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Trace determination of β-blockers and β_2-agonists in distilled and waste-waters using liquid chromatography–tandem mass spectrometry and solid-phase extraction

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ABSTRACT

A highly sensitive method for simultaneous determinations of eleven β-blockers and β-agonists in distilled and waste-waters using liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI-MS–MS) was developed, optimized and validated. The method was used for trace determinations of acebutolol, atenolol, metoprolol, propranolol, timolol, nadolol, labetalol, oxprenolol, pindolol, alprenolol and terbutaline. Oasis MCX and Clean Screen cartridges were used for solid phase extractions and an alkaline mixture of dichloromethane–propanol was used as mobile phase. Matrix effect was reduced by using methanol as a pre-eluant for removing co-extractives on the SPE cartridges and by applying the internal standard method for quantification. Using Oasis MCX-SPE cartridges, developed method gave average recoveries of 77.20–97.30% for drugs spiked at 150.00–500.00 pg/ml. Intra-day precisions gave RSD of 3.367–12.489% while as inter-day precisions gave RSD of 6.425–19.768%. Detection limits of 0.11–6.74 pg/ml and quantification limits of 0.14–22.88 pg/ml were obtained. Signal's suppression in the range of 4.50–24.50% was recorded due to the matrix effect. Drugs spiked in wastewater at 500.00 pg/ml concentrations level and stored at 4 °C for 6 days, showed insignificant degradation. Developed method was successfully applied to the analysis of pharmaceutical residues in effluents wastewaters. Five β-blockers and one β-agonists were detected in Al-Ain and Abu Dhabi wastewaters at average concentrations of 3.44–19.05 pg/ml. Atenolol was detected at higher average concentration ranged in 125.60–234.28 pg/ml. Results obtained suggest that adopted wastewater treatment processes are not enough to degrade these compounds.

1. Introduction

Pharmaceuticals in environment represent a category of pollutants called "emerging contaminants" that have raised concerns during the last decade [1]. Drugs are generally lipophilic to pass through the cell membranes and persistent to avoid being inactive before administration. They enter the environment via different routes, bioaccumulate and subsequently affect the aquatic and terrestrial ecosystems [2]. Fates of drugs in wastewater treatment plants have been discussed by Jorgensen and Sorensen [3]. Drugs and their metabolites are continuously introduced into sewage waters through excreta, disposal of unused/expired drugs or directly from pharmaceutical discharges [4,5]. Three types of harmful effects have been reported for drugs in environment that include (1) normal toxic effects impact cells, organs, ecosystems and ecosphere, (2) endocrine disruption effects that disturbs the normal functions of hormones and (3) environmental damaging effects disturbs the hormone balance in organisms. Out of more than three thousands reported human and veterinary drugs, less than 5.00% have been studied for their presence in environment [6,7]. Antibiotics received the highest concerns because of their wide use and responsibility for genetic selection of more harmful bacteria [8]. Anti-inflammatory, lipid regulators and β-blockers have also been investigated because of their intensive use, inefficient removal by wastewater treatment processes and poor degradability [9–11]. Other pharmaceutical residues reported in wastewater included anti-epileptic carbamazepine, analgesic anti-inflammatory, the analgesic opiate codeine, antidepressant, antibiotic, anti-ulcer ranitidine, lipid regulators, bronchodilators, histamine-2-blockers, anti-inflammatory agents, calcium channel blockers, angiotensin-II antagonists, antidepressants, illicit and psychiatric drugs [12–16]. These drugs can possibly end up in drinking waters sources via different routes and cause risks to humans upon long-term exposures [17,18].

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Cardiovascular diseases have become the second cause of death worldwide. In United Arab Emirates (UAE), overall consumption of β-blockers has reached more than one million units in 2010. β-Blockers are the first choice to treat abnormal heart rhythms, angina, hypertension, tachycardia, thyrotoxicosis, hypertrophic subaortic stenosis and as prophylactics against heart attacks [19,20]. They are also used in treating ocular pressure in glaucoma and ocular hypertension, physical symptoms associated with anxiety, migraine and symptoms associated with hyperthyroidism [21,22]. β-Blockers act by blocking the β-receptors in human body, slowing nerve impulses and reducing heart workload [15].

On the other hand, β_2-agonists are used to treat asthma, airway narrowing, pulmonary disorders and to promote growth in livestock production. They prevent bronchospasms by activating the β-receptors and relaxing the airway's smooth muscles [23–25]. The UAE overall consumption of β_2-agonists has reached one million units in 2010.

Due to the potential harmful impact of β-blockers and β_2-agonists – even if exist at very low concentrations – in aquatic environment, search for developing sensitive and selective analytical methods for their determinations has been in continuous process. Several β-blockers include propranolol, nadolol, atenolol, metoprolol, sotalol, bisoprolol have been reported in different aquatic environments [26–31]. Methods based on GC–MS, LC–MS and LC–tandem MS have been reported [32]. LC–MS is the technique of choice for determining pharmaceuticals and their metabolites in environmental samples because of its selectivity and sensitivity. β-Blockers in wastewater have been separated on C-18 columns using water–methanol–acetonitrile as mobile phase. Ammonium acetate, acetic acid, formic acid or methylammonium acetate have been used to improve sensitivity of the mass spectrometric detection [33–36]. β-Blockers and β_2-agonists in sewage, river and drinking waters were determined using GC–MS and LC–ESI-MS/MS. Average recoveries >70.00% and SD ≤ 12.00% were reported. Using PTFE vials and organic solvents have been recommended to reduce β-blockers' adsorption on active glass surfaces caused by the secondary amino group and results in low recoveries [37]. Atenolol, nadolol, metoprolol, bisoprolol and betaxolol were determined in wastewater using HPLC with fluorescence detection [38] and chiral LC–MS/MS [39]. Seventy-six pharmaceutical agents of nine classes included β-blockers, tetracyclines, macrolides, fluoroquinolones, diuretics, sedatives, sulfonamides and chloramphenicol were measured in wastewater using LC–ESI-MS/MS in positive and negative ion modes [40]. Laven used Oasis MCX and MAX solid phase extraction cartridges with LC–MS time-of-flight for detecting pharmaceuticals in wastewater [41]. Thirty pharmaceuticals were analyzed in surface and ground waters using positive and negative ion modes of LC–MS/MS [42].

The challenge in detecting β-blockers and β_2-agonists in wastewaters is attributed to their mixed occurrences, very low concentrations, complexity of the wastewater matrix and interference caused by co-extracted and/or co-eluted compounds from wastewater. Interfering substance with target analytes affect the ionization performance of the MS system and result in erroneous quantifications. Various extraction protocols have been recommended for reducing matrix effects, but not yet completely effective due to the different physicochemical properties of target analytes. Sensitivity of the analytical method is also challenging due to the presence of β-blockers and β_2-agonists in wastewaters at relatively low concentrations.

Therefore, this work aims to develop a sensitive and selective analytical method for the determinations of β-blockers and β_2-agonists in wastewater effluents using liquid chromatography–tandem mass spectrometry in conjunction with solid-phase extractions. The work also aims to open a door for subsequent studies on other pharmaceuticals and toxic chemicals in waste and drinking waters and to alert authorities about the potential harmful effects of such drugs on recycled water and on aquatic ecosystems. This study is the first to explore the presence of pharmaceuticals in wastewaters from United Arab Emirates.

2. Experimental

2.1. Materials and reagents

The β-blockers and β_2-agonists involved in this study were purchased from Sigma Aldrich with ≥98% purity. They included acebutolol: (*RS*)-*N*-{3-acetyl-4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl}butanamide (CAS: 34381-68-5); atenolol: (*RS*)-2-{4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl}acetamide (CAS: 29122-68-7); pindolol: (*RS*)-1-(1*H*-indol-4-yloxy)-3-(isopropylamino)propan-2-ol (CAS: 13523-86-9); oxprenolol: (*RS*)-1-[2-(allyloxy)phenoxy]-3-(isopropylamino)propan-2-ol (CAS: 6452-71-7); labetalol: (*RS*)-1-[2-(allyloxy)phenoxy]-3-(isopropylamino)propan-2-ol (CAS: 36894-69-6); metoprolol: (*RS*)-1-(isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol (CAS: 56392-17-7); propranolol: (*RS*)-1-(1-methylethylamino)-3-(1-naphthyloxy)propan-2-ol (CAS: 525-66-6); timolol: (*S*)-1-(*tert*-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol (CAS: 26839-75-8); nadolol: (2*R*,3*S*)-5-{[(2*R*)-3-(*tert*-butylamino)-2-hydroxypropyl]oxy}-1,2,3,4-tetrahydronaphthalene-2,3-diol (CAS: 42200-33-9); alprenolol: (*RS*)-1-(2-allylphenoxy)-3-(isopropyl-amino)propan-2-ol (CAS: 13707-88-5); terbutaline: (*RS*)-5-[2-(*tert*-butylamino)-1-hydroxyethyl]benzene-1,3-diol (CAS: 23031-25-6); and clenbuterol: (*RS*)-1-(4-amino-3,5-dichlorophenyl)-2-(*tert*-butylamino)ethanol (CAS: 37148-27-9). HPLC-grade methanol, dichloromethane, 2-propanol and ethyl acetate and analytical grades of formic acid, ammonium formate and ammonium hydroxide were also purchased from Sigma–Aldrich. Deionized water was used throughout.

Stock standard solutions (1.00 mg/ml) of each β-blocker were prepared in methanol and stored at 4 °C until use. Fresh working solutions were prepared daily. Mixtures of pharmaceutical standards at different concentrations were prepared by appropriate dilutions from the stock solutions.

2.2. Sample preparation and solid-phase extraction (SPE)

Into polyethylene bottles pre-rinsed with deionized water, sixty effluent samples were collected from two wastewater treatment plants in Abu Dhabi and Al-Ain over the period February 2009 to May 2009. Effluent samples were filtered under vacuum using 0.45 μm filters pre-washed with methanol and deionized water (GF6, Schleicher & Schuell) and stored at 4.00 °C until extraction.

A 200.00 ml sample was introduced into an Oasis MCX extraction cartridge (60.00 mg, 3.00 ml); pre-conditioned using 6.00 ml methanol followed by 10.00 ml of 0.001 M HCl at a flow rate of 10.00 ml/min. The cartridge was then dried for 10.00 min under vacuum, washed with 50.00 ml 0.001 M HCl and 6.00 ml methanol, respectively. After which, the cartridge was eluted with 8.00 ml dichloromethane–isopropanol–ammonium hydroxide IN 78:20:2% ratio. Resultant extract was evaporated till dryness on water bath under gentle nitrogen steam and re-constituted into 1.00 ml ammonium formate–methanol in 70:30% ratio. Resultant solution was loaded into 2.00 ml vials and mixed with 50.00 μl of clenbuterol (1.00 μg/ml) as an internal standard. A 20.00 μl aliquot was injected into the LC–MS–MS system.

Similarly 200.00 ml effluent was introduced at flow rate of 10.00 ml/min into a Clean Screen cartridge (130.00 mg, 3.00 ml) pre-conditioned with 6.00 ml methanol, 10.00 ml 1.00×10^{-6} M

HCl, 6.00 ml methanol and 2.00 ml phosphate buffer pH 6.00. The cartridge was then washed with 1.00 ml acetic acid (1.00 M), dried for 7.00 min under vacuum, washed again with 6.00 ml methanol and dried for 2.00 min. The cartridge was then eluted with 8.00 ml ethyl acetate containing 2.00% ammonium hydroxide. Resultant solution was evaporated till dryness on water bath under nitrogen, reconstituted into 1.00 ml ammonium formate–methanol (70:30%), loaded into 2.00 ml vial and mixed with 50.00 μl oxazepam (1.00 μg/ml) internal standard. A 20.00 μl portion was injected into the LC–MS–MS system.

2.3. Calibration curves

Standard solutions having concentrations of 5.00, 10.00, 25.00, 50.00, 100.00, 200.00, 400.00, 1000.00 and 2000.00 pg/ml were prepared by spiking deionized water with β-blockers and β_2-agonists. A 200.00 ml aliquot of each standard adjusted to pH 3.00 or 6.00 using 0.001 M HCl was extracted using Oasis MCX or Clean Screen cartridges, respectively as described in Section 2.2. A 50.00 μl of either clenbuterol (1.00 μg/ml) were added as internal standard. Samples were measured using the LC–MS. Calibration curves of concentration versus normalized areas were plotted. Curves were made in triplicate.

2.4. Conditions for LC separation and MS detection

LC analysis was performed using Thermo Finnigan-TSQ Quantum Discovery LC system equipped with triple-quadrupole mass spectrometer (Thermo Finnigan, San Jose, CA, USA) and supported with electrospray ionization source and thermostated oven.

A 20.00 μl sample aliquot was injected onto Hypersil gold C-18 HPLC column (50.00 mm × 2.10 mm × 3.00 mm; Thermo Finnigan, San Jose, CA, USA) at 10.00 °C. The column was eluted with methanol and 2.00 mM ammonium formate mixture at flow rate of 0.40 ml/min. A gradient elution started with 10:90% methanol:ammonium formate and increased linearly to 90:10% in 5.00 min and returned back to 10:90% within 0.50 min was applied. The system was allowed to equilibrate for 4.50 min before the next injection.

Flow from the column was transferred to the triple-quadrupole mass spectrometer using nitrogen gas for desolvation and nebulization at flow rates of 40.00 and 12.00 arbitrary units, respectively. Argon was used for collision at pressure of 1.50×10^{-3} Torr. The ion source and capillary were kept at 500.00 and 300.00 °C, respectively. Positive ions were acquired in multiple reactions monitoring (MRM) mode with a dwell time of 0.05 s. The electrospray needle voltage was set at 4.700 kV and the vaporization temperature was set at 250.00 °C. The auxiliary and the sheath gas pressures were 4.00×10^{-3} and 49.00×10^{-3} Torr, respectively. Choices of precursor ions, product ions, cone voltages and collision energies were optimized using the atmospheric pressure ionization (API) source operated in positive electrospray ionization (ESI) mode.

Detection of the drugs was performed in the MRM mode with a single time segment. Peak widths for the precursor and its corresponding product ions in Q_1 and Q_3 were both set at 0.70 amu (FWHM). The scan width for the selected product ions was set at 0.50 amu and the scan time at 0.05 s per transition. Argon was used as the collision gas at 1.5×10^{-3} Torr. Data processing was performed using the Finnigan Xcalibur Version 1.3 software.

2.5. Method validation

Validation parameters included linearity, accuracy, recovery, precision, selectivity, matrix effect, limit of detection, limit of quantification and stability were evaluated using β-blockers and β_2-agonists spiked in distilled water (Sections 2.2 and 2.3). At least, triplicate samples were measured for each data point.

Linearity was tested in the concentration range 1.00–100.00 pg/ml. Calibration curves were plotted as the area ratio of analyte to the internal standard versus concentration. Linearity in the concentration range 50.00–2000.00 pg/ml was also tested. Linearity was considered acceptable with correlation coefficient of $\geq$0.99.

Accuracy and recovery were evaluated using spiked samples at 150.00 and 500.00 pg/ml. Results obtained using Oasis MCX and Clean Screen cartridges were compared. Accuracies $\geq$75% were also considered acceptable.

Precision was evaluated based on intra-day assays using 150.00 and 500.00 pg/ml and inter-day assays using 20.00 and 80.00 pg/ml. Variance coefficients based on at least seven replicates were calculated. Precisions $\leq$20.00% were considered accepted.

Selectivity was evaluated by investigating the interferences of some common acidic and basic drugs on the analytes' signals. Matrix effect caused by co-elution of endogenous components from wastewater was estimated by measuring signals' suppressions compared to the neat standard having the same concentrations. Signal suppression is given by: % suppression = $[1 - (A_s/A_{abs})] \times 100$, where A_s is the analyte peak area in the spiked wastewater and A_{abs} is the analyte peak area of the neat standard at the same concentration.

Detection and quantification limits (LOD and LOQ) were determined using the statistical and empirical approaches. Statistically, LOD was estimated as the mean of the blank concentration plus there times the standard deviation of the blank. LOQ was estimated as the mean of the blank concentration plus ten times the standard deviation of the blank. According to the empirical definition, LOQ is defined as the concentration at which changes in retention time is within 2.00% and the quantitation value is within 20.00% of the target concentration in 90% of replicate samples [47].

Stabilities of drugs in wastewater were evaluated spiking drugs in wastewater samples at a concentration of 500.00 pg/ml. Samples were incubated at room temperature, extracted and analyzed at days 1st, 4th and 6th. Recoveries at the 4th and 6th days were compared with the 1st day using the statistical Student's *t*-test. Four replicates were used for each data point.

3. Results and discussion

β-Blockers and β_2-agonists are the basic compounds with structural formula represented by R—CH(OH)—CH_2—NH—R\. They contain secondary amino groups and hydroxyl groups on two adjacent carbon atoms. The names of β-blockers are ended by "olol" and its R is bonded though a —CH_2—O— linkage while as the names of β_2-agonists are ended by "alol" and its R is bonded through a C—C linkage (Fig. 1).

3.1. Solid phase extraction

Extractions of β-blockers and β_2-agonists using C-18-based stationary phases such as Envi-18, Isolute C-18, Oasis HLB, Empore SDB-XC and others have been reported [43]. These extractions suffered from low recoveries and/or presence of co-extractives resulted in matrix effects during ESI-MS–MS detections. To overcome these shortcomings, extraction approaches based on converting the amino groups to quaternary ammonium salts in strong acidic solution followed by selective extraction of the formed ionic compounds on strong cation-exchangers, were suggested. Under these conditions, neutral and acidic compounds in effluent wastewaters will be weakly bound to the SPE ion exchange cartridges and can be eluted first with methanol while as the basic

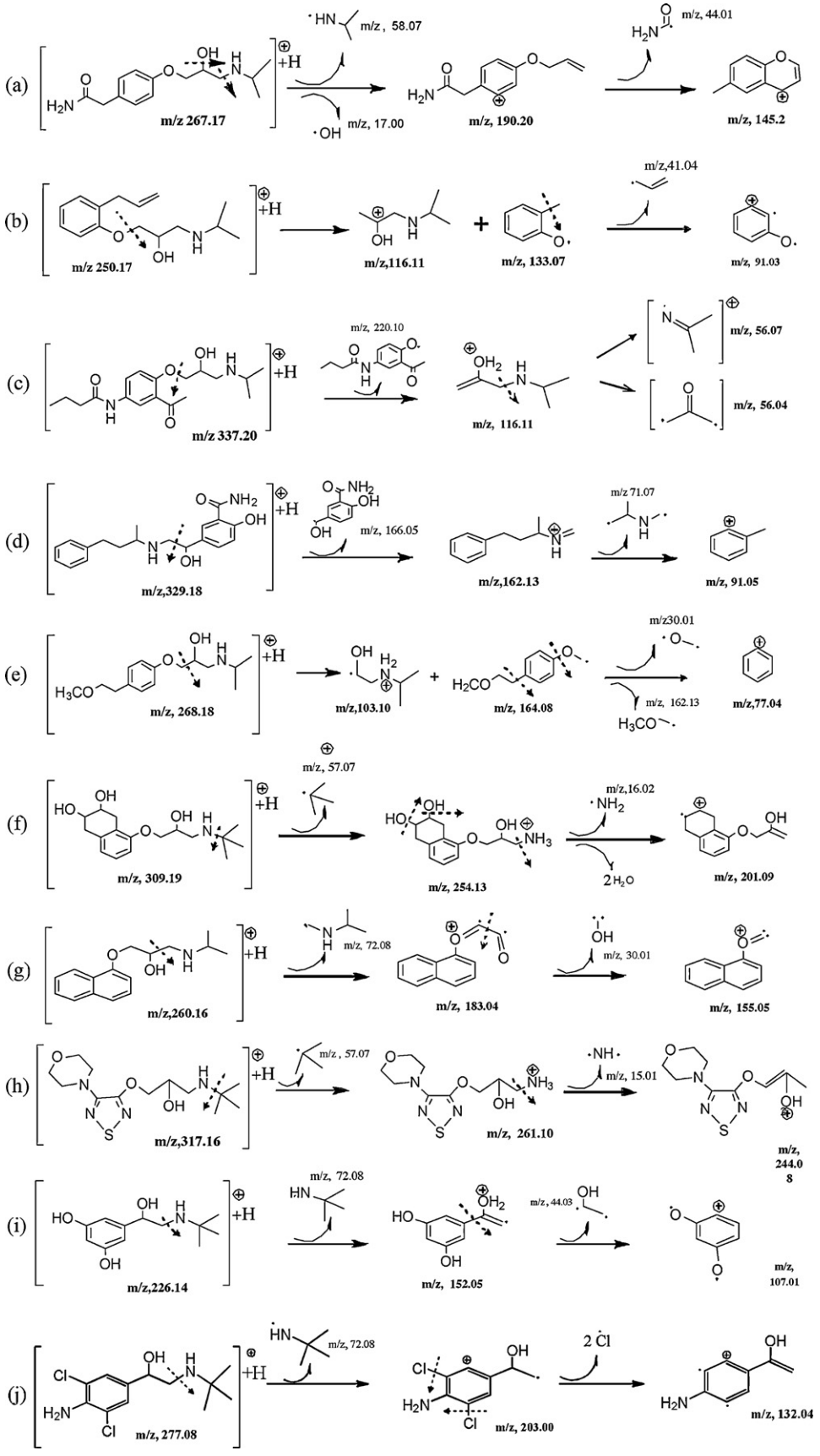

Fig. 1. Structural formulae and fragmentation schemes for atenolol (a), alprenolol (b), acebutolol (c), labetalol (d), metoprolol (e), nadolol (f) propranolol (g), timolol (h), terbutaline (i), clenbuterol (j) pindolol (k) and oxprenolol (l).

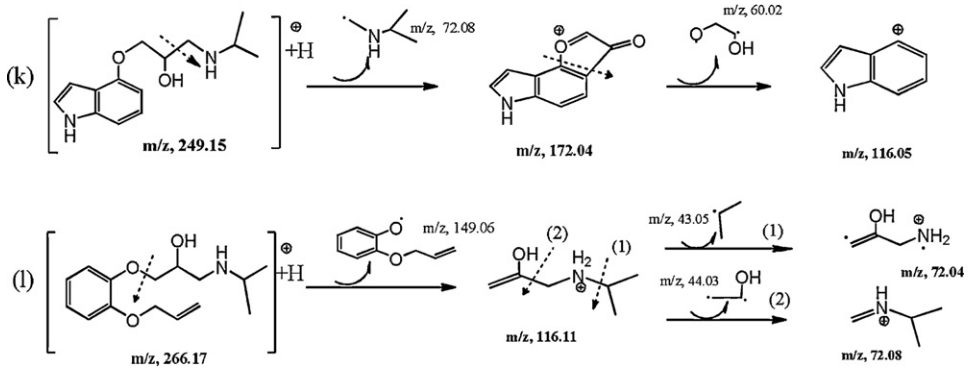

Fig. 1. (*Continued*.)

Table 1
Absolute recoveries of extracted β-blockers spiked in distilled water at different concentrations using Oasis MCX cartridges (A) and Clean Screen cartridges (B). SD, standard deviation; relative error% = ((measured value − true value)/true value) × 100.

Compound	Spiking level (pg/ml)	% Recovery (Mean ± SD) (n = 7)	Spiking level (pg/ml)	% Recovery (Mean ± SD) (n = 5)
(A) Using Oasis MCX extraction cartridges				
Alprenolol	150.00	83.00 ± 2.72	500.00	83.00 ± 5.30
Acebutolol	150.00	83.00 ± 2.05	500.00	86.00 ± 2.26
Metoprolol	150.00	89.00 ± 2.78	500.00	90.00 ± 1.89
Nadolol	150.00	78.00 ± 3.77	500.00	82.00 ± 3.12
Propranolol	150.00	81.00 ± 6.16	500.00	77.00 ± 7.59
Timolol	150.00	79.00 ± 1.47	500.00	86.00 ± 4.13
Pindolol	150.00	25.00 ± 8.79	500.00	30.00 ± 6.33
Oxprenolol	150.00	97.00 ± 3.09	500.00	87.00 ± 1.46
(B) Using Clean Screen extraction cartridges				
Atenolol	600.00	48.30 ± 11.60	1600.00	45.90 ± 6.48
Alprenolol	600.00	72.30 ± 12.30	1600.00	97.10 ± 1.72
Acebutolol	600.00	79.80 ± 8.40	1600.00	90.30 ± 2.93
Metoprolol	600.00	82.00 ± 7.95	1600.00	92.30 ± 1.56
Nadolol	600.00	32.00 ± 3.68	1600.00	51.80 ± 2.61
Propranolol	600.00	61.80 ± 14.10	1600.00	83.40 ± 3.81
Timolol	600.00	80.30 ± 8.60	1600.00	91.80 ± 1.79
Terbutaline	600.00	49.20 ± 6.11	1600.00	48.50 ± 4.04

ionic β-blockers and β_2-agonists will be strongly bound and eluted second by alkaline organic solvents [44–46].

In our work, samples were acidified with 0.001 M HCl, followed by solid phase extractions using Oasis MCX or Clean Screen cartridges. Effect of pH on extraction efficiency was tested. Dichloromethane–2-propanol–ammonium hydroxide mixture (78:20:2%) was found optimum for extracting β-blockers and β_2-agonists from acidified wastewater effluents. Oasis MCX has shown higher extraction efficiency compared to Clean Screen cartridges. The reason could be attributed to the fact that Clean Screen cartridges are more efficient for extracting drugs at pH ≥ 6.00. Table 1 shows the recoveries of investigated β-blockers and β_2-agonists using Oasis MCX and Clean Screen cartridges.

3.2. LC separation and MS detection of β-blockers and β_2-agonists

LC–MS–MS is advantageous in determining β-blockers and β_2-agonists since it does not need derivatization and can achieve detection limits down to 1.00 pg/ml. In our work, separation of investigated drugs was accomplished using C-18 Hypersil Gold column (50.00 mm × 2.10 mm × 3.50 μm, Thermo Finnigan, San Jose, CA, USA) and a mobile phase consisted of methanol and ammonium formate. The latter was used to maintain good peak shape and facilitate the production of the precursor ion for LC–MS–MS analysis. Separation of hydrophilic drugs such as atenolol from lipophilic ones such as labetalol and propranolol was achieved using gradient elution varied from 10:90% to 90:10% methanol:ammonium formate mixtures (Table 2). Peaks at retention times between 1.22 and 7.34 min were identified for investigated β-blockers and β-agonists confirming the optimization of separation condition (Table 3). Standard deviations in the range 0.03–0.09 min were obtained for retention times from more than twenty samples (n = 20) recorded over 5 days (Table 3).

Table 2
Gradient elution program and flow rate used for chromatographic separation of investigated β-blockers and β_2-agonists.

Time (min)	Flow (ml/min)	Solvent A% (methanol)	Solvent B% (2 mM ammonium formate)
0.00	0.40	10.00	90.00
1.00	0.40	10.00	90.00
5.00	0.40	90.00	10.00
5.50	0.40	10.00	90.00
10.00	0.40	10.00	90.00

Using ESI as ionization source, precursor ions were monitored as their protonated species. Optimization of our measurements has been performed by recording spectra of each compound in full scan mode. Once the $[M+H]^+$ precursor ion was identified, measurement parameters include number of scans, collision energies, cone voltages, voltage on tube lens and others were optimized. Table 3 also shows the retention times, precursor and MRM transitions, collision energies, cone voltages and tube lens for each investigated drug. The precursor ion, the most abundant and a third MRM transitions were found sufficient to confirm the identity of investigated drugs. Additional confirmations for the presence of investigated drugs were inferred from the ratio between the abundances of the

Table 3
Precursor and product ions, retention times and optimization parameters for MS–MS analysis of β-blockers and β_2-agonists observed under ESI LC–MS/MS conditions positive polarity. C.E., collision energy; T.L., tube lens; R.T., retention time (min). Standard deviations for recorded retention times over 5 days for $n = 20$, are given.

Analyte	Precursor ion	R.T. (min)	Product 1 (mass, name)	C.E. (V)	T.L. (V)	Product 2 (mass, name)	C.E. (V)	T.L (V)
Atenolol	267 $[M+H]^+$	1.22 ± 0.03	190.2, [5-(allyloxy)-2-(2-amino-2-oxoethyl) benzene-1-ylium]$^+$	28	114	145.2, [6-methyl-4H-chromen-4-ylium]$^+$	35	114
Alprenolol	250 $[M+H]^+$	7.34 ± 0.03	116.11, [2-hydroxy-1-(isopropylamino)propan-2-ylium]$^+$	25	112	91.03, [3-hydroxybenzene-1-ylium]$^+$	43	112
Acebutolol	337 $[M+H]^+$	6.58 ± 0.04	116.11, [2-hydroxy-1-(isopropylamino)propan-2-ylium]$^+$	30	119	56.07, [propan-2-imine]$^+$/[propan-2-one]$^+$	37	119
Labetalol	329 $[M+H]^+$	7.07 ± 0.03	162.13, [N-methylene-4-phenylbutan-2-aminium]$^+$	29	135	91.05, [2-methylbenzene-1-ylium]$^+$	34	135
Metoprolol	268 $[M+H]^+$	6.52 ± 0.03	103.10, [2-methylbenzene-1-ylium]$^+$	49	122	77.04, [benzene-1-ylium]$^+$	48	122
Nadolol	310 $[M+H]^+$	6.01 ± 0.04	254.13, $[[M+H]^+$–*tert*-butyl$]^+$	26	115	201.09, [254-NH_2–H_2O]$^+$	32	115
Propranolol	260 $[M+H]^+$	7.28 ± 0.03	183.04, [(Z)-naphthalen-1-yl(2-oxoethylidene)oxonium]$^+$	26	124	155.05, [methylene(naphthalen-1-yl)oxonium]$^+$	34	124
Timolol	317 $[M+H]^+$	6.48 ± 0.04	261.10, $[[M+H]^+$–*tert*-butyl$]^+$	28	111	244.08, $[[M+H]^+$–*tert*-butylamine$]^+$	27	111
Terbutaline	226 $[M+H]^+$	1.65 ± 0.04	152.05, [(1-(3,5-dihydroxyphenyl) vinyl) oxonium]$^+$	27	116	107.01, [2,4-dihydroxybenzene-1-ylium]$^+$	41	116
Pindolol	249 $[M+H]^+$	3.17 ± 0.09	172.04, [3-oxo-3,6-dihydrofuro[2,3-e]indol-1-ium]$^+$	27	114	116.05, [1H-indol-4-ylium]$^+$	28	114
Oxprenolol	266 $[M+H]^+$	6.92 ± 0.03	116.11, [2-hydroxy-N-isopropylprop-2-en-1-aminium]$^+$	26	114	72.08, [N-methylenepropan-2-aminium]$^+$ or [2-hydroxyprpenyl-1-amine]$^+$	27	114
Clenbuterol[a]	277 $[M+H]^+$	6.28 ± 0.05	203.00, $[[M+H]^+$–*tert*-butylamine$]^+$	40	114	132.04, [5-amino-2-(1-hydroxy vinyl)benzene-1-ylium]$^+$	25	114

[a] Clenbuterol was used as internal standard.

MRM transitions and small shifts (≤3%) in the retention times of investigated peaks.

Fig. 1 shows schematic representations for the fragmentation patterns of investigated β-blockers and β_2-agonists (a–l). Electrospray ionization results in the formation of $[M+H]^+$ ions, subsequently fragmented into different fragments (Table 3). Fragmentations of atenolol, metoprolol, nadolol, propranolol, timolol, terbutaline, clenbuterol and pindolol are initiated by losing the isopropyl or isobutyl amine moieties. Further fragmentation result in ions with smaller masses based on benzene moieties. The parent ions, and the two most abundant transitions and their expected fragments are listed in Table 3.

3.3. Method validation

Performance of developed method for the determination of β-blockers and β_2-agonists was validated in distilled water using accuracy, linearity, detection limit, quantification limit, precision, selectivity, matrix effect and stability.

3.3.1. Accuracy

Spiked distilled water samples having drugs' concentrations in the range 150.00–1600.00 pg/ml were extracted and analyzed using the developed method. Oasis MCX SPE cartridges gave recoveries in the range of 77.20–97.30% for β-blockers spiked at 150.00 and 500.00 pg/ml. Pindolol gave recoveries between 24.60 and 26.70%. The reason could be attributed to its high adsorption affinity on the SPE cartridges. Using Clean Screen SPE cartridges, recoveries in the range of 45.90–90.80% were obtained for β-blockers spiked at 600.00 and 1600.00 pg/ml. Atenolol and terbutaline gave recoveries between 45.50 and 49.20% (Table 1).

Thus, with the exception of pindolol, acceptable recoveries were obtained for investigated drugs. Oasis MCX cartridges gave better accuracy with 2.70–22.8% relative error compared to 7.70–68.00% relative error obtained using Clean Screen cartridges. Therefore, Oasis MCX cartridges were used for further investigations.

3.3.2. Calibration and linearity

Spiked distilled water with ten β-blockers and one β_2-agonist were extracted using Oasis MCX cartridges and analyzed using the experimental conditions given in Section 2.4. Linear calibration graphs in the concentration range 1.00–100.00 pg/ml were obtained. Calibration graphs for labetalol (1), timolol (2), metoprolol (3), acebutolol (4), oxeprenolol (5), propranolol (6), alprenolol (7), nadolol (8), atenolol (9), pindolol (10) and terbutaline (11) are shown in Fig. 2a. Correlation coefficients $R^2 > 0.99$ were obtained for all compounds.

Calibration graphs in the concentration range 50.00–2000.00 pg/ml were also established. Correlation coefficients of $R^2 > 0.99$ were obtained (Fig. 2b).

These dynamic ranges are four times wider than previously published reports and indicated the suitability of developed method for determining β-blockers and one β_2-agonist in distilled and waste waters at different concentration ranges [15].

3.3.3. Quantification and detection limits

Detection and quantification limits were determined using the statistical and empirical definitions given in Section 2.5 [47]. Using the statistical definitions, detection limits in the range 0.11–6.74 pg/ml and quantification limits in the range 0.14–22.88 pg/ml were obtained for the target drugs (Table 4). Using the empirical definition based on ten replicates at a concentration of 10.00 pg/ml, quantification limits in the range 8.72–10.08 pg/ml were obtained (Table 4) These LOD and LOQ values are comparable to some reported values [15,48].

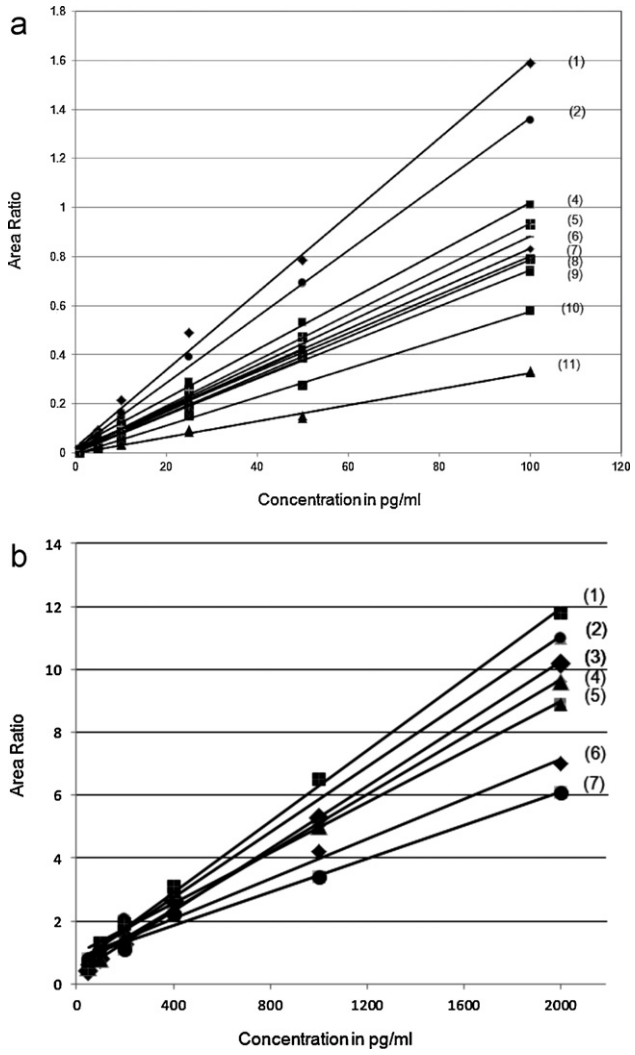

Fig. 2. Calibration curves of spiked distilled water samples with a mixture of β-blockers and β_2-agonist at (a) concentration range of 1.00–100.00 pg/ml. (1) labetalol, (2) timolol, (3) metoprolol, (4) acebutolol, (5) oxeprenolol, (6) propranolol, (7) alprenolol, (8) nadolol, (9) atenolol, (10) pindolol and (11) terbutaline. (b) concentration range of 100.00-2000.00 pg/mL. (1) alprenolol, (2) oxeprenolol, (3) nadolol, (4) acebutolol, (5) metoprolol, (6) timolol and (7) propranolol. Correlation coefficient, $R^2 > 0.99$ were obtained for all curves.

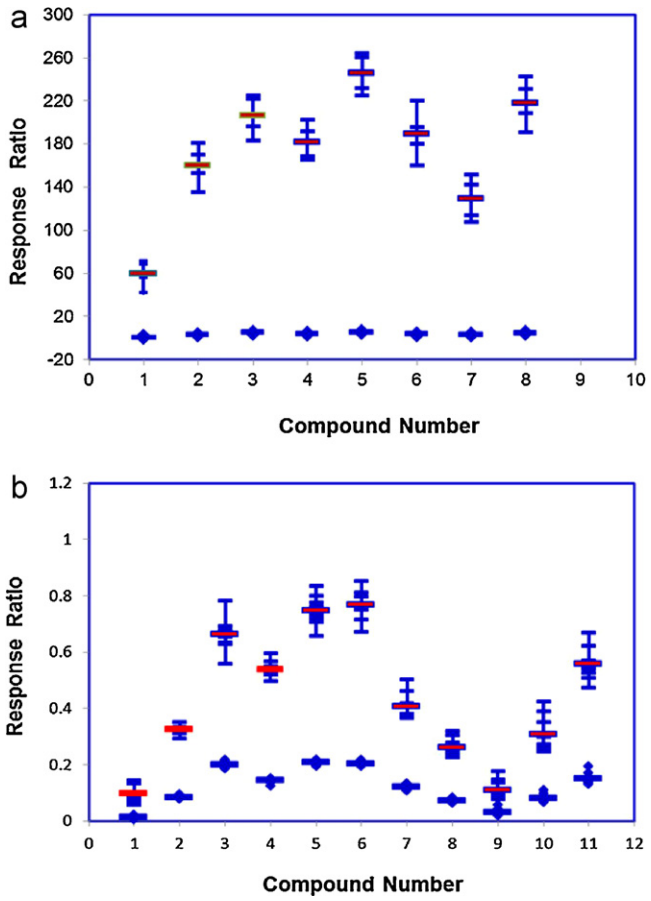

Fig. 3. (a) Scattered dot plot for intra-day precision using drugs' concentration of 150.00 pg/ml (lower group) and 500.00 pg/ml (upper group). The middle points represent the average values for each data set. Compounds' numbers are 1: pindolol, 2: nadolol, 3: timolol, 4: acebutolol, 5: metoprolol, 6: oxeprenolol, 7: propranolol, 8: alprenolol. (b) Scattered dot plot for intra-day precision using drugs' concentration of 20.00 pg/ml (lower group) and 80.00 pg/ml (upper group). The middle points represent the average values for each data set. Compounds' numbers are 1: pindolol, 2: nadolol, 3: timolol, 4: acebutolol, 5: metoprolol, 6: oxeprenolol, 7: propranolol, 8: alprenolol, 9: terbutaline, 10: atenolol, 11: labetalol.

3.3.4. Precision

Precision of the developed method was evaluated using spiked water samples. Samples were extracted using Oasis MCX cartridges and analyzed using the conditions given in Section 2.4. Intra-day precision was evaluated by analyzing eight replicate standards spiked at 150.00 and 500.00 pg/ml. Pindolol, nadolol, timolol, acebutolol, metoprolol, oxprenolol, propranolol and alprenolol gave variance coefficients in the range 0.034–0.125 and RSD in the range 3.367–12.489% (Table 5). Intra-day precision was evaluated by analyzing ten replicate standards spiked at 20.00 and 80.00 pg/ml. Pindolol, nadolol, timolol, acebutolol, metoprolol, oxprenolol, propranolol, alprenolol, terbutaline, atenolol and labetalol gave variance coefficients in the range 0.035–0.173 and RSD in the range 3.454–17.284% (Table 5).

Table 4
Limits of detection (LOD) and limits of quantification (LOQ). LOQ_1 is calculated based on the statistical definition and LOQ_2 is based on the empirical definition given in Section 2.5.

Analyte	LOD (pg/ml)	LOQ_1 (pg/ml)	LOQ_2 (pg/ml)
Atenolol	0.76	4.35	8.77
Alprenolol	6.74	16.80	–
Acebutolol	1.31	6.31	8.96
Metoprolol	1.89	8.27	9.77
Nadolol	0.41	1.62	9.26
Propranolol	5.04	22.88	10.08
Timolol	0.06	0.22	8.72
Terbutaline	0.11	0.14	–
Oxprenolol	–	–	9.92

Fig. 3 shows the scattered dot plots for the relative response ratio calculated by dividing the area of chromatogram's peaks specific for each drug on the area of the internal standard. Fig. 3a shows the scattered plot for intra-day precision at drugs' concentration of 150.00 pg/ml (lower group) and at 500.00 pg/ml (upper group). Fig. 3b shows the scattered plot for intra-day precision at drugs' concentration of 20.00 pg/ml (lower group) and 80.00 pg/ml (upper group). These results indicated high precisions for our developed method.

3.3.5. Selectivity

Selectivity of the developed method was ascertained by studying the interference of twelve basic and ten acidic drugs at concentrations of 1.00 μg/ml. These encompassed cocaine,

Table 5
Intra-day and inter-day precisions of β-blockers and β_2-agonist spiked in distilled water. Mean of the response ratios; standard deviations (SD), relative standard deviations and coefficient of variation (CV) are given at *n* equals 8 and 10.

(A) Intra-day precision using eight replicate samples ($n = 8$)

Drug	Spiking level (150.00 pg/ml)				Spiking level (500.00 pg/ml)			
	Mean response ratio	SD	RSD	CV	Mean response ratio	SD	RSD	CV
Pindolol	0.850	0.108	12.733	0.127	60.490	10.938	18.084	0.181
Nadolol	2.685	0.133	4.961	0.050	159.850	18.344	11.476	0.115
Timolol	4.633	0.403	8.692	0.087	206.603	18.574	8.990	0.090
Acebutolol	3.625	0.306	8.431	0.084	182.045	16.396	9.007	0.090
Metoprolol	5.048	0.481	9.527	0.095	245.510	18.334	7.468	0.075
Oxprenolol	3.123	0.105	3.367	0.034	189.130	23.621	12.489	0.125
Propranolol	2.753	0.324	11.769	0.118	128.934	19.611	15.209	0.152
Alprenolol	4.098	0.294	7.177	0.072	218.333	21.518	9.856	0.098

(B) Inter-day precision using ten replicate samples ($n = 10$)

Drug	Spiking level (20.00 pg/ml)				Spiking level (80.00 pg/ml)			
	Mean response ratio	SD	RSD	CV	Mean response ratio	SD	RSD	CV
Pindolol	0.028	0.005	17.898	0.179	0.086	0.005	6.285	0.063
Nadolol	0.084	0.005	6.425	0.064	0.326	0.018	5.490	0.055
Timolol	0.200	0.011	5.496	0.055	0.662	0.056	8.524	0.085
Acebutolol	0.145	0.009	6.191	0.062	0.538	0.028	5.190	0.052
Metoprolol	0.208	0.008	3.849	0.039	0.749	0.051	6.748	0.068
Oxprenolol	0.204	0.007	3.454	0.035	0.767	0.050	6.530	0.065
Propranolol	0.121	0.009	7.427	0.074	0.407	0.044	10.741	0.107
Alprenolol	0.073	0.005	7.320	0.073	0.262	0.030	11.610	0.116
Terbutaline	0.030	0.005	17.284	0.173	0.139	0.023	17.071	0.171
Atenolol	0.081	0.014	16.950	0.017	0.355	0.061	17.156	0.172
Labetalol	0.150	0.019	12.970	0.130	0.557	0.055	9.879	0.099

theophylline, tripelennamine, promethazine, antipyrine, heptaminol, dipyrone, methcarbamol, prilocaine, procaine, ephedrine, atropine ibuprofen, paracetamol, caffeine, ketorolac, phenacetin, flunixin, diclofenac, tolfenamic, vedaprofen and flufenamic. Interferences from these compounds at the retention times of investigated β-blockers and β_2-agoinsts and change in response factors were not observed. In addition, the base line resolution for all drugs was obtained in presence of interfering compounds. This indicated the high selectivity of the developed method.

3.3.6. Matrix effect

Matrix effect is attributed to the high susceptibility of the ESI ion source to interfering components co-extracted from the wastewater matrix and result in suppressing the measured MS signals leading to erroneous results. Approaches to reduce matrix effect include selective extraction, effective sample cleanup, improvement of separation conditions, external calibration using matrix-matched samples, standard addition, internal standard and dilution of sample extracts [49–51].

In our measurements, selective extraction and internal standard calibrations were adopted to reduce the matrix effect. Suppression of MS signal by matrix effect was quantified by comparing peak areas from spiked wastewaters with that of neat standards having the same concentrations as given in Section 2.5. Low signals' suppressions ranged in 4.50–6.25% were observed for labetalol and alprenolol. Mild signals' suppressions of 24.50%, 14.50%, 20.00%, 12.75% and 21.70% were observed for nadolol, timolol, acebutolol, metoprolol, and propranolol, respectively.

Our results at zero fold dilution are quite acceptable compared to previously published signals' suppressions amounted 40.00–90.00% using zero fold dilution [48] and amounted to zero using more than five folds dilution [15]. Thus, matrix effect has significantly reduced in our in our developed method and signal suppression is quite acceptable. This indicated that the method could be quite useful for the analysis of β-blockers and β_2-agonists in wastewater.

3.3.7. Stability of β-blockers and β_2-agonists in wastewater

Since wastewater matrix is extremely degrading environment, investigating drugs' stabilities in such environment is essential for the validation of the analytical method used. The developed method in this work is based on measuring the parent drug molecules and not its degraded or metabolic products. Therefore, confirmation on stabilities and integrities of drugs is important for the reliability of validation parameters.

In this study, stabilities of seven β-blockers spiked in wastewater at concentrations of 500.00 pg/ml and stored at 4.00 °C for up to 6 days were investigated. Samples were extracted and analyzed for their drugs' contents immediately after spiking, after 4 days and after 6 days in three replicates. Stability expressed as recovery% of each drug was recorded. Recoveries ≥80% were obtained for all investigated drugs stored up to 6 days (Table 6). Applying the statistical Student's *t*-test, insignificant differences in drugs' contents of the wastewater samples analyzed at day 1st, day 4th and day 6th were obtained ($P > 0.05$).

These results suggest that investigated β-blockers are potentially stable in waste effluents for several days at 4.00 °C (Table 6).

3.4. Analysis of wastewater samples

Two wastewater treatment plants (WWTP) were involved in this study in Abu Dhabi and Al-Ain cities. The former receives more than 500,000.00 m^3 while the latter receives more than 140,000.00 m^3 of influent wastewaters per day. The two WWTPs respectively produce 480,000.00 and 123,000.00 m^3 of treated effluent wastewater per day. On the other hand, around one million dosage units were reported as UAE annual consumption for each of β-blockers and β-agonists. This high consumption relative to the population has pulled our attention to the possibility of finding significant concentrations of β-blockers and β-agonists in wastewaters.

β-blockers and β_2-agonists are metabolized in liver and kidney and excreted as unchanged in different percentages ranging in 5.00–20.00% for metoprolol, oxprenolol and timolol; 35.00–40.00%

Table 6
Inter-days stability of β-blockers in waste water samples spiked at 500.00 pg/ml and extracted using Oasis MCX cartridge. Student's *t*-test is used to calculate differences in recoveries between days 0 and 4 and days 0 and 6. *P* is the probability difference between the experimental and the hypothetical values.

Compound	Spiked level (pg/ml)	Recovery % ($n = 5$)			*P* value	Significance
		0 days	4 days	6 days		
Alprenolol	500.00	83.00	86.00	86.00	0.852	No
Acebutolol	500.00	86.00	88.00	89.00	0.172	No
Metoprolol	500.00	90.00	92.00	92.00	0.949	No
Nadolol	500.00	82.00	89.00	86.00	0.240	Yes
Propranolol	500.00	77.00	80.50	83.00	0.587	No
Timolol	500.00	86.00	88.00	83.00	0.070	No
Oxprenolol	500.00	87.00	107.00	104.00	0.080	No

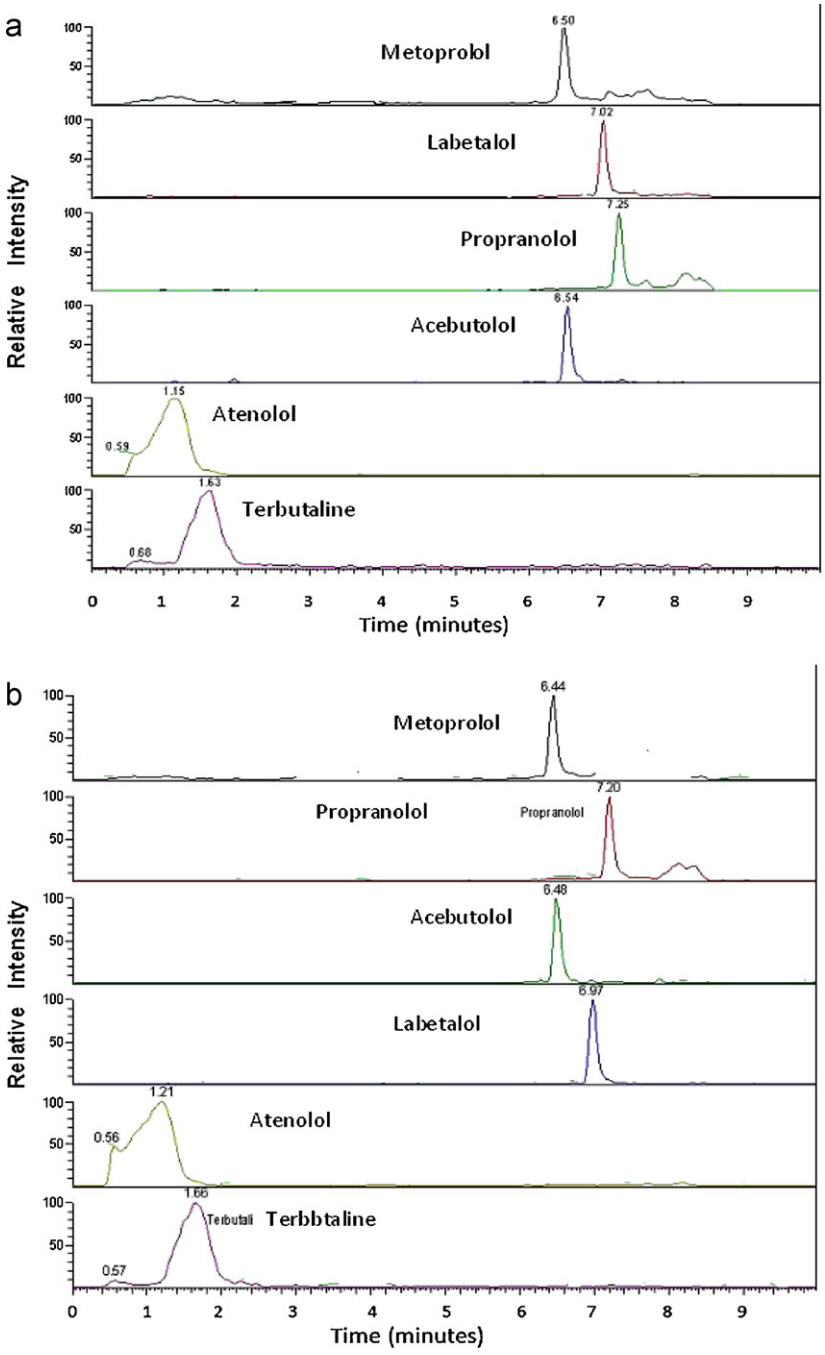

Fig. 4. LC–ESI-MS–MS chromatograms of the five β-blockers and the one β_2-agonist found in the waste water treatment plants of Al Ain city (a) and Abu Dhabi city (b). Chromatograms were collected using the MRM transitions of the corresponding compounds.

Table 7
Concentrations (pg/ml) of β-blockers and β_2-agonists in sewage effluent samples found in Al-Ain wastewater treatment works.

Samples	Terbutaline	Atenolol	Metoprolol	Propranolol	Labetalol	Acebutolol
AN-1	14.94	164.02	10.71	21.17	11.81	14.10
AN-2	25.25	172.81	15.28	7.13	11.22	14.03
AN-3	18.59	196.76	15.81	8.03	8.03	14.01
AN-4	15.70	134.19	16.27	7.37	5.32	13.48
AN-5	23.80	179.90	17.71	20.43	3.49	5.02
AN-6	5.07	201.90	3.10	10.35	3.13	2.38
AN-7	4.85	249.12	5.03	13.85	3.08	2.37
AN-8	6.32	226.26	4.48	10.80	3.04	2.31
AN-9	6.65	267.40	5.35	9.82	3.03	2.29
AN-10	5.02	266.29	5.32	10.38	3.01	2.28
AN-11	6.55	282.85	5.13	9.68	3.00	2.21
AN-12	5.10	252.30	4.29	8.96	2.88	2.21
AN-13	5.74	258.87	5.59	12.08	2.87	2.21
AN-14	2.57	258.03	5.07	10.06	2.85	2.17
AN-15	4.80	257.84	5.45	11.14	2.81	2.15
AN-16	5.93	257.50	4.92	12.09	2.77	2.12
AN-17	3.34	256.05	4.65	9.93	2.61	2.11
AN-18	3.84	242.65	2.94	10.31	2.54	1.98
AN-19	4.28	241.27	5.79	10.16	2.49	1.59
AN-20	4.51	205.03	4.07	9.83	2.44	1.55
AN-21	4.09	282.01	4.84	10.35	2.37	1.52
AN-22	6.10	316.40	5.47	12.33	2.36	1.38
AN-23	5.65	248.66	4.12	9.96	2.32	1.34
AN-24	4.85	291.37	5.70	12.99	2.27	1.22
AN-25	6.33	210.51	3.76	12.52	2.17	1.19
AN-26	6.44	248.19	5.17	18.94	2.04	1.18
AN-27	3.43	222.22	4.95	19.95	1.90	1.11
AN-28	4.97	194.52	4.73	11.88	1.90	1.03
AN-29	5.24	208.60	5.32	14.91	1.80	1.02
AN-30	5.80	234.77	5.73	17.92	1.71	14.10
SUM	225.74	7028.29	196.74	365.31	103.20	117.62
Av	7.53	234.28	6.56	12.18	3.44	3.92
SD	5.77	40.24	4.02	3.78	2.45	4.54

Table 8
Concentrations (pg/ml) of β-blockers and β_2-agonists in sewage effluent samples found in Abu Dhabi wastewater treatment works.

Samples	Terbutaline	Atenolol	Metoprolol	Acebutolol	Labetalol	Propranolol
AD-1	12.37	76.24	16.71	9.96	334.02	26.69
AD-2	14.56	108.48	13.26	6.78	11.99	25.51
AD-3	14.48	85.43	10.84	11.44	11.77	25.09
AD-4	14.80	80.94	14.56	9.19	11.41	24.66
AD-5	13.32	113.78	15.01	10.13	11.02	23.29
AD-6	16.07	93.48	14.29	7.42	10.59	23.16
AD-7	15.83	96.99	10.58	10.02	10.57	20.86
AD-8	19.36	93.67	9.82	9.89	10.27	20.69
AD-9	16.34	97.56	11.72	10.47	10.14	19.68
AD-10	16.67	108.36	12.32	3.03	9.99	16.06
AD-11	1.37	196.13	5.41	2.79	9.23	12.13
AD-12	N/F	149.28	5.58	2.30	8.99	12.05
AD-13	2.28	167.29	6.29	2.26	8.70	11.84
AD-14	1.80	206.08	6.28	1.50	8.62	11.57
AD-15	N/F	140.53	5.82	1.23	8.12	9.34
AD-16	7.23	202.61	5.39	1.53	7.77	9.19
AD-17	N/F	177.63	4.75	2.30	7.75	8.83
AD-18	2.31	155.84	4.50	2.14	7.51	8.49
AD-19	4.93	206.60	5.50	3.06	7.50	7.50
AD-20	1.81	154.04	3.25	3.03	7.45	7.26
AD-21	5.71	180.83	4.68	11.37	7.34	6.91
AD-22	10.42	72.07	20.87	10.73	6.99	6.87
AD-23	21.88	117.75	9.28	10.74	6.11	6.03
AD-24	12.01	109.35	13.53	12.85	6.07	5.87
AD-25	8.16	63.63	21.54	11.32	5.83	5.61
AD-26	12.15	96.67	14.68	11.13	5.67	5.57
AD-27	16.43	136.19	10.22	12.63	5.47	3.17
AD-28	9.48	84.13	20.58	10.97	4.99	2.92
AD-29	12.84	99.81	14.58	11.32	4.86	2.08
AD-30	15.36	96.68	16.94	N/F	4.81	1.79
SUM	299.96	3768.07	328.73	213.53	571.54	370.69
Av	11.11	125.60	10.96	7.36	19.05	12.36
SD	5.78	43.04	5.25	4.15	58.53	7.87

for pindolol and acebutolol; 55.00–60.00% for labetalol and terbutaline and in 85.00–90.00% for atenolol and alprenolol [24,52,53].

Application of the developed method for analyzing wastewater was demonstrated by analyzing sixty composite effluent samples from Abu Dhabi and Al-Ain WWTPs collected over three months' time period. Fig. 4 shows the chromatograms depict the MRM transitions of drugs found in wastewater. Tables 7 and 8 summarize the concentrations of the five β-blockers and one β_2-agonist found in both WWTPs. Terbutaline, atenolol, metoprolol, propranolol, acebutolol and labetalol were detected in all effluent samples at average concentrations of 7.53, 234.28, 6.56, 12.18, 3.92 and 3.440 pg/ml respectively for samples collected from Al-Ain WWTP. Corresponding average concentrations of 11.11, 125.60, 10.96, 7.36, 12.36 and 19.05 pg/ml were found respectively in samples collected from Abu Dhabi WWTP. Atenolol showed the highest detected concentration average concentrations in both WWTPs (234.00 and 125.00 pg/ml, respectively). Average concentrations of 3.44–12.36 pg/ml were detected for all detected compounds in both WWTPs. The average concentration of labetalol was approximately five times higher in Abu Dhabi's WWTP relative to Al-Ain's WWTP. Similarly, average concentration of acebutolol was found three times higher in Abu Dhabi's WWTP relative to Al-Ain's WWTP. The reasons could be attributed to differences in consumptions in both cities, quantity of excreted unchanged drugs and half-life in sewer system. However, exact prediction for the concentration patterns of drugs in wastewaters has been uneasy task.

Further information are needed to confirm whether the 10–20 fold higher concentrations in atenlol compared to other detected drugs is due to its hydrophilicity, daily consumption or their metabolic rates. Reasons could be attributed to the facts that atenolol is largely excreted unchanged and more heavily used. Propranolol and metoprolol are the most hydrophobic while atenolol and metoprolol are the least. Consequently, propranolol is expected to be highly adsorbed on sewage and give low concentration in wastewater compared to atenolol which will be less adsorbed and give higher concentrations (Tables 7 and 8).

According to Petrovic et al. [4], propranolol, metoprolol, acebutolol and oxprenolol were found in wastewater effluents from France, Greece, Italy and Sweden at concentrations of 0.01–0.09, 0.08–0.39, 0.06–0.13 and 0.02–0.05 μg/l, respectively. Propranolol was found at concentration ranges of 0.17–0.29 and 0.08–0.28 μg/l in Germany and UK, respectively while as metoprolol was found at concentration range of 0.73–2.2 μg/l in Germany [4]. Scheurer et al. reported concentrations of 0.4, 1.9, 2.0 and 0.062 μg/l for atenolol, sotalol, metoprolol, and propranolol respectively in effluent wastewater from treatment plant in Koblenz, Germany [54]. They also reported average concentrations of 0.36–1.15, 0.32–0.70, 0.00–0.16, 0.02–0.06 and 0.01–0.04 μg/l for atenolol, metoprolol, sotalol, propranolol and nadolol in effluent wastewaters from Ontario, Canada, respectively. Variations in concentrations were attributed to different drugs consumptions rates [54]. Our findings are much lower than the above results and those reported for Canadian wastewaters, EU and other parts of the world [55–57]. This might be attributed to patients' consumptions and population.

These results suggest that β-blockers and β_2-agonists are able to survive against wastewater treatment processes and perhaps could go to surface and drinking waters. Further study is needed to explore this option and to oversee other pharmaceuticals in wastewaters in both cities.

4. Conclusion

In this work, a highly sensitive and selective method for simultaneous trace determinations of eleven β-blockers and β_2-agonists in distilled and waste-waters was developed and optimized using liquid chromatography tandem mass spectrometry with electrospray ionization source and solid phase extraction. The method was validated using linearity, accuracy, selectivity, selectivity, detection limit, quantification limit, precisions, stability and matrix effect parameters. The method was used for trace determinations of acebutolol, atenolol, metoprolol, propranolol, timolol, nadolol, labetalol, oxprenolol, pindolol, alprenolol and terbutaline. Oasis MCX and Clean Screen cartridges were used for solid phase extractions and an alkaline mixture of dichloromethane–propoanol was used as mobile phase.

Average recoveries in the range 77.20–97.30 ± (1.46–8.79)% were obtained for drugs spiked at 150.00–500.00 pg/ml using Oasis MCX-SPE cartridges. Drugs spiked in wastewater gave intra-day precisions RSD of 3.367–12.489% and inter-day precisions RSD of 6.425–19.768% at concentration levels of 150.00–500.00 pg/ml and 20.00–80.00 pg/ml, respectively. Detection and quantification limits of 0.11–6.74 pg/ml and 0.14–22.88 pg/ml were respectively obtained for investigated drugs. Co-extractives from wastewater could be reduced by adopted extraction protocol. Signal's suppression caused by matrix effect ranged in 4.50–24.50% at zero dilution of samples' extracts was recorded for different investigated drugs. Drugs spiked in wastewater at 500.00 pg/ml concentrations level and stored at 4.00 °C for 6 days showed insignificant degradation.

Developed method has proved efficient and invaluable for the analysis of β-blockers and β-agonists in effluent wastewaters from Al-Ain and Abu-Dhabi WWTPs. Five β-blockers and one β-agonist were detected at average concentrations of 3.44–19.05 pg/ml. Atenolol was detected at average concentration of 125.60–234.28 pg/ml. Results obtained suggest that wastewater treatment processes adopted are not enough to degrade these compounds.

Occurrence and stability of investigated drugs in the two investigated WWTPs necessitates further work on their fate and impact on such aquatic ecosystem. Further research is needed to detect other pharmaceuticals and to evaluate strategy and efficiency of treatment processes adopted in wastewater treatment plants.

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References

[1] C.G. Daughton, A.T. Ternes, Environ. Health Perspect. 107 (1999) 907.
[2] B.H. Sorensen, S.N. Nielsen, P.F. Lanzky, F. Ingerslev, H.C.H. Luetzhoft, S.E. Jorgensen, Chemosphere 36 (1998) 357.
[3] S.E. Jorgensen, B.H. Sorensen, Chemosphere 40 (2000) 691.
[4] M. Petrovic, M.D. Hernando, M.S. Diaz-Cruz, D. Barcelo, J. Chromatogr. A 1067 (2005) 1.
[5] S. Diaz Cruz, D. Barcelo, Handb. Environ. Chem. 5 (2004) 227.
[6] K. Fent, A.A. Weston, D. Caminada, Aquat. Toxicol. 76 (2006) 122.
[7] S.D. Richardson, T.A. Thernes, Anal. Chem. 77 (2005) 3807; S.D. Richardson, T.A. Thernes, Anal. Chem. 83 (12) (2011) 4614.
[8] B.E. Erickson, Environ. Sci. Technol. 36 (2002) 140A.
[9] M. Petrovic, S. Gonzalez, D. Barcelo, Trends Anal. Chem. 22 (2003) 685.
[10] K. Reddersen, T. Haberer, U. Dunnbier, Chemosphere 49 (2002) 539.
[11] T.A. Ternes, M. Meisenheimer, D. McDowell, F. Sacher, H.J. Brauch, B.H. Gulde, G. Preuss, U. Wilme, N.Z. Seibert, Environ. Sci. Technol. 36 (2002) 3855.
[12] M. Gomez, M. Poetrovic, A. Alba, D. Bracelo, J. Chromatogr. A 1114 (2006) 224.
[13] N. Vieno, T. Tuhkanen, L. Kronberg, J. Chromatogr. A 1134 (2006) 101.
[14] B.K. Hordern, R. Dinsdale, A. Guwy, J. Chromatogr. A 1161 (2007) 132.
[15] H.B. Lee, K. Sarafin, T.E. Peart, J. Chromatogr. A 1148 (2007) 158.
[16] T.A. Ternes, Water Res. 32 (1998) 3245.
[17] T. Heberer, J. Hydrol. 266 (2002) 175.
[18] P.E. Stackelberg, E.T. Furlong, M.T. Meyer, S.D. Zaugg, A.K. Henderson, D.B. Reissman, E.T. Furlong, Sci. Total Environ. 329 (2004) 99.
[19] J.J. McMurray, M.J. Kendall, Beta-Blockers in Heart Failure, Martin Dunitz Ltd., London, UK, 2002.
[20] L.C. Silva, M.G. Trevisan, R.J. Poppi, M.M. Sena, Anal. Chim. Acta 595 (2007) 282.
[21] S.M. Al-Ghannam, J. Pharm. Biomed. Anal. 40 (2006) 151.

[22] J.M. Cruickshank, B.N.C. Prichard, Beta-Blockers in Clinical Practice, Churchill Livingstone, Edinburgh, New York, USA, 1988.
[23] M.J. Paik, J. Lee, K.R. Kim, Anal. Chim. Acta 601 (2007) 230.
[24] P. Gonzalez, C.A. Fente, C. Fronco, B. Vazquez, E. Quinto, A. Cepeda, J. Chromatogr. B 693 (1997) 321.
[25] J. Cai, J. Henion, J. Chromatogr. B 691 (1997) 357.
[26] N.M. Vieno, T. Tuhkanen, L. Kronberg, Environ. Sci. Technol. 41 (2007) 5077.
[27] T.G. Kibbey, R. Paruchuri, D.A. Sabatini, L. Chen, Environ. Sci. Technol. 41 (2007) 5349.
[28] S. Wiegel, A. Aulinger, R. Brockmeyer, H. Harms, J. Loffler, H. Reincke, R. Schmidt, B. Stachel, W. von Tumpling, A. Wanke, Chemosphere 57 (2004) 107.
[29] D. Calamari, E. Zuccato, S. Castiglioni, R. Bagnati, R. Fanelli, Environ. Sci. Technol. 37 (2003) 1241.
[30] F. Sacher, F.T. Lange, H.-J. Brauch, I. Blankenhorn, J. Chromatogr. A 938 (2001) 199.
[31] D.B. Huggett, I.A. Khan, C.M. Foran, D. Schlenk, Environ. Pollut. 121 (2003) 199.
[32] T.A. Ternes, Trends Anal. Chem. 20 (2001) 419.
[33] X.S. Miao, B.G. Koenig, C.D. Metcalfe, J. Chromatogr. A 952 (2002) 139.
[34] M.J. Hilton, K.V. Thomas, J. Chromatogr. A 1015 (2003) 129.
[35] J.B. Quintana, T. Reemtsma, Rapid Commun. Mass Spectrom. 18 (2004) 765.
[36] X.S. Miao, C.D. Metcalfe, J. Chromatogr. A 998 (2003) 133.
[37] T.A. Ternes, R. Hirsch, J. Muëller, K. Haberer, Fresenius J. Anal. Chem. 362 (1998) 329.
[38] P.P. Vázquez, M.M. Galera, A.S. Guirado, M.M.P. Vázquez, Anal. Chim. Acta 666 (2010) 38.
[39] L.N. Nikolai, E.L. McClure, S.L. MacLeod, C.S. Wong, J. Chromatogr. A 1131 (2006) 103.
[40] B. Shao, D. Chen, J. Zhang, Y. Wu, C. Sun, J. Chromatogr. A 1216 (2009) 8312.
[41] M. Laven, T. Alsberg, Y. Yu, M.A. Erici, H. Sun, J. Chromatogr. A 1216 (2009) 49.
[42] C.K. Meng, Application Note # 5989-5319EN, Agilent Technologies, Inc., Wilmington, Delaware, USA, 2008, http://www.agilent.com/chem
[43] M.D. Hernando, M. Petrovic, A.R. Fernandez, D. Barcelo, J. Chromatogr. A 1046 (2004) 133.
[44] M.K. Angier, R.J. Lewis, A.K. Chaturvedi, D.V. Canfield, J. Anal. Toxicol. 29 (2005) 517.
[45] L. Damasceno, R. Ventura, J. Ortuno, J. Segura, J. Mass Spectrom. 35 (2000) 1285.
[46] G.D. Branum, S. Sweeney, A. Palmeri, L. Haines, C. Huber, J. Anal. Toxicol. 22 (1998) 135.
[47] D.A. Armbruster, M.D. Tillman, L.M. Hubbs, Clin. Chem. 40 (7) (1994) 1223.
[48] M. Gros, M. Petrovi, D. Barcelo, Talanta 70 (2006) 678.
[49] A. Kloepfer, J.B. Quintana, T. Reemtsma, J. Chromatogr. A 1067 (2005) 153.
[50] T. Benijts, R. Dams, W. Lambert, A.D. Leenheer, J. Chromatogr. A 1029 (2004) 153.
[51] A. Alder, S. Luderitz, K. Lindtner, H.J. Stan, J. Chromatogr. A 1058 (2004) 67.
[52] S. Ollers, H.P. Singer, P. Fassler, S.R. Muller, J. Chromatogr. A 911 (2001) 25.
[53] U. Borchard, J. Clin. Basic Cardiol. 1 (1998) 5.
[54] M. Scheurer, M. Ramil, C.D. Metcalfe, S. Groh, T.A. Ternes, Anal. Bioanal. Chem. 396 (2010) 845.
[55] M.L. Farre, I. Ferrer, A. Ginebreda, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova, D. Barcelo, J. Chromatogr. A 938 (2001) 187.
[56] S. Yang, J. Cha, K. Carlson, J. Chromatogr. A 1097 (2005) 40.
[57] A.A.M. Stolker, W. Niesing, E.A. Hogendoorn, F.M. Versteegh, R. Fuchs, U.T. Brinkman, Anal. Bioanal. Chem. 378 (2003) 955.