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Potential of capillary-column-switching liquid chromatography–tandem mass spectrometry for the quantitative trace analysis of small molecules Application to the on-line screening of drugs in water

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Abstract

We have investigated the potential of capillary-column-switching liquid chromatography coupled to tandem mass spectrometry (cLC–MS–MS) for the quantitative on-line trace analysis of target compounds in aqueous solutions. The technical design of the nano-scale cLC system developed at our Institute for peptide and protein identification has been tested and evaluated for the direct trace analysis of drugs in water samples. Sulphametoxazole, bezafibrate, metoprolol, carbamazepine and bisoprolol occurring frequently in Dutch waters, were selected as test compounds. Adequate conditions for trapping, elution and MS–MS detection were investigated by employing laboratory made 200 μm i.d. capillary columns packed with 5 μm aqua C_{18} material. In the final cLC–MS–MS conditions, a 1 cm length trapping column and a 4 cm length analytical column were selected. Under these conditions, the target compounds could be directly determined in water down to a level of around 50 ng/l employing only 25 μl of water sample. Validation was done by recovery experiments in ground-, surface- and drinking-water matrices as well as by the analysis of water samples with incurred residues and previously analyzed with a conventional procedure involving off-line solid-phase extraction and narrow-bore LC with MS–MS detection. The new methodology provided recoveries (50–500 ng/l level) between 50 and 114% with RSDs ($n = 3$, each level) below 20% for most of the compounds. Despite the somewhat less analytical performance in comparison to the conventional procedure, the on-line approach of the new methodology is very suitable for screening of drugs in aqueous samples.

Keywords: Water analysis; Environmental analysis; Column switching; Screening; Drugs

1. Introduction

Capillary-column liquid chromatography (cLC) coupled to MS detection has been successfully applied in the field of structural elucidation of biomolecules such as proteins and peptides [1–6]. The resulting extremely low peak volume of the compounds (nano liters) obtained by this technique makes it possible to detect a very low (absolute) amount of compounds using MS detection.

In order to improve the efficiency of the miniaturization process in LC, column switching systems are used to overcome the limited injection volumes [1,4,7,8]. In this approach a short capillary column, typically 1 cm length, is used as a first column, enabling relatively high flow rates during trapping of analytes.

Despite the advantages of high sensitivity and low consumption of sample, cLC coupled to MS has not been applied frequently for the quantitative trace analyses of small molecule target compounds. The main reason possibly is the limitation in the volume of sample that can be injected without excessive band broadening of the analytes. Additionally, special demands and experience are necessary in order to apply adequate connections and solvent deliveries in capillary LC as well as the hyphenation to the MS.

As an example, cLC coupled to UV detection has been used for the determination of drugs in human plasma, leading to increased sensitivity of three-to-five-fold in comparison to conventional HPLC methods [9]. This technique coupled

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to an ion trap mass spectrometer has been applied for the determination of drugs in urine using electrospray ionization (ESI) mode [10] and in pure water operating under atmospheric pressure chemical ionization (APCI) [11].

A sophisticated, robust and fully automated nano-scale cLC column-switching system has been developed at our Institute [4,12] and used for the structural elucidation of peptides and proteins employing multiple MS (MS^n) detection. Exploiting this experience, the potential of this technique towards quantitative analysis of small target molecules was investigated. For this purpose, we selected the analysis of a number of drugs frequently encountered in Dutch surface, ground and waste water.

The presence of pharmaceuticals in the environment has been of growing concern in the last years [13–16]. During 2002–2003, two surveys were carried out at our laboratory with a developed robust procedure [17] employing off-line SPE and narrow bore LC with ESI tandem mass spectrometric detection (MS–MS). Hence, the performance of the capillary-column-switching LC system with MS–MS detection was investigated for the trace determination of five human drugs in environmental water samples. Beside the feature of consuming a very small volume of aqueous sample, the on-line approach offers the possibility to the development of less laborious analytical methodologies in relation with other conventional HPLC methods.

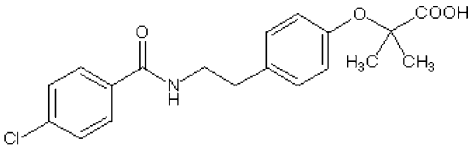
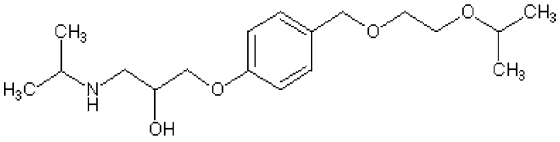
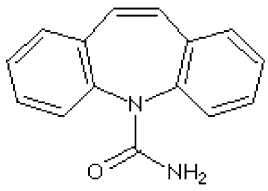
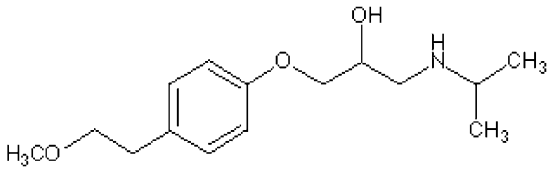
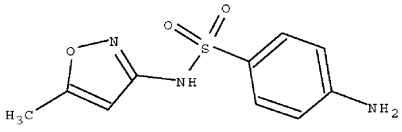
2. Experimental

2.1. Chemicals and samples

Metoprolol tartrate, sulphamethoxazole and carbamazepine were obtained from Bufa Pharmaceutical Products (Uitgeest, The Netherlands) with a purity between 98 and 102%. Bezafibrate was supplied from Sigma Chemical Co. (St. Louis, MO, USA) and bisoprolol fumarate from Merck (Amsterdam, The Netherlands) with a purity >99%. Standards were dissolved in methanol (1000 μg/ml) and diluted in HPLC-grade water containing 0.01% (v/v) formic acid. Table 1 shows the characteristics of the selected drugs.

Acetonitrile and methanol were HPLC grade (Biosolve, Valkenswaard, The Netherlands). Ammonium acetate (98%) and formic acid (89–91%) (both analytical-reagent grade) were obtained from Merck (Darmstadt, Germany). HPLC-grade water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Bedford, MA, USA).

Table 1
Characteristics of the selected drugs

Compounds	Therapy category	Structures
Bezafibrate	Antihyperlipoproteinemic	
Bisoprolol	Antihypertensive	
Carbamazepine	Analgesic, anticonvulsant	
Metoprolol	Antihypertensive, antianginal, antiarrhythmic	
Sulphamethoxazole	Antibiotic	

Different types of water samples (drinking, ground, surface and waste water) were analyzed. Samples with suspended particles and/or containing organic matter were acidified (0.01% formic acid content) and then filtered through 0.2 μm disposable nylon 4 mm filters purchased from Bester (Amstelveen, The Netherlands) before direct injection in cLC.

2.2. Columns and connections

Fused silica capillary columns (200 μm i.d., undeactivated) were obtained from J&W Scientific (Folsom, CA, USA) or from Polymicro Technology (Phoenix, AZ, USA). Tubing connections were made with MicroTight fittings and unions (Upchurch Scientific, Oak Harbor, WA, USA). Capillary tubing connections to the HPLC pump, to the injection valve and to the mass spectrometer were made with 1/16 in. (1 in. = 2.54 cm) Valco connections with short polyether ether ketone (Peek) sleeves (Alltech, Breda, The Netherlands).

Porous ceramic frits were prepared in 200 μm i.d. undeactivated, fused silica capillaries (each 30 cm long), as described by Meiring et al. [4]. Briefly, a mixture of 300 μl of potassium silicate solution and 100 μl of formamide were thoroughly mixed at room temperature. The mixture was deposited immediately inside the capillary for a few seconds by capillary action. Typically, a bundle of 10 fused silica capillaries were filled at a time. The material was polymerized by heating it in the oven of a gas chromatograph, ramped from 25 to 100 °C in 15 min and kept at 100 °C for an additional 4 h. The resulting porous frits were stored and used without any further treatment (e.g. deactivation) and, finally, cut to a length of 0.5–1 mm with a fused silica precision cutter just prior to use.

HPLC capillary columns were prepared using the procedure described by Meiring et al. [4] with a 50 bar high-pressure vessel. Basically, the stationary phase was suspended in isopropanol in a 2 ml vial and placed into the high-pressure vessel. Using a ferrule of 0.4 mm, the fused silica capillary was connected into the vial and packed with the stationary phase at a pressure of 50 bar.

The packing materials tested for trapping and/or analytical columns were aqua C_{18} (5 μm, Phenomenex, Torrance, CA, USA) and Vydac 214 C_4 (5 μm, The Separations Group, Hesperia, CA, USA). Additionally, 3 and 10 cm length columns packed with Vydac 214 C_4, and 1, 3, 4 and 10 cm length columns packed with aqua C_{18} were used in method development.

In the selected procedure, capillary columns packed with aqua C_{18} were used with a length of 1 and 4 cm for the trapping and the analytical column, respectively.

2.3. Instrumentation

The complete design is depicted schematically in Fig. 1, which shows the three steps of the sample analysis (sample injection, analyte trapping, and analytical separation).

The capillary-column-switching LC system consisted of a LC 250 binary pump (Perkin-Elmer, Norwalk, CT, USA) operated at a flow rate of 1 ml/min. The flow was split by means of a VICI (Valco, Schenkon, Switzerland) micro Tee union using 50 μm i.d. fused silica tubing of appropriate length. A six-port injection valve fitted with a 25 μl injection loop was used for manual injection of samples. For column switching operations, a VICI six-port valve controlled by a micro electric two-position valve actuator was used.

The flow rates through both the trapping and analytical column were daily adjusted by measuring the flow with a capillary 5 μl volumetric tube. In the loading step position (see Fig. 1), the flow rate of the trapping column was checked after the fused silica restrictor. The flow through the analytical column was measured in the analytical separation step position just before the connection with the detector.

A triple quadrupole mass spectrometer (Waters, Micromass Quatro Ultima, Almere, The Netherlands) operating in positive-ion ESI mode was used for MS–MS detection. Drying gas as well as nebulising gas was nitrogen. The cone gas flow was set to approximately 90 l/h and the desolvation gas flow to 200 l/h. Infusion experiments were performed using a single syringe pump 11 (Harvard Apparatus, Holliston, MA, USA) directly connected to the interface. For operation in MS–MS, the multiple reaction monitoring (MRM) mode was chosen. Collision gas was argon with a pressure of 5.19×10^{-3} mbar in the collision cell. Desolvation temperature was set to 300 °C and the source temperature to 120 °C. The capillary voltage was set to 3.2 kV, maintaining the cone voltage at 30 V for all the transitions. Collision energies were optimized for every compound and a dwell time of 0.18 s per scan was chosen for all of them. Table 2 shows the optimized parameters for each compound selected for the MS–MS method. The mass spectrometer was controlled by the MassLynx NT data software.

A UV detector set at wavelength of 220 nm was used in preliminary experimental work. It consisted of a variable-wavelength detector model ABI 759A (Applied Biosystems, Ramsey, CA, USA) equipped with a U-Z capillary cell (LC Packings, San Francisco, CA, USA) with 8 mm optical path length and 35 nl illuminated volume.

Table 2
LC–MS–MS conditions for the determination of drugs

Compounds	*m/z*		Collision energy (eV)
	Parent ion	MS–MS ion	
Carbamazepine	236.95	194.00	20
Sulphamethoxazole	254.00	156.00	16
Metoprolol	268.00	116.05	19
Bisoprolol	326.10	116.05	19
Bezafibrate	362.00	316.10	15

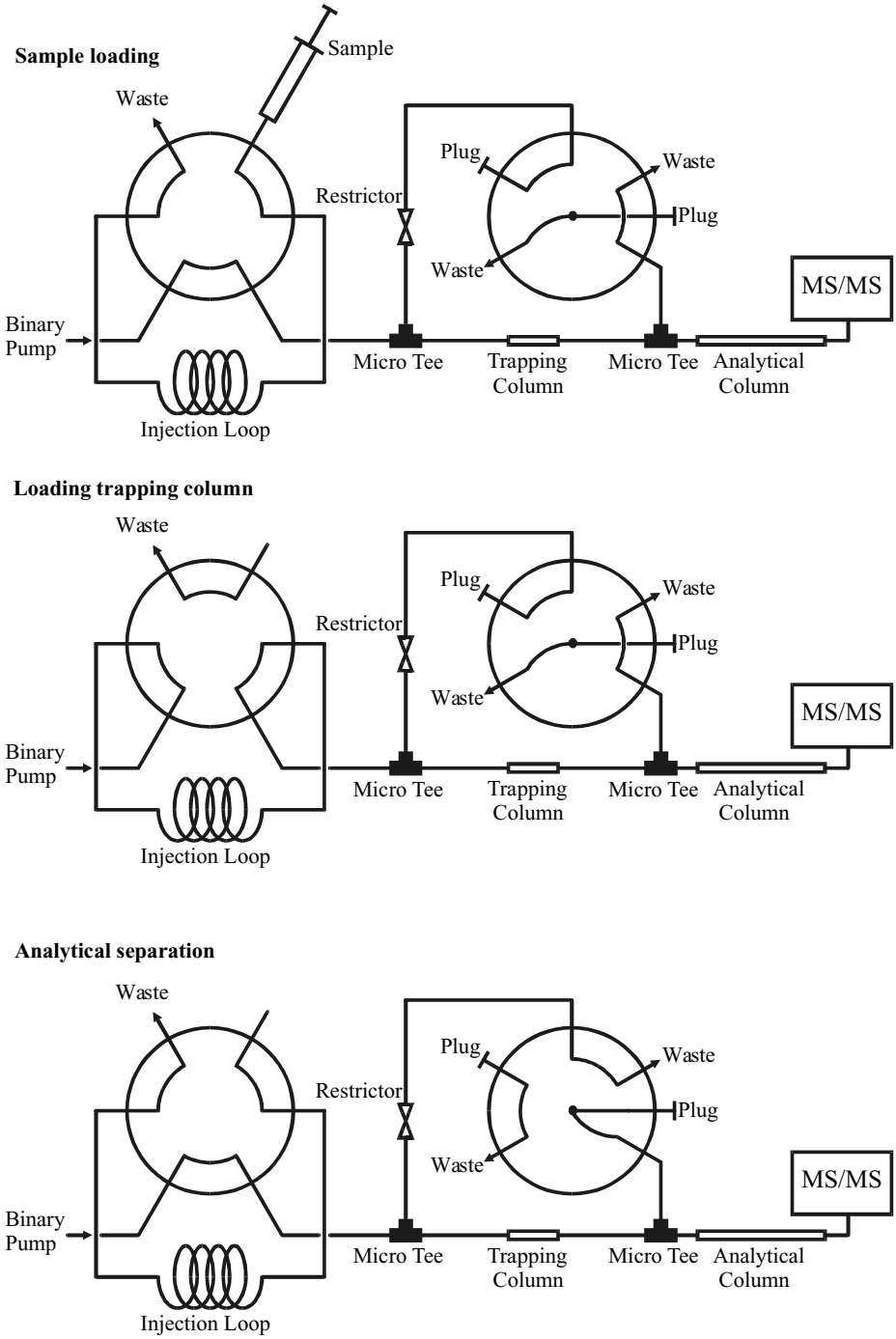


Fig. 1. Scheme of the cLC–MS system. Valve positions are given for each individual step in the sample analysis. At the moment of sample injection and loading of the trapping column, the flow rate is around 30 μl/min through the trapping column only. During these stages, no flow passes through the analytical column. During analytical separation, the flow rate through both the trapping column and the analytical column is around 4 μl/min.

2.4. Procedure

Acidified and filtered water samples were directly injected (25 μl) in the cLC system. During injection, the analytes were trapped on the first column using 100% of solvent A (water containing 0.01% formic acid) as the mobile phase with a flow rate adjusted to around 30 μl/min. After 1 min, the valve was switched to connect the trapping column on-line with the analytical column. Simultaneously, a linear gradient (in 5 min) to 100% solvent B (methanol containing 0.01% formic acid) was performed with a flow rate of around 4 μl/min, followed by a 10 min isocratic elution with 100% solvent B. For the adjustment of the flow rate, a 25 cm × 50 μm i.d. fused silica tubing was used as flow restrictor. During analytical separation, the injection loop was bypassed. Before injecting the next sample, both

the trapping and the analytical columns were equilibrated in 100% solvent A for 10 min.

3. Results and discussion

3.1. General considerations

An important aspect of the approach of the capillary column switching system is that the whole solvent delivery is performed with only one conventional binary pump, viz. not specially designed for low-flow applications. Obviously, this approach is both simple and cost effective. However, one must be aware that with a one-pump system, a heart-cutting cleanup as applied in the trace analysis of pesticides in combination with non-selective UV detection [18] is not possible. This approach is optimally designed for the efficient (rapid) large volume injection at an elevated flow rate and for the removal of very hydrophilic and ionic matrix compounds, e.g. salts and surfactants.

In this paper, the selection of test compounds was made on terms of frequency of findings in Dutch waters and also on available methodology [17] in order to compare the performance of cLC column switching. The existing procedure includes extraction, clean-up and concentration (factor 200) on solid-phase-extraction cartridges and LC–MS–MS analysis using. a 100 × 2.1 mm i.d. C_{18} LC column and a binary linear gradient with the solvents water and methanol containing 2 mM ammonium acetate at a flow of 200 μl/min. Limits of detection (LODs; $S/N = 3$) for this procedure ranged between 2 and 10 ng/l.

The main functions of the first capillary column are (i) to trap (concentrate) the analytes during the large volume sample injection, (ii) to remove hydrophilic interference's by washing, and (iii) to avoid as much as possible band broadening of the compounds during trapping and transfer to the analytical separation column. Obviously, a 100% aqueous solvent will be most suitable as the mobile phase. However, the conventional apolar C_{18} stationary phases can hamper good compatibility with pure water as solvent (collapsing of octadecyl chains), loosing retention and decreasing the performance of the column. Therefore, based on experienced fully compatibility with a 100% aqueous mobile phase, the commercial available packing materials 5 μm aqua C_{18} and 5 μm Vydac 214 C_4 were chosen as stationary phases.

The present study comprises two parts. The first part involved the study and selection of suitable cLC column-switching conditions to be used for the on-line determination of the selected drugs aiming at a sensitivity of about 50 ng/l for each compound. In the second part, validation of the cLC–MS–MS method was performed by means of calibration plots, recovery experiments and the analysis of various types water samples with incurred residues.

3.2. Selection of capillary-column-switching conditions

As explained in a previous review on column-switching LC and large volume injection (LVI) of aqueous samples [18], the capacity factor (k) of the analyte and the separation power of the column are important parameters in method development. Sufficient retention, typically $k > 1$ in a mobile phase with 50% methanol, will be required to obtain top-column focussing of the analyte during injection, while efficient columns (e.g. plate number, $N > 30{,}000$ plates/m) minimize the elution volume of the analyte, and, hence, favor sensitivity.

In capillary LC, extra-column band broadening is an important aspect to be considered. The contributions of the peak profile of the analyte can be expressed as the sum of their variances due to the column and extra-column dispersion (injector, detector cell and connecting tubing).

The chromatographic performance of the selected stationary phases—aqua C_{18} and Vydac 214 C_4—was firstly tested by injecting 1.7 μl of individual standard solutions in HPLC water using UV detection at 220 nm and an isocratic elution with three different mobile phases (IM-1, IM-2 and IM-3). The results expressed in terms of capacity factor (k) and plate number (N, total number of plates per meter) are given in Table 3. The void volume (V_0) of the columns were calculated using the time at which the first disturbance of the baseline in the chromatogram caused by the solvent front occurred after injection. Most of the analytes showed an adequate retention ($1 < k < 10$) for both capillary columns indicating that both type of columns could be suitable for trapping purposes.

However, the N values were relatively low as can be expected by the effect of extra-column dispersion. To approximately determine this contribution, the column was removed from the system and the injection valve was directly connected to the detector connection. For approximately 1 s, acetone was injected into the system at a flow rate of approximately 7 μl/min. The estimated value of extra-column dispersion was 0.0992 μl^2 (0.0136 μl^2 corresponded to injector variance). The plate number values, corrected for the estimated extra-column dispersion, clearly show a significant increase for all analytes.

The next step was to test LVI with column–switching cLC. First experiments were carried out with UV detection. A sample volume of 25 μl was selected for injection in order to get the aimed sensitivity. In first instance, 3 cm length columns were tested as trapping columns and 10 cm length columns as analytical columns using the three mobile phases given in Table 4. Employing 100% aqueous media for trapping and rising, and a linear gradient after column switching (conditions, see Section 2), it appeared that the more polar compounds, bisoprolol and metropolol, were not completely trapped on Vydac 214 C_4 material, whereas aqua C_{18} material provided full recovery for all compounds. Regarding the mobile phases, GM-3 with acetonitrile as modifier provided slightly favorable elution conditions, e.g. reduced col-

Table 3
Elution performance of compounds (1 μg/ml) on packed capillary columns (10 cm × 200 μm i.d.; injection, 1.7 μl standard in water)

Packing material	V_0 (μl)	Compounds	IM-1[a]			IM-2[b]			IM-3[c]		
			k	N[d] (n/m)	N[e] (n/m)	k	N[d] (n/m)	N[e] (n/m)	k	N_1[d] (n/m)	N_2[e] (n/m)
5 μm aqua C_{18}	3.6	Sulphamethoxazole	0.7	10,240	56,530	1.1	11,560	91,900	1.4	17,800	118,860
		Bezafibrate	2.2	11,700	90,820	16.7	17,980	18,810	3.6	23,910	185,250
		Metropolol	2.6	8,230	16,030	0.9	13,540	74,760	1.0	14,750	81,410
		Carbamazepine	3.3	12,030	23,450	3.6	13,460	26,230	1.8	19,970	158,610
		Bisoprolol	4.3	10,760	15,200	0.9	9,410	74,760	1.0	14,750	81,410
5 μm Vydac 214 C_4	3.4	Sulphamethoxazole	0.9	6,550	64,000	1.1	12,250	76,560	1.1	10,240	81,000
		Bezafibrate	1.5	7,840	110,250	4.6	5,100	5,920	1.7	8,010	36,830
		Metropolol	1.4	3,540	7,860	1.2	7,630	90,250	1.6	4,290	9,520
		Carbamazepine	1.8	3,070	3,390	1.8	7,200	38,450	1.2	11,420	90,250
		Bisoprolol	2.4	8,410	16,530	1.6	3,820	7,520	1.8	5,760	15,130

[a] Mobile phase IM-1: mixture of methanol–water (52:48, v/v) containing 2 mM ammonium acetate.
[b] Mobile phase IM-2: mixture of methanol–water (52:48, v/v) containing 0.01% formic acid.
[c] Mobile phase IM-3: mixture of acetonitrile–water (40:60, v/v) containing 0.01% formic acid.
[d] N: plate number $(t_r/\sigma)^2$ without estimated correction for external dispersion.
[e] N: plate number with estimated correction for the external dispersion.

umn pressure and improved chromatographic efficiency of some compounds, in comparison to the other mobile phase compositions. Using both acidic mobile phases (GM-2 and GM-3), further optimization was carried out by reducing the length of both columns in order to maximize flow rate under the allowable pressure. Satisfactory results were obtained with 1 cm length trapping column and 4 cm length analytical column, providing a flow rate of 30 and 4 μl/min, respectively. The flow was daily checked before and after about 8 h of measurement and did not vary significantly (less than 5%), providing a good reproducibility of retention times.

3.3. cLC–MS–MS

The column-switching cLC system was transferred and coupled to the tandem mass spectrometer. As regards MS–MS detection, similar conditions were used as applied for the existing procedure [17]. Unfortunately, the use of acetonitrile did not provide sensitive MS detection of the drugs. Despite the somewhat less elution performance of the drugs, improved sensitivity was obtained when methanol was used as modifier in stead of acetonitrile, a phenomena that has been reported in literature [17,19]. Hence, water–methanol containing 0.01% formic acid (GM-2, Table 4) was chosen as the mobile phase for further cLC–MS–MS experiments. In comparison to the conventional LC–MS procedure [17], the gain in (absolute) sensitivity of the new approach is clearly shown in Table 4, allowing the direct analysis of the compounds in water at the sub-ppb level in a short analysis time (less than 20 min).

Table 4
Comparison of height response ($\times 10^5$) obtained after direct injection of 2.5 ng aqueous standard solution using conventional LC–MS–MS and cLC–MS–MS applying a linear gradient elution (conditions, see Section 2)

	Capillary LC (GM-2)[a]	Capillary LC (GM-3)[b]	Conventional LC (GM-1)[c]
Carbamazepine	636	593	63
Sulphamethoxazole	96	66	5
Metoprolol	160	92	3
Bisoprolol	687	428	27
Bezafibrate	82	50	6

[a] GM-2: solvent A, water containing 0.01% formic acid; and solvent B, methanol containing 0.01% formic acid.
[b] GM-3: solvent A, water containing 0.01% formic acid; and solvent B, acetonitrile containing 0.01% formic acid.
[c] GM-1: solvent A, water containing 2 mM ammonium acetate; and solvent B, methanol containing 2 mM ammonium acetate.

The linearity of the procedure was studied by analyzing five concentrations of standard solutions in HPLC-grade water in the range of 10–1000 ng/l in duplicate. Calibration curves provided satisfactory linearity ($0.977 < r < 0.999$) for the compounds studied.

Firstly, validation of the procedure was carried out by recovery experiments in drinking water at two concentration levels (100 and 500 ng/l; $n = 3$ each level). The samples used as blanks were previously analyzed without finding any of the compounds studied. The recoveries ranged between 50 and 114% with RSD values below 20%. LODs ($S/N = 3$) were estimated to be at least to be 30 ng/l for each compound in this type of water.

Secondly, recovery experiments were performed by spiking five natural water samples of different origin at the aimed sensitivity level of 50 ng/l. These stored samples were part of a monitoring program and analysed before with the conventional LC–MS procedure and did not contain any of the drugs. Table 5 shows that for bezafibrate, bisoprolol and carbamazepine, mean recoveries were in the range of 70–105% with a RSDs below 20%. Metoprolol was distinctly lower recovered (18%), indicating the presence of a matrix effect on the ionization at this low concentration level. Differences between the different types of water are low, empha-

Table 5
Recoveries (%) of various type of water samples spiked with the drugs at a level of 50 ng/l ($n = 3$)

Compounds	Surface water	Ground water	Ground water	Drinking water	Drinking water	Mean recovery (%)	RSD (%)
Bezafibrate	68	70	75	76	65	71	7
Bisoprolol	69	61	61	78	77	69	12
Metoprolol	13	21	16	19	20	18	18
Sulphamethoxazole	16	89	nd[a]	69	nd[a]	–	–
Carbamazepine	81	86	118	113	130	105	20

[a] nd: not detected; and (–): not calculated.

sizing the good precision of the approach for this type of analysis.

However, the results for sulphamethoxazole were not consistent, and the drug could not be recovered in two samples as a result of a significant decrease in sensitivity. An example chromatogram for the cLC–MS–MS analysis of a ground water sample spiked at 50 ng/l is given in Fig. 2.

Based on a signal-to-noise ratio of 3, the LODs in various type of water samples were calculated to be approximately 10 ng/l for bisoprolol, metoprolol and carbamazepine, and about two times higher metoprolol. Considering RSDs below 20%, the limits of quantification (LOQ) were estimated to be 50 ng/l for all compounds except for sulphamethoxazole. Encountered in this set of samples, the accurate analysis of this compound depends on the type of sample.

It can be noticed that the performance of the new methodology using external calibration for quantification, is lower in comparison to the existing procedure providing reproducibility's below 10% for all compounds at this concentration level. As can be expected, analyses of crude

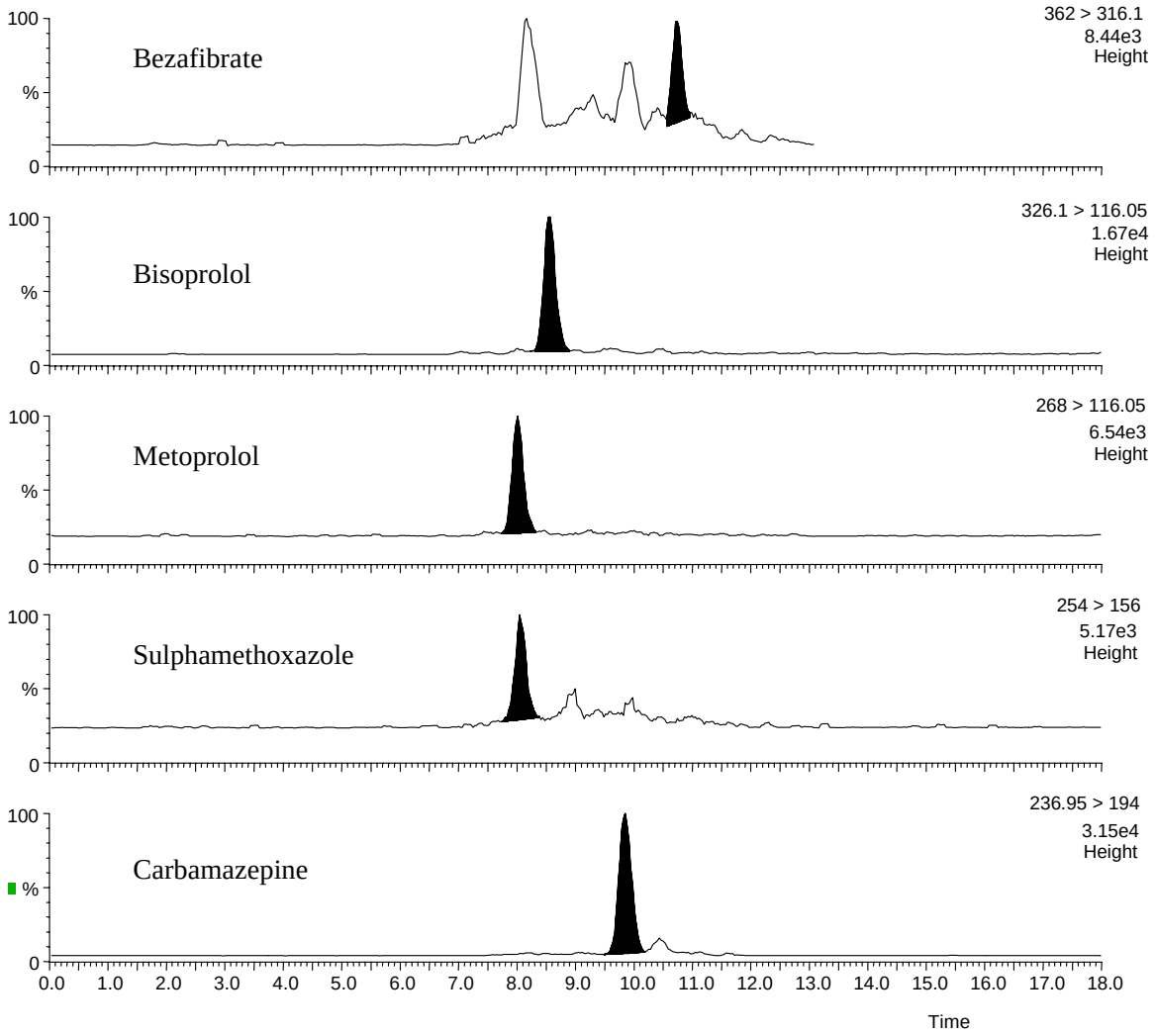


Fig. 2. Chromatograms for the on-line cLC–MS–MS analysis of 25 μl of ground water spiked at a level of 50 ng/l for each compound.

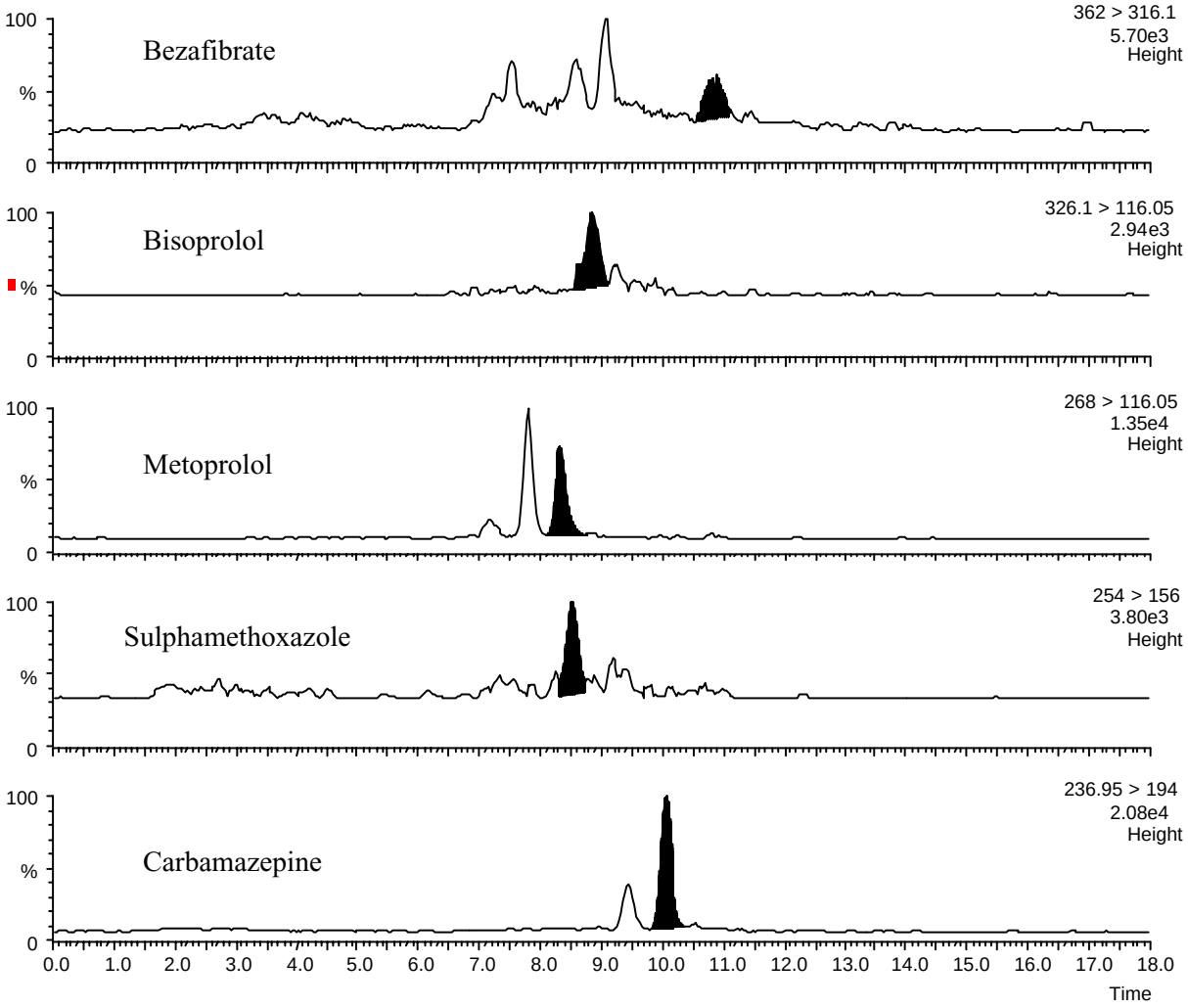


Fig. 3. Chromatograms for the on-line cLC–MS–MS analysis of 25 μl of a waste water sample containing bezafibrate (<50 ng/l), bisoprolol (<50 ng/l), metoprolol (840 ng/l), sulphamethoxazole (67 ng/l), and carbamazepine (238 ng/l).

samples contain more matrix compounds influencing the degree of ionization of the compounds.

Finally, the new methodology was tested by the analysis of water samples with incurred residues. The samples were part of a monitoring program on the occurrence of drugs in Dutch drinking water and related sources and analyzed about 1 year ago. Seven samples were selected including various types of water and containing residues of one or more drugs at concentration level of at least 50 ng/l. Between these samples, the program included the sampling of one wastewater taken from a plant treatment nearby Bilthoven (Groenekan, The Netherlands). As regards contamination and (possible) matrix effects, such a sample presents a worse case situation, and, was therefore also included in this study.

As regards the finding of drugs, there was no difference between the two methods. With both methods bezafibrate (detected in one sample), bisoprolol (in four samples), metoprolol (in five samples), sulphamethoxazole (in two samples) and carbamazepine (in seven samples) were found, at concentrations ranging between 10 and 840 ng/l. As an example, the on-line cLC–MS–MS analysis of the waste water sample is shown in Fig. 3.

In terms of the quantitative performance of the two methods, the calculated overall relative standard deviation of the duplicate data ($n = 19$) was 38%. In most cases, the concentrations found with the on-line cLC–MS–MS method were lower. Besides ion suppression effects, the difference might be caused by the time of storage of samples between analyses.

4. Conclusions

The use of capillary-column-switching liquid chromatography coupled to tandem mass spectrometric detection has been successfully applied for the direct, selective and sensitive screening of drugs in water. Beside fully automated analysis, the methodology enables the determination of such

compounds at the sub-ppb level consuming only 25 μl of sample. For four out of five compounds tested, the repeatability was below 20% and recoveries ranged between 50 and 100% indicating the suitability of this technique for screening purposes.

The analysis of crude water samples, without removing matrix components, could lead in some cases to a certain degree of ionization suppression of analytes. Enhanced reliable quantification can be expected using internal standards, e.g. stable isotope labeled compounds, or to extend the cLC system with an additional LC pump to perform an efficient cleanup on the first column.

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