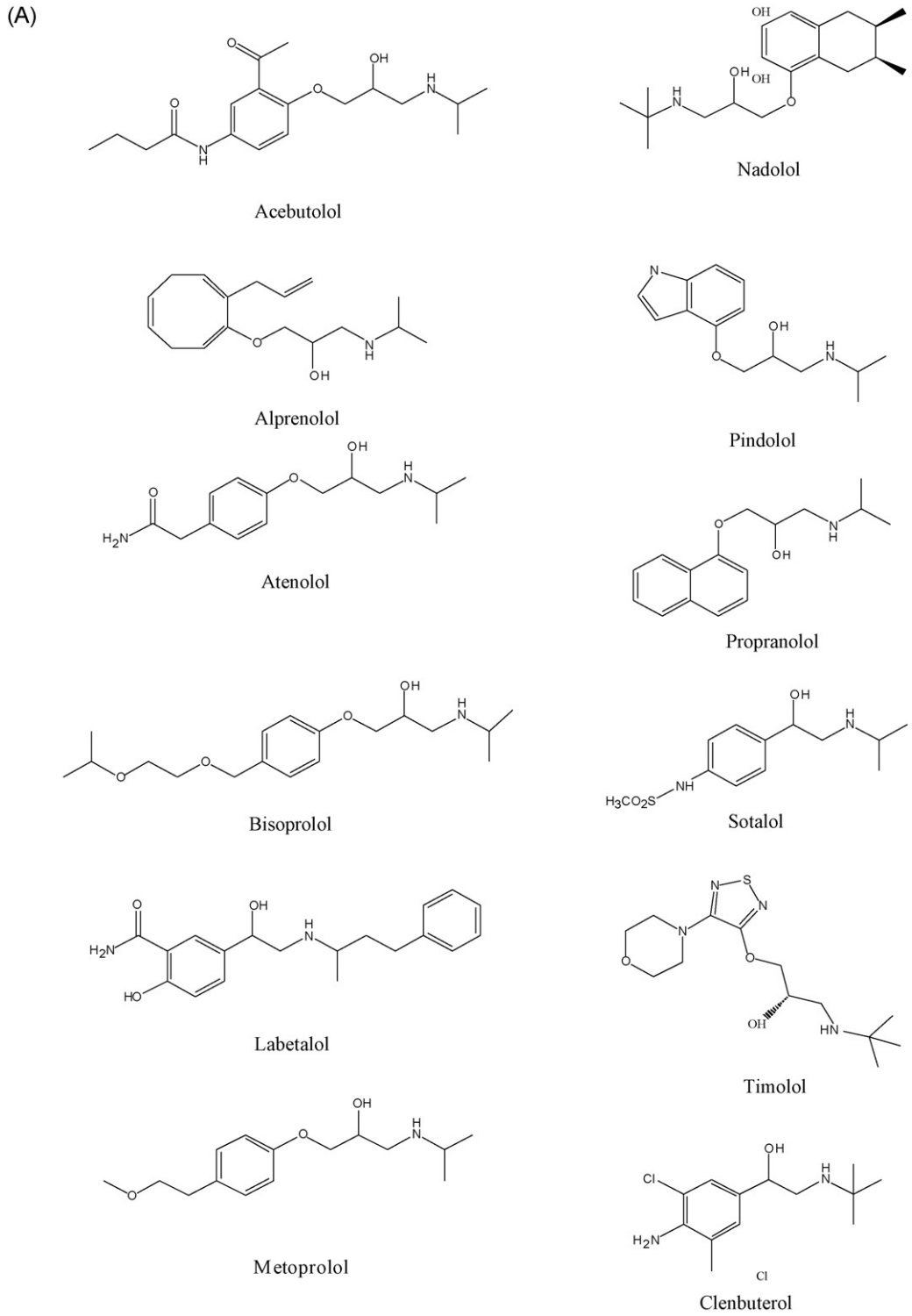
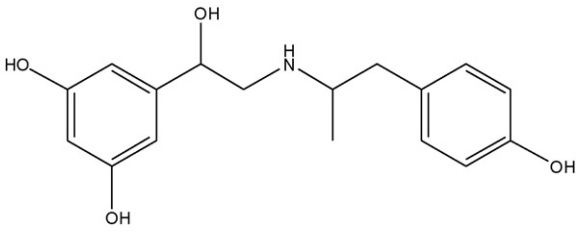
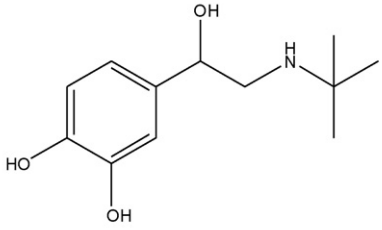


Fig. 1. Chemical structures of the β-blockers and β_2-agonists.

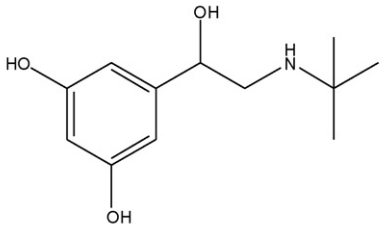
(B)



Fenoterol



Salbutamol



Terbutaline

Fig. 1. (*Continued*).

There are many existing methods for the determination of β-blockers and β_2-agonists at trace levels. One of the more popular methods is based on GC–MS detection which requires chemical derivatization of the drugs to improve chromatographic properties. The derivatization may be accomplished by acylation (mainly pentafluoroacylation) [11,12], cyclic boronation [13,14], or trimethylsilylation [14], or a combination of them (trimethylsilylation followed by acylation) [14–16]. However, so far, there has not been a single derivatization procedure that works well for all of the most commonly used β-blockers and β_2-agonist, thus the GC methods are not the most suitable for the screening of environmental samples. In some cases, a derivatization procedure may produce more than one product, making quantitation of a compound more difficult [14]. In contrast, LC methods require no derivatization. In combination with a sensitive and selective mass spectrometer, LC–MS and LC–MS/MS methods have been a favorite choice for the detection of these pharmaceuticals in sewage extracts [6,7,17–20]. Extraction of β-blockers and other drugs from wastewater and surface water samples has usually been accomplished by solid-phase extraction (SPE) using the Isolute C_{18} [18], Strata X [21], and Oasis HLB [22,23] cartridges with reversed phase adsorbents. Using a selective extraction technique with the Oasis MCX cartridge and a LC–MS/MS procedure, this paper discusses the development of an analytical method for the determination of 12 β-blockers and β_2-agonists in sewage samples. These drugs are all currently in use in Canada and are thus potential contaminants in our aquatic environment.

2. Experimental

2.1. Chemicals and reagents

Organic solvents (acetonitrile, dichloromethane, methanol, and 2-propanol) were distilled-in-glass grade available from Burdick and Jackson and used without further purification. All pharmaceutical standards (Table 1), mostly with purity between 95 and 99%, were obtained from Sigma (Sigma-Aldrich Canada, Oakville, Canada). They are available in either pure form or as a salt in one of the following forms: hydrochloride, hydrobromide, sulfate, maleate, and fumarate. Distilled water was purified by a Milli-Q unit (Millipore, Billerica, USA). Formic acid (ACS reagent grade, 98%), and Celite 545 were products of EMD Chemicals (Darmstadt, Germany). Hydrochloric acid (37%) and ammonium hydroxide (30% as NH_3), both in ACS reagent grade, were purchased from Aldrich.

Individual stock solutions of the pharmaceuticals were prepared in acetonitrile or methanol at 1000 μg/mL with the exception of salbutamol, which was made up at 500 μg/mL. Stock solutions stored in PTFE-lined screw cap test tubes were kept at −20 °C in the dark. Mixtures of the 15 pharmaceuticals, at concentrations of 50, 200, and 500 pg/μL, were prepared in

Table 1
Quantitation and confirmation MRMs, cone voltage, and collision energy for β-blockers and β_2-agonists

Drug	Form	Quantitation MRM	Confirmation MRM	Cone energy (V)	Collision energy (kV)
Acebutolol[a]	Hydrochloride	337 > 116	337 > 260	60	17
Alprenolol[a]	Hydrochloride	250 > 116	250 > 173	50	17
Atenolol[a]	Pure	267 > 145	267 > 190	50	25
Bisoprolol[a]	Hemifumarate	326 > 116	326 > 326	60	25
Clenbuterol[b]	Hydrochloride	277 > 203	277 > 259	19	10
Fenoterol[b]	Hydrobromide	304 > 135	304 > 107	50	15
Labetalol[a]	Hydrochloride	329 > 311	329 > 207	30	14
Metoprolol[a]	Tartrate	268 > 116	268 > 133	60	20
Nadolol[a]	Pure	310 > 254	310 > 236	50	15
Pindolol[a]	Pure	249 > 116	249 > 172	50	17
Propranolol[a]	Hydrochloride	260 > 116	260 > 183	50	20
Salbutamol[b]	Pure	240 > 148	240 > 166	30	15
Sotalol[a]	Hydrochloride	273 > 255	273 > 213	30	12
Terbutaline[b]	Hemisulfate	226 > 152	226 > 125	32	17
Timolol[a]	Maleate	317 > 261	317 > 244	35	17

[a] β-Blocker.
[b] β_2-Agonist.

solvent A (Section 2.4) and used as working standards for LC and LC–MS/MS work.

2.2. Collection of STP sewage influent and effluent samples

Composite primary and final sewage effluent samples, over a 24-h period, were collected in May and June 2006, by the staffs of the sewage treatment plants from the following cities in southern Ontario, Canada: Brantford, Galt, Guelph, and Toronto (with plants located at Ashbridges Bay, Highland Creek, Humber River, and North Toronto). All plants above use primary (sedimentation) and secondary (activated sludge) treatments, while the Guelph plant also uses tertiary treatment (sand filtration) to treat wastewaters. In addition, they employ disinfection processes such as chlorination (in the summer months) and/or ultraviolet (UV) treatment to further improve the quality of the effluent. The samples, in 1-L amber glass bottles, were shipped immediately to our laboratory and were kept at 4 °C in the dark until extraction. No preservative was added to the samples during the storage period.

2.3. Extraction of sewage samples

All sewage samples were extracted within 48 h after collection. Just prior to extraction, each sample was filtered through a GF/C filter paper (9.0 cm diameter, particle retention 1.2 μm) with a layer of Celite to reduce clogging of the filter in an all-glass apparatus. An aliquot of 250 mL of the filtered sample was subsequently acidified to a pH of ca. 3 with 1 M HCl. Meanwhile, for each sample, a 6-mL, 150-mg Oasis MCX cartridge (30 μm, Waters Part No. 186000256) was conditioned by elution with 6 mL of methanol, followed by 10 mL of water at pH 3. Via a siphon tube, each sample was applied to the MCX cartridge using an SPE manifold (Supelco Visiprep DL 5-7044 or equivalent), at a flow rate between 10 and 15 mL/min. After extraction, the cartridge was dried for 10 min under vacuum. It was then rinsed with 100 mL of pH 3 water (rinse discarded) and 6 mL of methanol (also discarded) and eluted with 8 mL of a mixture of dichloromethane/2-propanol/ammonium hydroxide (78/20/2, v/v). The extract was evaporated in a water bath (40 °C) by a stream of nitrogen just to dryness then redissolved in 1.0 mL of solvent A (Section 2.4) for LC–MS/MS analysis. The sample extract was filtered through a 13 mm, 0.45 μm nylon syringe filter to remove particulate matters before LC analysis, if needed.

2.4. LC with fluorescence and ultraviolet detection

Liquid chromatographic analyses of β-blocker and β_2-agonist standards were performed by an Agilent 1100 series LC system equipped with a degasser (G1322A), a quaternary pump (G1311A), a liquid sampler (G1313A), a column compartment (G1316A), a variable-wavelength UV detector (G1314A), and a fluorescence detector (G1321A). A Zorbax SB-C_8 column (150 × 2.1 mm, 3.5 μm) together with a guard column (SB-C_8, 12.5 × 2.1 mm, 5 μm), also obtained from Agilent Technologies, was used to separate the β-blockers and β_2-agonists. Optimal resolution of the pharmaceuticals was achieved by gradient elution using two mixtures, namely, water/acetonitrile/formic acid 94.5/5.0/0.5 (v/v) (solvent A) and acetonitrile/formic acid 99.5/0.5 (v/v) (solvent B). The gradient began with 100% A and was linearly programmed to 25% B in 13 min. The solvent composition remained at 75% A and 25% B for the next 13 min. A post time of 14 min at 100% A was used to equilibrate the column for the next injection. A constant flow rate of 0.2 mL/min, a column temperature of 35 °C, and a sample volume of 10 μL were used for all injections. The UV detector was set at 220 nm and fluorescence detection was carried out at 230 nm excitation and 310 and 425 nm emission. Total chromatographic and equilibration time of each run was ca. 40 min.

2.5. LC–MS/MS analysis

Sample extracts were also analyzed by a Micromass Quattro Ultima triple quadrupole mass spectrometer (Manchester, UK) equipped with an electrospray ionization (ESI) interface operated in the positive mode. A Waters 2695 Separations Module was used to generate a gradient profile as described above for the UV and fluorescence detection. Instrument control, as well as data acquisition and manipulation, was carried out by the MassLynx 4.1 software operating in the Windows XP environment. Nitrogen (+99% purity), generated by an on-line system, was used as the nebulizing and desolvation gases with typical flow rates of ca. 50 and 500 L/h, respectively. Source and desolvation temperatures of 120 and 350 °C, respectively, were used for analyses. The capillary voltage was held at 3.45 kV.

Compound-dependent parameters were established by infusing standard solution of individual pharmaceutical, at 1 μg/mL, into the ion source using a Harvard syringe pump (Harvard Apparatus, Holliston, MA, USA) at a flow rate of 10 μL/min. With the collision gas off and the source and desolvation temperatures set to 100 and 225 °C, respectively, the cone voltage as well as aperture, hexapoles 1 and 2 voltages were optimized for the maximum production of the precursor ion, MH^+. The collision energy was then applied and optimized, after the collision gas (argon at 2×10^{-3} mbar) was turned on, for the maximum production of the product ions. Quantitative analysis of the pharmaceuticals was set up in multiple-reaction monitoring (MRM) mode, with one transition (precursor > product ion) used for quantitation and another for confirmation (Table 1). For bisoprolol, no significant fragment ion other than *m/z* 116 was observed and therefore single-reaction monitoring (SRM) was used in this case. A sample volume of 10 μL was used for all injections.

2.6. Quantification

Quantification of the β-blockers and β_2-agonists using LC–MS/MS was carried out in MRM mode by an external standard method. Based on the areas recorded for the quantitative MRM transition (Table 1), a linear, three-point calibration curve was generated for each compound at the concentrations of 50, 200, and 500 pg/μL. These levels correspond to 200, 800, and 2000 ng/L of the drugs in the original samples after a concentration factor of 250. Sample extracts with pharmaceutical concentrations higher than 500 pg/μL were diluted and analyzed again. Sample results were not corrected for recovery.

2.7. Stability study

Approximately 16 L of a grab effluent sample was collected from a local sewage treatment plant. The sample was filtered through a 1.2 μm GF/C filter and split into six 2-L sub-samples, with numbers from 1 to 6. Bottles 1–5 were spiked with a mixture of the β-blockers and β_2-aginists (bisoprolol was not included at the time of the stability study) to a nominal concentration of 250 ng/L for each compound. Aliquots (250 mL) from bottles 1 and 6 (unspiked) were analyzed immediately for the drugs. Bottles 2–5 were stored in the dark at 4 °C and aliquots from bottles 2–5 were extracted and analyzed 24 h, 2 days, 4 days, and 7 days after spiking, respectively.

3. Results and discussion

3.1. Liquid chromatographic separation and LC–MS/MS detection of β-blockers and β_2-agonists

Due to the shortage of instrument time for the LC–MS/MS, a combination of UV and fluorescence detection was initially used in this study to establish the chromatographic separation of the β-blockers and β_2-agonists. Since many of these compounds are very sensitive to fluorescence detection, this technique has been used for the simultaneous determination of eight β-blockers in corneal permeability studies in vitro [24]. In our case, an excitation wavelength of 230 nm was used for all compounds. As the emission maxima for most compounds occurred between 300 and 320 nm, a value of 310 nm was selected, except for labetalol where a value of 425 nm was used. However, due to their very low fluorescence responses, chromatographic data for acebutolol, timolol, pindolol, labetalol, and clenbuterol were obtained by UV detection at 220 nm.

Separation of the 15 pharmaceuticals in Table 1 was accomplished by a Zorbax SB-C_8 column with the exception of metoprolol and clenbuterol. Formic acid was used to maintain good peak shape and facilitate the production of the precursor ion MH^+ for LC–MS/MS analysis (see later discussion). Adequate resolution of the more polar or hydrophilic β-blockers and β_2-aginists (e.g. atenolol and salbutamol) from the less polar or lipophilic ones (e.g. labetalol and propranolol) was achieved by a linear solvent program varying from 5 to 25% of acetonitrile. Only one peak was observed for each compound, although all of them had at least one chiral center on the alkyl side chain. However, separation of the enantiomers of β-blockers, for example, could have been achieved by using chiral columns with either a Pirkle-type α-Burke 1 stationary phase under normal-phase conditions [25] or with a vancomycin-based phase under reversed-phase conditions [10,26].

Under collision-induced dissociation conditions, several major fragment ions were observed for the β-blockers and β_2-agonists (Table 2). The species $[MH\text{-}18]^+$, arising from the neutral loss of a water molecule from the pseudo-molecular ion, was observed for acebutolol, labetalol, sotalol, clenbuterol, and salbutamol. The neutral loss of isobutene (56 U) followed by the loss of water from the pseudo-molecular ion to produce the $[MH\text{-}74]^+$ ion was observed for nadolol, timolol, clenbuterol, salbutamol, and terbutaline. This is consistent with the structures of the five compounds above as they all have the necessary *tert*-butyl group (Fig. 1) to generate the isobutene fragment. The loss of 77 U, generated from the elimination of water and isopropylamine (18 + 59 U), was noted for the β-blockers bearing the $-NH-CH(CH_3)_2$ side chain, including acebutolol, alprenolol, atenolol, metoprolol, pindolol, and propranolol. Another major ion common to nearly all β-blockers with the exception of nadolol and timolol was *m/z* 116, arising from the loss of the $-CH_2-CH(OH)-CH_2-NH-CH(CH_3)_2$ fragment.

Table 2
Retention times (t_R, in min), precursor and major product ions of β-blockers and β_2-agonists observed under ESI LC–MS/MS conditions

Drug	t_R (min)	MH^+	$[MH-18]^+$	$[MH-56]^+$	$[MH-74]^+$	$[MH-77]^+$	Others
Acebutolol	17.36	337	*319*			260	116
Alprenolol	24.32	250				173	116
Atenolol	8.96	267				190	145, *116*
Bisoprolol	21.77	326					116
Labetalol	22.11	329	311				207, *116*
Metoprolol	18.02	268				*191*	133, 116
Nadolol	13.60	310		254	236		*201*
Pindolol	14.15	249				172	116
Propranolol	23.87	260				183	*155*, 116
Sotalol	7.85	273	255				213
Timolol	17.02	317		261	244		
Clenbuterol	17.91	277	259		203		
Fenoterol	11.50	304					135, 107
Salbutamol	6.97	240	*222*		166		148
Terbutaline	6.53	226		*170*	152		125

Product ions not used for MRM are shown in italics.

3.2. Solid-phase extraction of drugs in sewage samples

Several SPE procedures were available in the literature for the extraction of β-blockers and β_2-agonists from wastewater samples. Many of them involved the use of C_{18}-based stationary phases such as the ENVI-18, the Strata-X, the Isolute C_{18}, the Oasis HLB, and the Empore SDB-XC disks. However, very few procedures included a cleanup step prior to the elution of the compounds of interest, resulting in an extract containing large amounts of coextractives. The latter would likely create problems (matrix effects) in the quantification of drugs by LC–ESI–MS/MS. Some of the reported SPE procedures also suffered from low recoveries (<50%) of the target compounds [16,18,21]. In order to alleviate these two problems, a different approach in the extraction step has been attempted in our laboratory.

In every β-blocker and β_2-agonist, an amino group is present. As most of these compounds have pK_a values above 9, conversion into the corresponding cation, $NH_2RR'^+$, can easily be achieved under acidic conditions. Selective extraction of such compounds is thus possible provided that the SPE is carried out with a strong cation-exchange phase such as the Oasis MCX cartridges. In this case, most of the neutral and acidic compounds in a sewage sample which were retained by the reversed phase functional groups of the cartridge could be desorbed by a methanol elution step, without losing any of the basic drugs. Meanwhile, the basic β-blockers and β_2-agonists that held strongly onto the solid phase by the interaction with the $-SO_3^-$ groups in the MCX phase could be quantitatively desorbed by elution with dichloromethane and 2-propanol in the presence of a base such as ammonium hydroxide. Although few workers have applied this technique to wastewater samples, the use of strong cation-exchange phases such as the Bond Elut Certify [12,14,15], the DAU (drugs of abuse) [13], or the Isolute SCX [27] SPE columns has been successfully demonstrated in the extraction of β-blockers and β_2-agonists from biological matrices.

Table 3
Percent recovery (mean and standard deviations for a replicate of six determinations) and method detection limits (MDL) for β-blockers and β_2-agonists spiked to distilled water

Compound	Spiking level (ng/L)	% recovery (mean ± SD)	Spiking level (ng/L)	% recovery (mean ± SD)	MDL (ng/L)
Acebutolol	500	95 ± 5	50	88 ± 6	9
Alprenolol	500	85 ± 5	50	82 ± 5	7
Atenolol	500	96 ± 4	50	90 ± 4	6
Bisoprolol	500	88 ± 6	50	91 ± 5	8
Clenbuterol	500	90 ± 5	50	93 ± 6	9
Fenoterol	500	44 ± 7	50	<2	NA
Labetalol	500	93 ± 3	50	88 ± 7	11
Metoprolol	500	91 ± 5	50	85 ± 5	8
Nadolol	500	88 ± 4	50	82 ± 6	9
Pindolol	500	<5	50	<2	NA
Propranolol	500	91 ± 7	50	93 ± 6	10
Salbutamol	500	87 ± 4	50	73 ± 5	6
Sotalol	500	95 ± 7	50	90 ± 6	8
Terbutaline	500	81 ± 10	50	<2	NA
Timolol	500	89 ± 5	50	83 ± 5	7

Table 4
Percent recoveries and standard deviations (four replicate extractions and determinations) of β-blockers and β_2-agonists from spiked sewage effluent after cold storage

Compound	Initial concentration[a] (ng/L)	% recovery			
		1 day	2 days	4 days	7 days
Acebutolol	852	91 ± 11	81 ± 12	80 ± 14	102 ± 7
Alprenolol	209	97 ± 7	115 ± 5	105 ± 9	100 ± 10
Atenolol	1782	100 ± 7	108 ± 19	100 ± 18	122 ± 18
Clenbuterol	260	90 ± 13	83 ± 10	76 ± 10	95 ± 5
Labetalol	309	89 ± 4	78 ± 14	80 ± 10	91 ± 3
Metoprolol	726	86 ± 9	82 ± 5	80 ± 16	99 ± 12
Nadolol	267	107 ± 7	121 ± 21	104 ± 16	109 ± 13
Propranolol	245	108 ± 9	128 ± 11	110 ± 20	110 ± 6
Salbutamol	253	86 ± 13	76 ± 13	79 ± 16	97 ± 7
Sotalol	881	86 ± 16	73 ± 8	73 ± 16	94 ± 5
Timolol	247	91 ± 7	86 ± 14	89 ± 13	96 ± 5

[a] The initial concentrations were determined immediately after fortification of the sample with a mixture of the drugs to a nominal concentration of 250 ng/L. The concentration of each drug was the sum of the blank in the effluent sample and the spike.

3.3. Method performance—precision, accuracy, detection limits, and linearity runs

Distilled water samples fortified to either 500 or 50 ng/L for each β-blocker and β_2-agonist were extracted and analyzed by the present procedure in order to evaluate the performance of the method. As shown in Table 3, more than 80% recovery and acceptable precision were obtained for all drugs except for fenoterol, pindolol, and terbutaline. Pindolol was not extracted at all at both concentrations and the recoveries of fenoterol and terbutaline were very low at the 50 ng/L level. It was therefore concluded that our present method did not apply to these three compounds and they were then excluded in all further studies. The method detection limits, estimated by three times the standard deviation obtained at the lower spiking level, ranged from 6 to 11 ng/L for the drugs (Table 3). The LC–MS/MS responses were linear over a range from 10 to 500 pg/μL, with an $r^2 > 0.99$ for each compound.

3.4. Matrix effects

In order to study the effect of sample coextractive on the LC–ESI–MS/MS responses, we have analyzed the extracts undiluted and after a twofold dilution with solvent A for all the

Table 5
Concentrations (ng/L) of β-blockers and β_2-agonists found in Ontario (Canada) sewage primary and final effluent samples

	Propranolol	Sotalol	Metoprolol	Atenolol	Acebutolol	Labetalol	Bisoprolol	Timolol	Nadolol	Salbutamol
Daily dose (mg)[a]	80–320	160–320	50–400	25–100	200–800	100–400	2.5–40	20	80–320	0.1–0.4
log K_{ow}[b]	3.21	−0.62	1.88	0.16	1.77	2.55	2.15	1.91	0.93	NA
STP/sample										
A. Primary effluent	19	270	344	1880	1090	70	47	<7	139	14
A. Final effluent	30	257	297	987	662	77	71	<7	36	<6
B. Primary effluent	79	463	268	1650	646	279	74	<7	146	17
B. Final effluent	50	280	177	642	303	69	43	<7	26	8
C. Primary effluent	49	180	246	1350	562	185	59	<7	81	7
C. Final effluent	27	162	185	729	308	64	41	<7	47	<6
D. Primary effluent	39	312	243	1180	221	87	26	<7	76	9
D. Final effluent	45	284	244	839	184	84	20	<7	76	7
E. Primary effluent	15	321	214	2210	380	114	25	7	129	8
E. Final effluent	35	264	274	1680	463	175	23	<7	66	10
F. Primary effluent	23	567	664	1570	256	83	32	<7	76	9
F. final effluent	22	429	402	1080	219	81	21	<7	56	8
G. Primary effluent	34	297	308	1720	367	83	38	7	74	9
G. Final effluent	29	219	243	1380	310	90	24	<7	70	7
Range overall	15–79	162–567	177–664	642–2210	184–1090	64–279	20–74	<7–7	26–146	<6–17
Median overall	32	282	257	1370	339	84	35	7	75	9
Median primary	34	312	268	1650	380	87	38	7	81	9
Median final	30	264	244	987	308	81	24	<6	56	8
% removal	12	15	9	40	19	7	37	–	31	11

Alprenolol and clenbuterol were not detected in the given samples.
[a] Adult, for treatment of hypertension and prevention of cardiac infarctions, from Ref. [29].
[b] From Refs. [30–32].

β-blockers and salbutamol. With an overall concentration factor of 250, none of the detected compounds in all test samples exhibited a change in the response factor after dilution, suggesting the absence of matrix effect in our case. We have further analyzed the extracts for the four more abundant β-blockers (acebutolol, atenolol, metoprolol, and sotalol) after a 5- and a 10-fold dilution, and again no change in the response factors have been observed. However, if the methanol fraction (which was discarded in this procedure) and the elution fraction were combined and analyzed, the response factors of all drugs were severely reduced (by 50% to over 90%). The response factors would not remain constant until the extracts were four-fold diluted or more. Although, with a limited database, it was impossible for us to conclude that the present procedure would eliminate signal suppression for β-blockers in all wastewater samples. This optimized extraction and cleanup method has, at least, made a significant contribution in the reduction of the matrix effect that was frequently observed in the LC–ESI–MS and LC–ESI–MS/MS analyses of organics in highly contaminated wastewater extracts.

3.5. Stability study of spiked sewage effluent samples

As it is often impractical to extract a sample immediately after collection, the validity of analytical results relies entirely on the integrity of the compounds of interest in the sample matrix. This situation is particularly crucial for sewage as the sample is extremely active biologically. However, such vital information is rarely available in the literature. We have investigated the stability of the β-blockers and β_2-agonists in sewage samples under cold storage conditions (4 °C in the dark in amber bottles). In this case, a bulk sewage effluent sample was collected from a local sewage treatment plant. Aliquots of the spiked effluent were extracted and analyzed for the drugs, in replicates of four,

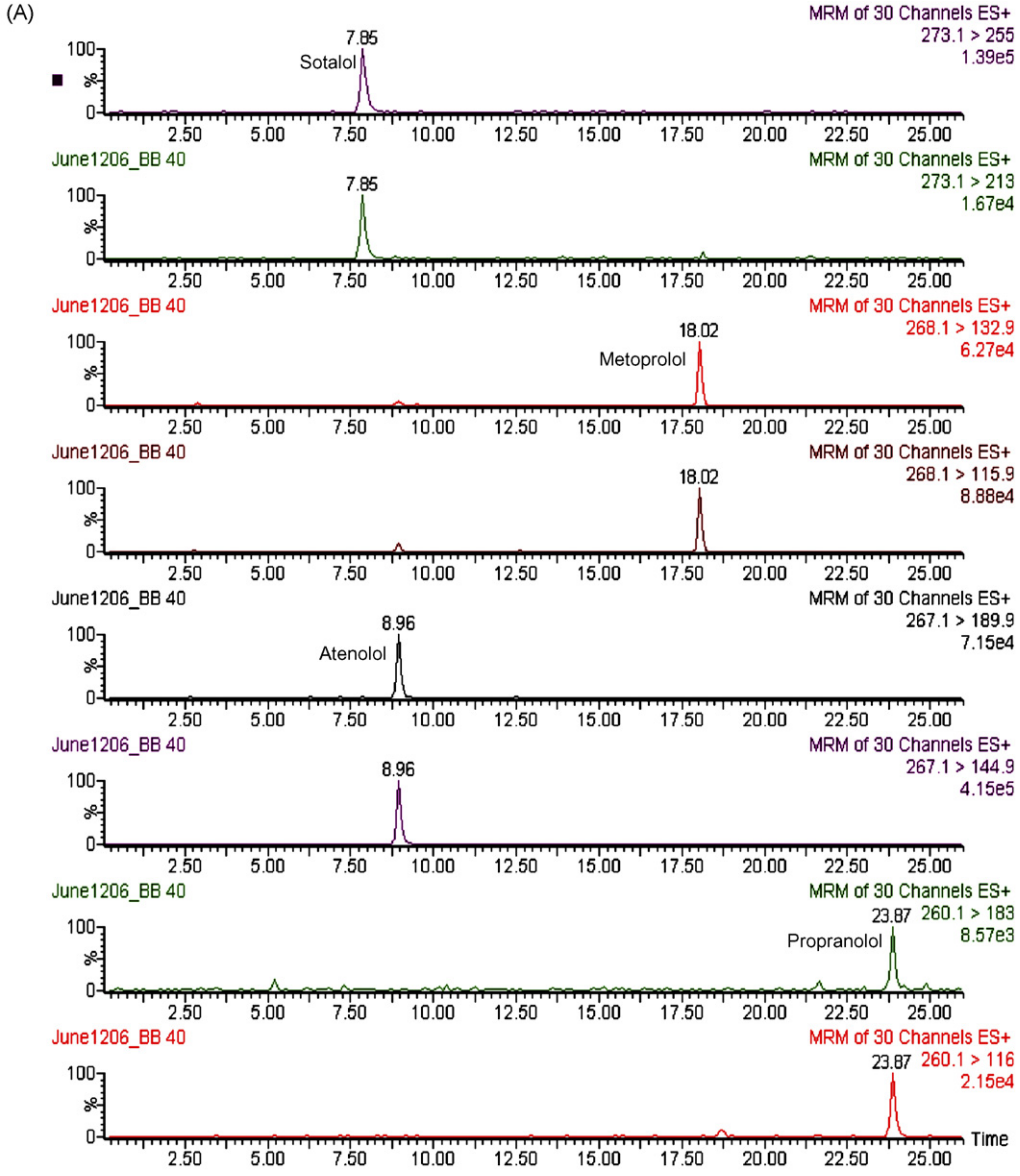


Fig. 2. MRM transitions of the β-blockers found in a sewage effluent.

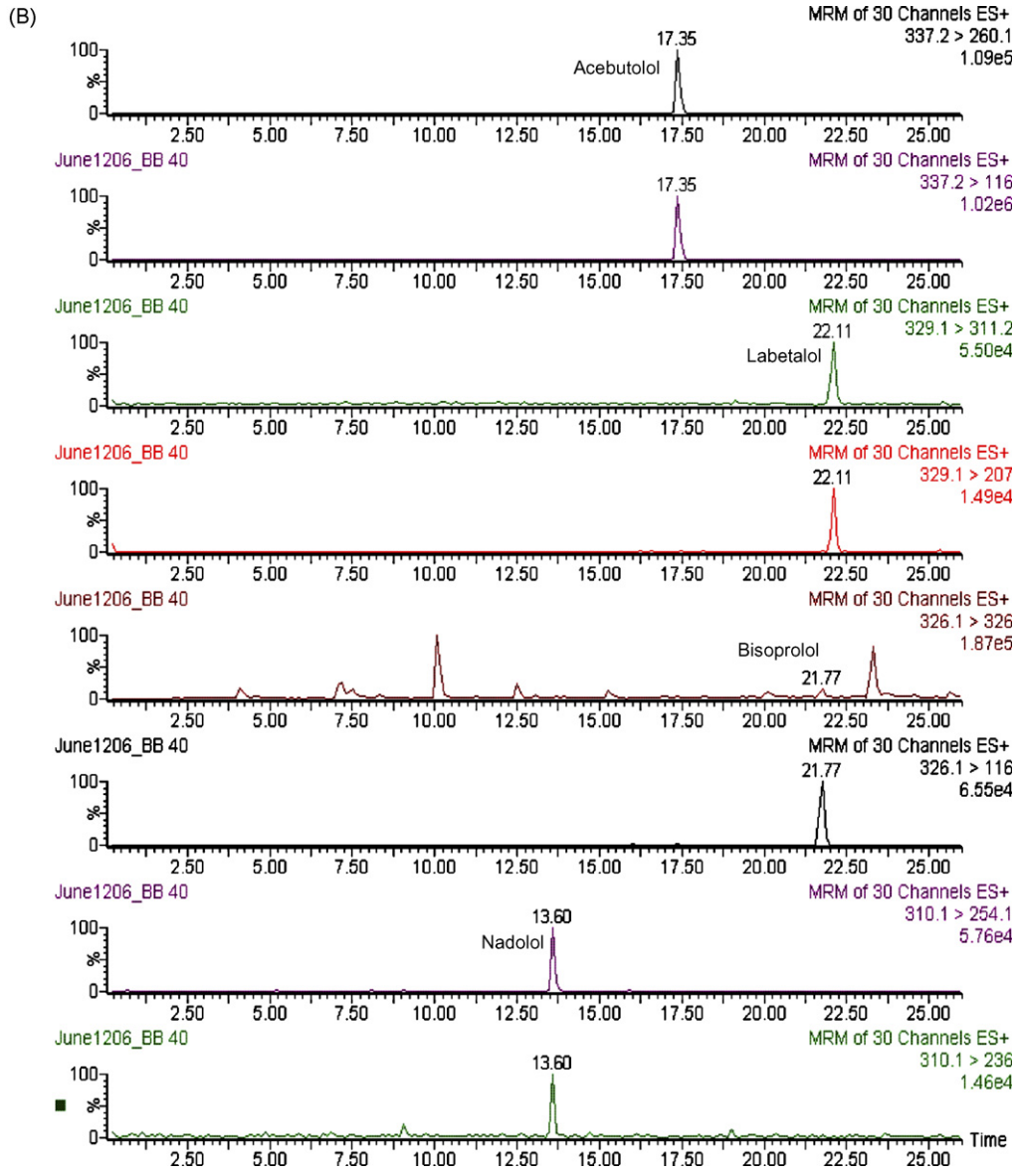


Fig. 2. (*Continued*).

immediately after spiking, as well as 24 h, 2, 4, and 7 days after spiking. As indicated by the results summarized in Table 4, all drugs were >80% recovered at all time intervals except for a few cases in this stability study. The stability of the β-blockers and the β_2-agonists in spiked sewage effluents under cold storage conditions suggested that the drugs are potentially stable for a few days at 4 °C in a similar matrix after the samples have been collected.

3.6. Occurrence of β-blockers and β-agonists in sewage samples

Of the 12 β-blockers and β_2-agonists that could be extracted by the present method, alprenolol and clenbuterol were not detected in any one of the wastewater samples tested (Table 5). Timolol was detected, at 7 ng/L, in only two of the 14 samples. Salbutamol was found in all but two samples, with a median concentration of 9 ng/L. In contrast, atenolol, acebutolol, sotalol, and metoprolol were found in all samples at much higher levels, and their overall median concentrations were 1370, 339, 282, and 257 ng/L, respectively. Labetalol, nadolol, bisoprolol, and propranolol were also present in all samples, with median concentrations ranging from 84 to 32 ng/L. Our findings are therefore consistent with the results reported previously for samples collected in Canada [10] and other parts of the world [6,17,19,21]. A chromatogram depicting the MRM transitions of the β-blockers and salbutamol found in a final effluent is shown in Fig. 2.

The concentration of a drug occurring in a 24-h composite sewage sample is proportional to the amount of daily consumption, the quantity of unmetabolized drug (or sometimes its conjugate) excreted, its half-life once it entered our sewer system, as well as the amount of wastewater generated during the day. Since most of the above information is not available to us, it

is impossible to generate predicted concentrations of these drugs for comparison with the observed levels. However, it was noted that those β-blockers that occurred at higher concentrations in our samples (Table 5) were either more hydrophilic (e.g. sotalol and atenolol, with log K_{ow} − 0.62 and 0.16, respectively, which were largely excreted unchanged) or more heavily used (e.g. acebutolol and metoprolol which were partially reflected by the higher daily doses). Further information is needed to confirm whether or not the near 10-fold difference in median concentrations (34 vs. 312 ng/L in the primary effluent) observed, respectively, for propranolol and sotalol, the two β-blockers with comparable recommended daily doses, was actually due to their large difference in hydrophilicity (log K_{ow}), i.e. their metabolic rates. It was also noted that the median concentrations of nearly all the β-blockers and salbutamol, the only β_2-agonist found in wastewaters in this study, were similar in both primary and final effluents, with the overall removal rates ranging from 7 to 40%. In a recent study on the removal of selected pharmaceuticals in wastewater treatment plants from five EU countries [28], the removal rates for metoprolol and atenolol were both <10%. In contrast, our observed removal rates were 9 and 40%, respectively, for the two drugs. Regardless, these results suggest that β-blockers are stable enough to survive the conventional wastewater treatment techniques and perhaps that was the reason why such compounds were readily detected in surface water samples collected near the outfall of sewage treatment plants in an earlier study [9].

4. Conclusions

Due to its sensitivity and the highly specific precursor/product transitions, LC–ESI–MS/MS is an invaluable tool for the positive identification and quantification of β-blockers and β_2-agonists occurring in sewage samples. Using a selective solid-phase extraction procedure, matrix coextractives can be greatly reduced, so that quantitative results are apparently not affected by the matrix effect. Our results suggested that at least eight β-blockers and salbutamol, ranging from low to high nanogram per liter levels, were present in all municipal wastewater samples collected in southern Ontario, Canada. Due to their frequent occurrence and stability in the sewer system, further work on the fate and effects of β-blockers and β_2-agonists in environmental samples is needed in order to assess the impact of these drugs on the aquatic ecosystem.

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