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Multi heart-cutting two-dimensional liquid chromatography–atmospheric pressure photoionization-tandem mass spectrometry method for the determination of endocrine disrupting compounds in water

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ABSTRACT

The multi-heart-cutting two-dimensional liquid chromatography–tandem mass spectrometry method using atmospheric pressure photoionization has been developed and successfully validated for the determination of nine endocrine disrupting compounds in river water. The method is based on the use of two different reverse-phase columns connected through a six-port two-position switching valve equipped with a 200 μl loop. An orthogonal separation was achieved by proper selection of stationary phases, mobile phases, and the use of a gradient elution in both dimensions. The method shows excellent performance in terms of accuracy (86.2–111.1%), precision (intra-batch: 6.7–11.2%, inter-batch: 7.2–13.5%), and sensitivity (1.2–7.1 ng l^{-1}). Twenty real samples collected from the Loučka and the Svratka rivers were analyzed, the studied compounds were found in all Svratka samples (9.7–11.2 ng l^{-1} for β-estradiol, 7.6–9.3 ng l^{-1} for estrone, and 24.6–38.7 ng l^{-1} for bisphenol A).

1. Introduction

Endocrine disrupting compounds (EDCs) have become crucial emerging contaminants due to their presence in environmental waters giving rise to concerns over possible adverse effects to wildlife and humans. Various groups of chemicals, natural or man-made, have endocrine-disrupting activity and hence they are of environmental relevance. These chemicals include estrogens, pesticides, dioxins, certain polychlorinated biphenyls, and plasticizers such as bisphenol A or alkylphenol ethoxylate surfactants and their degradation products.

In 2009, the U.S. EPA published the final contaminant candidate list-3 (CCL-3) which specifies the priorities for regulatory decision making and information collection. The final CCL-3 now includes several natural and synthetic estrogens such as α-estradiol, β-estradiol, 17α-ethinylestradiol, estriol, and estrone [1]. Consequently, a new EPA method was introduced in 2010 for measuring 7 hormones in drinking water (estriol, β-estradiol, 17α-ethinylestradiol, estrone, testosterone, 4-androstene-3,17-dione, and equilin): EPA Method 539, determination of hormones in drinking water by solid phase extraction (SPE) and liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) [2].

The off-line SPE and liquid chromatography–tandem mass spectrometry represent the gold standard for analysis of estrogens and xenoestrogens in various matrices, as can be extracted from the reading of some revision articles on this subject [3–6]. Substantial improvements in EDCs analysis were achieved by using on-line connection of SPE to liquid chromatography. Petrovic et al. successfully used column switching LC–MS for an integrated sample clean-up and analysis of EDCs in sediment samples. A restricted access material (RAM) pre-column with various modifications (C_4, C_8 or C_{18}) was employed [7]. In contrast, Watanabe et al. successfully integrated a surface modified MIP pre-column as a pre-treatment device with the on-line LC–MS detection of E2 in water samples [8]. Salvator et al. explored the possibility of on-line sample cleanup with derivatization (utilizing dansyl chloride) directly on the solid-bed of a pre-column. In the optimized protocol, LOQs of 1 ng l^{-1} were achieved with only 1 ml of sample. Estradiol, estrone, and 17α-ethynylestradiol were under study [9]. Instead of commonly used ESI-MS, Viglino et al. employed atmospheric pressure photoionization-mass spectrometry (APPI-MS) for the detection of estrogens and progestogens during a fully automated on-line SPE method development. Surprisingly, no dopant was used and the authors state that comparative APPI experiments with a dopant (toluene and acetone) showed no improvement in sensitivity [10]. The extensive study of both separation systems (ultra-performance LC with or without post-column split, employing a mixed-mode column and on-line SPE) and MS ionization modes (ESI, APCI, APPI, and APCI/APPI) were performed by Lien et al. The best

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combination of LC systems and ion sources was UPLC coupled with an ESI source operated in positive mode when analytes were detected as appropriate dansylated derivatives (unfortunately, an ionization efficiency of native estrogens in positive and negative mode was not compared) [11].

In the solid-phase extraction, in either on-line or off-line mode, the first column serves only as a trap; therefore, the method suffers from several drawbacks, such as poor selectivity/efficiency of separation, resulting in co-extraction of interfering matrix components which can negatively affect the final qualitative and quantitative analysis. Consequently, higher demands are placed on the separation efficiency of HPLC. The lack of selectivity can sometimes be improved by using an analyte-selective sorbent based on immunoaffinity extraction [12,13] or a molecularly imprinted polymer [14,15]. An alternative approach is represented by the use of the high resolving power of multidimensional chromatography, preferably in on-line connection, which effectively utilizes the selectivity and separation efficiency available from both chromatographic columns.

In my previous work [16], the heart-cutting 2D-chromatography–tandem mass spectrometry method was developed to improve separation of four estrogens from the sediment matrix. The method was based on the direct transfer of only one fraction from the first dimension to the second. Pascoe et al. used a similar configuration of 2D-LC and demonstrated that 2D-LC–MS single-compound analysis (i.e. one compound per injection) can be more effective in reducing matrix-related signal suppression than multiple-compound analysis [17]. The aim of this work is to develop a method for the determination of several endocrine disrupting compounds in water based on multi-heart-cutting two-dimensional liquid chromatography–tandem mass spectrometry using atmospheric pressure photoionization source. Four natural or synthetic estrogens (β-estradiol, 17α-ethinylestradiol, estriol, and estrone), plastics-derived xenoestrogen (bisphenol A), and degradation products of alkylphenol ethoxylate surfactants (linear and branched octyl- and nonylphenol) were under study.

2. Experimental

2.1. Chemicals

Estriol (E3) (min. 99.7%), estrone (E1) (min. 99.3%), β-estradiol (β-E2) (min. 98.9%), 17α-ethynylestradiol (EE2) (min. 99.3%), and 4-*n*-nonylphenol (*n*-NP) (99.9%) were purchased from Fluka (Steinheim, Germany). 4-*tert*-Octylphenol (*t*-OP) (99%) was obtained from Supelco (Bellefonte, PA, USA). Bisphenol A (BPA) (99%), 4-octylphenol (OP) (min. 99%), and nonylphenol (NP) (analytical standard, technical mixture of ring and chain isomers) were obtained from Aldrich (Steinheim, Germany). Each compound was dissolved in MeOH to make a stock solution at a concentration of 50 μg ml^{-1} and was stored at −10 °C. Working solutions were prepared by mixing and diluting the stock solutions with methanol prior to use.

Acetonitrile (ACN) and methanol (MeOH) were obtained from Riedel-de Haën (LC/MS CHROMASOLV® grade, Seelze, Germany). Anisole (purum, 99%) was purchased from Fluka (Steinheim, Germany). Formic acid and ammonium hydroxide solution were purchased from Fluka Chemie (puriss p.a., eluent additive for LC–MS grade, Buchs, Switzerland). Demineralized water was purified using a Milli-Q-RG (Millipore, Bedford, MA, USA).

2.2. Sample collection and preparation

Water samples were collected at 20 randomly chosen sites along the river Svratka (Brno, Czech Republic) and a relatively unspoiled area of the river Loučka (the Bohemian-Moravian Highlands, Czech Republic) in September of 2011. The samples were preserved with formaldehyde (1%, v/v) to prevent biological degradation and stored in amber glass bottles at 4 °C. The Loučka samples were used in all SPE and 2D-LC preliminary experiments.

Oasis WAX (150 mg, weak anion exchanger) cartridges (Waters, Milford, MA, USA) were used for a concentration and a clean-up step of the water samples using an Alltech (Deerfield, IL, USA) manifold system. The cartridges were conditioned with 5 ml of MeOH and 5 ml of water prior to use. Thereafter, the samples (500 ml) were passed through the cartridge at a rate of 10 ml min^{-1}. The cartridge was rinsed with 5 ml of 5% formic acid in water:MeOH (80:20, v/v), followed by 5 ml of 5% ammonium hydroxide in water:MeOH (80:20, v/v). All retained analytes were eluted with 5 ml of MeOH. After SPE, solvents were evaporated under a gentle stream of nitrogen, the residue was dissolved in 75 μl of MeOH, filtered through a 0.45 μm nylon membrane filter and a 25 μl aliquot together with 10 μl of water was injected into the chromatographic system.

2.3. Heart-cutting-2D-HPLC

A schematic of the column configuration and valve switching is shown in Fig. 1. After injection of the sample onto the column 1, the eluted fraction containing analyte(s) is trapped in the loop. By switching the valve, this fraction is then transferred onto the column 2 and separated. The chromatographic separation was performed using an Agilent 1100 chromatographic system (Agilent, Waldbronn, Germany, equipped with a vacuum degasser, a quaternary pump, an autosampler, a column thermostat, and a diode-array detector). The second mobile phase pump was a Knauer HPLC 100 pump (Dr. Ing. Herbert Knauer GmbH Berlin, Germany, equipped with a vacuum degasser). Two analytical columns were connected via an electronically controlled six-port two-position switching valve equipped with a 200 μl loop.

A reverse-phase Luna C5 (100 mm × 2.0 mm, 5 μm particle size, column 1) (Phenomenex, Torrance, CA, USA) column equipped with a HPLC Ultra Column in-line filter (porosity 0.5 μm, Phenomenex,

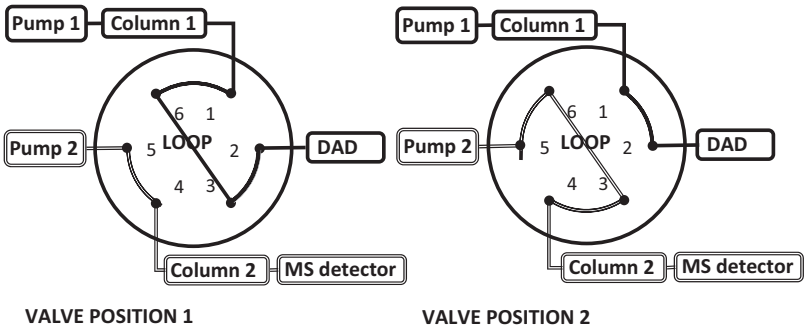

Fig. 1. Schematic of the heart-cutting 2D-HPLC-MS/MS system.

Table 1
The gradient conditions and the column switching program.

Column 1	Luna C5, 100 mm × 2.1 mm, 5 μm			Column 2	Zorbax Bonus RP, 100 mm × 4.6 mm, 3.5 μm	
Time (min)	Flow (ml min^{-1})	B (%) ACN	Valve 1 position	Time (min)	Flow (ml min^{-1})	B (%) MeOH
0.0	0.25	25	0.00 min–2	0.0	1.75	50
1.0	0.25	25	3.90 min–1	5.0	1.75	50
			4.40 min–2			
			8.80 min–1			
			9.30 min–2			
			9.50 min–1			
			9.90 min–2			
10.0	0.25	100	12.20 min–1	7.5	1.75	60
			12.40 min–2			
			12.70 min–1			
			13.40 min–2			
14.0	0.25	100		9.0	1.75	60
14.1	0.25	25		14.0	1.75	80
20.0	0.25	25		17.0	1.75	100
				20.0	1.75	100
				20.01	1.75	50

Torrance, CA, USA) with a linear gradient of water (solvent A) and acetonitrile (solvent B) was used in the first dimension. The flow rate was 0.25 ml min^{-1}. A Zorbax Bonus RP (100 mm × 4.6 mm, 3.5 μm particle size, column 2) (Agilent, Palo Alto, CA, USA) column was used in the second dimension. A linear gradient of water (solvent A) and methanol (solvent B) at a flow rate of 1.75 ml min^{-1} was used. The temperature in both dimensions was maintained at 35 °C. The time schedule for column 1, column 2, valve switching, and mobile phase gradient profiles are shown in Table 1.

2.4. APPI-MS

An ion-trap mass spectrometer (Finnigan LCQ Advantage MAX, Thermo Fisher Scientific, San Jose, CA, USA) equipped with an atmospheric pressure photoionization (APPI) interface was used. A KDS model 100 Series (KD Scientific, Holliston, MA, USA) syringe pump was used for anisole (dopant) infusion via a T-piece into the ion source at a flow rate of 0.015 ml min^{-1}. The flow was post-column split using a LC–MS splitter with a fixed 5:1 ratio (Supelco, Bellefonte, PA, USA).

All analytes were detected in the positive mode as the appropriate cation radicals $[M]^{+\bullet}$. The optimized MS conditions and the compound-dependent parameters are summarized in Table 2. Quantitative analysis was performed using a full scan MS/MS mode; two specific product ions were monitored per compound. Instrument control and data analysis were performed using XCalibur (version 2.0.7) software.

2.5. Method validation

Recovery of the whole method was evaluated by analyzing five replicate samples spiked with a concentration of 30 ng l^{-1} of each standard. The recovery was determined by comparison of the mean result for six analyses to the nominal concentration value. The recovery ranging from 80 to 120% was considered acceptable.

Calibration curves were constructed from triplicate analysis of the water extracts (previously probed not to contain the target analytes) spiked with the standard mixture of the analytes. Five solutions of different concentration (5, 7, 10, 40, and 100 ng l^{-1} for BPA, E2, E1; 7, 8, 10, 40, and 100 ng l^{-1} for EE2, OP, and *n*-NP; 9, 10, 15, 40, and 100 ng l^{-1} for E3; 25, 30, 40, 50 and 100 ng l^{-1} for *t*-OP and NP) were prepared for all analytes. The calibration curves were constructed based on the peak area of analytes versus the analyte concentration.

The limit of detection (LOD) was estimated as at least 3 × standard deviation of the blank measurement (sb)/slope of calibration curve (*b*). The limit of quantification (LOQ) was estimated as at least 10 × standard deviation of the blank measurement/slope of calibration curve. The calculated LODs and LOQs of the method were experimentally checked.

The precision was evaluated at three concentration levels (a concentration level similar to the LOQ, 50, and 100 ng l^{-1}). The intra-batch precision was evaluated by analyzing six samples spiked on the same day. Inter-batch precision was assessed by measuring six replicates per concentration level on three consecutive days. A precision as RSD of less than 15% was considered acceptable except for concentration levels similar to the LOQ, where it should not exceed 20% [18].

Table 2
The optimized conditions of APPI source operated in the positive mode.

Parameter	E3	BPA	E2	E1	EE2	*t*-OP	NP	OP	*n*-NP
Vaporiz. temp. (°C)	350	350	350	350	350	350	350	350	350
Capillary temp. (°C)	150	150	150	150	150	150	150	150	150
Sheat/aux. gas flow rate (units)	44/25	36/28	46/19	46/19	46/19	35/24	35/24	35/24	35/24
Capillary voltage (V)	24	22	30	30	30	19	19	19	19
Tube lens offset (V)	16	8	22	22	22	11	11	11	11
In-source CID (V)	8	8	8	8	8	8	8	8	8
Parent ion (*m*/*z*)	288	228	272	270	296	206	220	206	220
Product ions (*m*/*z*)	146 + 202	120 + 213	172 + 188	172 + 185	172 + 213	135 + 177	135 + 149	107 + 191	107 + 149
Relative collision energy (%)	36	40	36	34	36	36	38	38	39

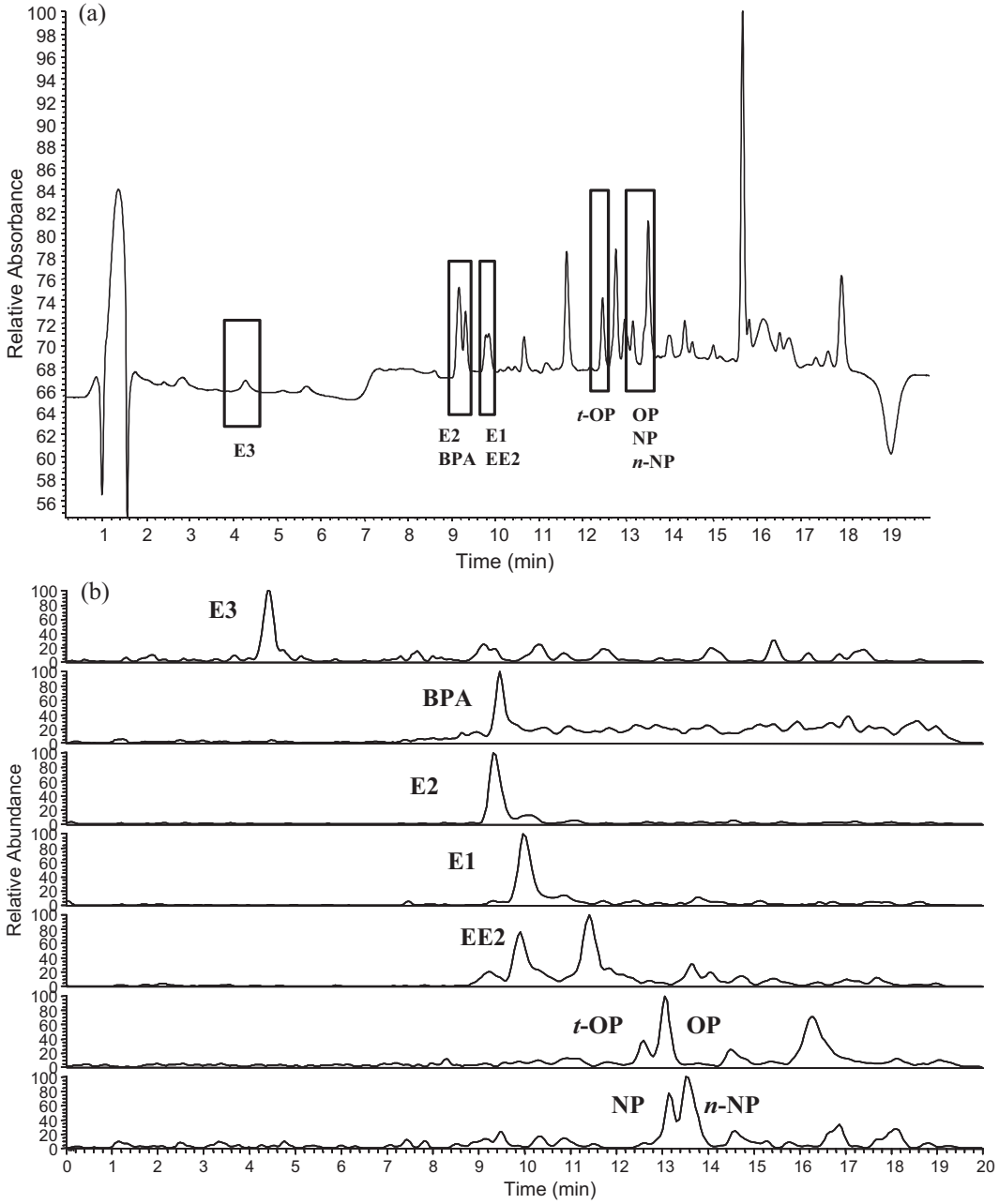

Fig. 2. (a) 1D-LC chromatogram of water extract enriched to a level of 200 pg μl^{-1} (25 μl injected). Data acquired with UV-DAD (λ = 280 nm) (rectangles define fractions transferred from the first to the second LC dimension). E3: estriol; BPA: bisphenol A; E2: β-estradiol; E1: estrone; EE2: 17α-ethynylestradiol; *t*-OP: 4-*tert*-octylphenol; OP: 4-octylphenol; NP: nonylphenol; *n*-NP: 4-*n*-nonylphenol. (b) 1D-LC chromatograms of water extract enriched to a level of 200 pg μl^{-1} (25 μl injected). Data acquired in MS/MS mode. E3: estriol; BPA: bisphenol A; E2: β-estradiol; E1: estrone; EE2: 17α-ethynylestradiol; *t*-OP: 4-*tert*-octylphenol; OP: 4-octylphenol; NP: nonylphenol; *n*-NP: 4-*n*-nonylphenol.

3. Results and discussion

3.1. Columns and mobile phase selection

The column is the heart of a good chromatographic system and therefore the selection of the column used in both the first and second dimension is essential for the development of any separation method. In 2D-LC the coupling of two similar phases may be experimentally simple; however, the correlation between retention mechanisms is usually high.

Two approaches for the selection of columns were chosen. Firstly, the available columns were evaluated in terms of "orthogonality" according to the work of Pellet et al. [19] and Snyder et al. [20]. The PQRI database was used to evaluate "differences" in selectivities of columns [21]. The highest F_s-value (32.27) was found for Luna Phenyl-hexyl (Phenomenex) and Zorbax Bonus RP (Agilent) columns. Some other F_s values are as follows: F_s (Zorbax Bonus RP–Luna C18) = 29.2; F_s (Zorbax Bonus RP–Luna C8) = 25.72; F_s (Zorbax Bonus RP and Luna C5) = 24.95.

Secondly, the "orthogonality" of the four column pairs was shown by using plots of log k (column 1) versus log k (column 2). A mobile phase consisting of water and MeOH or water and ACN was used. A high correlation coefficient (r^2 = 0.892–0.996) was found when different columns, but the same mobile phase in both dimensions were used. Correlation coefficient ranged from 0.867 to 0.931 when the same columns, but different mobile phases were

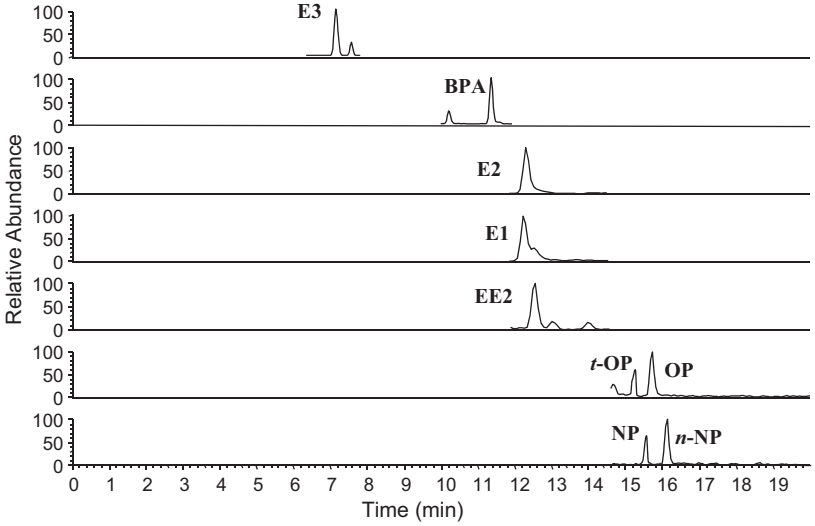

Fig. 3. 2D-LC chromatograms of water extract enriched to a level of 200 pg μl^{-1} (25 μl injected). Data acquired in MS/MS mode. E3: estriol; BPA: bisphenol A; E2: β-estradiol; E1: estrone; EE2: 17α-ethynylestradiol; *t*-OP: 4-*tert*-octylphenol; OP: 4-octylphenol; NP: nonylphenol; *n*-NP: 4-*n*-nonylphenol.

used. The lowest correlation coefficient (r^2 = 0.653) was found for the system of Luna C5 with water:ACN and Zorbax Bonus RP with water:MeOH as a mobile phase. Similar values (r^2 = 0.681–0.697) were found for the same system of a mobile phase and a reverse-phase column (Luna C18, C8, Phenyl-hexyl) with a Zorbax Bonus RP column.

Due to a better sensitivity of mass spectrometry detection of all analytes in MeOH than in ACN, the 2D-LC system consisting of a reverse-phase Luna C5 column with water:ACN as a mobile phase in the first dimension and the column with an embedded polar group Zorbax Bonus RP with water:MeOH as a mobile phase in the second dimension was selected.

3.2. Fraction transfer

In previous work, the direct transfer of the fraction of interest from the first column to the second was performed by simple valve switching [16]. However, this method could not be used for the transfer of multiple fractions due to the limitations in timing of the valve switching and a mobile phase composition. In this case, it appeared to be more convenient to use the transfer of fractions via a loop. This approach, however, required an appropriate "peak spacing" during the separation in the first dimension to avoid the loss (complete or partial) of a fraction of interest. While using two loops would be preferable, this approach was not applicable due to the unavailability of a ten-port two-position switching valve. A proper "peak spacing" was primarily achieved by proper column selection and by using a linear gradient of the mobile phase. Finally, five fractions (each having the elution volume of less than 180 μl) were completely transferred onto the second column without the loss of peak symmetry and width.

The stability of retention times was checked by analyzing 15 spiked extracts. The RSDs were in the range of 0.74–0.96%. A 200 μl loop was found sufficient to compensate for variations in retention times.

3.3. Multiple heart-cutting 2D-separations

Fig. 2a illustrates the separation of an extract enriched to a level of 5 ng of each analyte per injection using Luna C5 column with diode-array detection. Appropriate chromatograms obtained with mass spectrometry detector (MS/MS) are given in Fig. 2b. The quantification in MS/MS mode led to an overestimation of the results probably due to insufficient matrix separation. Recoveries were in the range of 65–286%. Inspection of the chromatograms of non-spiked extract obtained with UV detector revealed the matrix co-elution for later eluted analytes. A co-elution was also observed in MS/MS mode for later eluted analytes which correlates to the increase in MS response. In contrast, MS/MS spectra revealed no noticeable changes in the representation of fragments which varied only in their intensities (up to 18%), except for *t*-OP.

An improvement in the quality of quantitative data can be achieved by increasing the efficiency of the separation system. Higher resolution can be obtained by using gradient elution, increasing plate number or through a multidimensional approach. If a multidimensional separation is applied and the correlation between separation mechanisms is high, the technique is more akin to a column-switching process. However, the orthogonal separation can still be achieved by using a gradient elution in conjunction with column and solvent selectivity. Fig. 3 shows reconstructed 2D-LC chromatograms acquired in MS/MS mode. Employing of a linear gradient in the second dimension instead of an isocratic elution resulted in the separation of two extra peaks from EE2. The accuracy of EE2 changed from 152% (1D-LC–MS/MS) to 105% (2D-LC–MS/MS) which corresponds with the response of two extra peaks. Similarly, an extra peak with the same parent and product ions was separated from E3 and BPA. A co-elution between *t*-OP and a matrix compound with the same parent ion, but completely different MS/MS spectra, was still observed. Accuracy of the 2D-separation ranged from 86% (E3) to 111% (*t*-OP) and it was acceptable even without the use of internal standards. Match factors between the acquired MS/MS spectra and library spectra were higher than 950 except for *t*-OP. These results were achieved mainly due to selectivity tuning via column and solvent selection and use of a gradient elution. The increase of column efficiency (number of theoretical plates N) via use of two columns connected in series was clear for estriol (N = 1280 and 14,600 for 1D and 2D systems, respectively). For other analytes, the difference in N between 1D and 2D-LC was significantly lower. The number of theoretical plates ranged from 14,100 to 27,500 and from 17,800 to 49,700 for 1D and 2D-LC, respectively.

The gain in efficiency obtained by using a multidimensional separation system was illustrated during the analysis of EE2 by one-dimensional gradient separation employing a column which was different in the stationary phase, length, and particle size. Although the analysis was simplified by the presence of a single analyte, a

Table 3
Method validation.

	t_r (min)	Recovery[a] (%)	Linear range (ng l^{-1})	r^2	LOD[b] (ng l^{-1})	LOQ[c] (ng l^{-1})	Intra-batch precision (%)[d]			Inter-batch precision (%)[e]		
							c_{subLOQ}[f]	50 (ng l^{-1})	100 (ng l^{-1})	c_{subLOQ}[f]	50 (ng l^{-1})	100 (ng l^{-1})
E3	7.17	86.2 (8.1)	9–50	0.9911	2.6	8.7	8.9	8.0	7.5	12.4	10.4	10.2
BPA	11.25	99.5 (6.8)	4–50	0.9991	1.4	4.7	7.2	6.8	6.8	9.1	7.5	7.2
E2	12.36	100.8 (8.1)	4–50	0.9954	1.2	4.0	8.3	7.5	7.1	10.2	8.5	7.6
E1	12.30	98.7 (6.7)	4–50	0.9963	1.2	4.0	7.3	6.8	6.7	8.9	7.5	7.2
EE2	12.54	105.4 (7.8)	6–50	0.9921	1.7	5.7	9.5	7.9	7.8	9.8	8.6	8.4
t-OP	15.26	111.1 (9.5)	13–50	0.9908	6.0	20.0	11.2	11.0	9.3	13.5	10.6	10.2
NP	15.59	109.0 (7.9)	13–50	0.9928	7.1	23.7	10.6	10.7	8.8	12.2	10.5	10.3
OP	15.72	104.7 (7.1)	6–50	0.9978	1.9	6.3	8.5	7.5	7.3	9.9	8.4	8.4
n-NP	16.10	105.6 (6.8)	6–50	0.9986	1.9	6.3	7.9	7.1	7.1	9.3	8.2	8.0

[a] Recovery of the whole analytical procedure, RSDs are given in parentheses ($n = 6$).
[b] Limit of detection as 3 × standard deviation of blank measurement/slope of calibration curve.
[c] Limit of quantification as 10 × standard deviation of blank measurement/slope of calibration curve.
[d] $n = 6$.
[e] $n = 18$.
[f] Concentration level similar to the LOQ.

satisfactory accuracy was achieved only after complex and lengthy optimization (data not presented).

3.4. Method validation

All validation tests were done with water samples previously examined not to contain any target analytes. Because of the unavailability of reference materials, the method validation was performed with samples enriched to the required concentration level. Summarized results of the method validation are given in Table 3.

Estimated LODs and LOQs were calculated on the basis of calibration curves and experimentally checked by analyzing water samples enriched to the desired level. For LOD level, the signal-to-noise ratio (S/N) ranged from 7:1 to 13:1. Similarly, S/N on LOQ level was in the range 15:1 to 28:1. The criteria were 3:1 for LOD and 10:1 for LOQ.

3.5. Analysis of real water samples

The on-line 2D-liquid chromatography–tandem mass spectrometry method was successfully applied to the determination of nine endocrine disrupting compounds in water samples collected in the Czech Republic. Five samples collected at the Loučka sampling sites did not contain any analytes above detection limits. These samples were primarily used in preliminary experiments.

The analyses of 15 samples collected from the river Svratka showed the occurrence of bisphenol A and estrone in all samples. The concentration of BPA ranged from 24.6 to 38.7 ng l^{-1}. Estrone was found in the range of 5.6–7.3 ng l^{-1}. Estradiol was found in three samples at the concentrations of 9.7, 11.1, and 11.2 ng l^{-1}. Other analytes were below the detection limits. Fig. 4 shows a chromatogram of a real water sample analyzed using the proposed method.

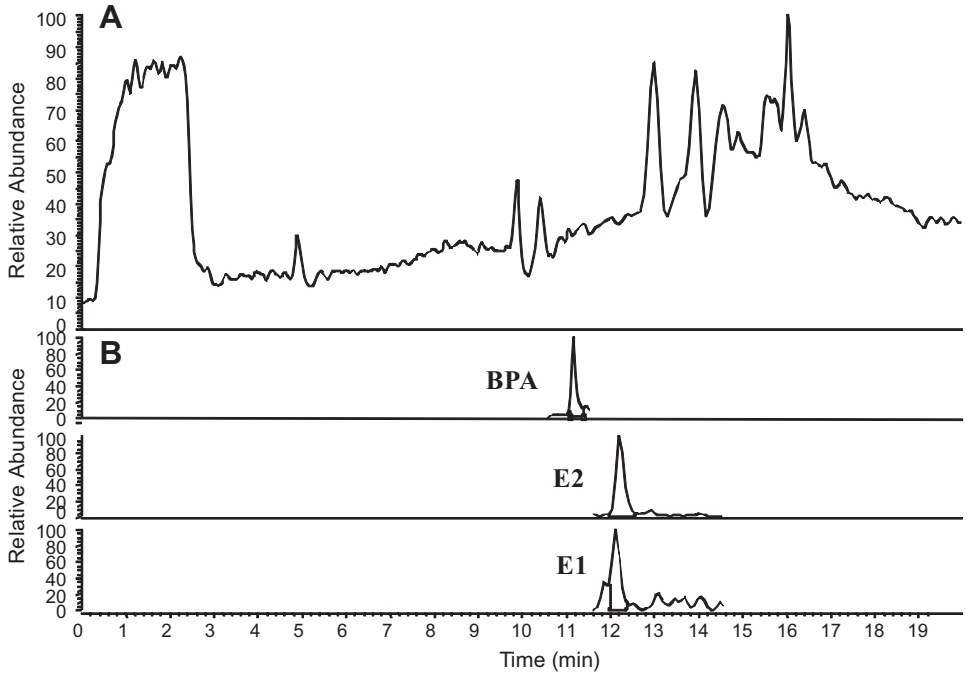

Fig. 4. Chromatograms of real water samples collected in the Svratka river. (A) 2D-LC total ion current MS chromatogram. (B) 2D-LC reconstructed ion current MS/MS chromatograms. BPA: bisphenol A (32.8 ng l^{-1}); E2: β-estradiol (11.2 ng l^{-1}); E1: estrone (7.3 ng l^{-1}).

4. Conclusion

The heart-cutting two-dimensional reverse-phase LC system for the separation of nine endocrine disrupting compounds in water samples was successfully developed and validated. The method was based on the proper selection of analytical columns used in both dimensions through a database search and a simple experimental column evaluation in connection with a mobile phase choice. The system is fully automated and was assembled exclusively from commercially available components.

The application of this system to the analysis of real river water samples demonstrated a significant decrease in matrix effect on quantitative data compared to a single-column chromatography. Accuracies of 1D-separation were found in the range of 65–286% and it was improved to 86–111% by employing 2D-chromatography. Viglino et al. evaluated matrix effects for the method consisting of on-line SPE connected to HPLC and APPI-MS/MS detection and presented results ranging from 85 to 103% and 75 to 95% in surface water and sewage water samples, respectively [10]. Lien et al. compared several chromatographic systems and MS ionization sources. For river water samples they found out that matrix effects of ESI and APPI sources were similar under the same LC conditions and none of the four LC systems was able to significantly eliminate ion suppression (e.g. dansyl ESI+, on-line SPE: 63.1–93.8%; dansyl ESI+, UPLC with split: 59.6–96.0%. Low values were found for alkylphenols) [11].

It was also showed that multi-compound analysis using the heart-cutting 2D-LC approach is applicable even to compounds with wide ranges of polarity due to the possibility to simply tune the selection of columns and tuning gradient conditions in both dimensions. Moreover, a substantial improvement in the orthogonality of the separation system can be achieved by using a gradient elution in the second dimension.

The recoveries for each compound ranged from 86.2 to 111.1% and the intra- and inter-batch precisions ranged from 6.7 to 11.2% and 7.2 to 13.5%, respectively. The LODs were in the range of 1.2–7.1 ng l^{-1}. Up to four times higher value of LOD was found for branched isomers in comparison to linear alkylphenols.

Not so commonly used cation radicals have been utilized in atmospheric pressure ionization-MS detection. AS was shown in my previous work, photoionization of estrogens via proton transfer showed better sensitivity than APCI operated in positive mode and even better when compared to ESI, APCI, and APPI operated in negative mode [16]. When toluene was used as a dopant for the detection of alkylphenols, poor ionization efficiencies were observed. Poor results were found also in APCI+; no signal was observed in ESI+. The use of anisole led to an appreciable improvement of ionization efficiency for alkylphenols, while ionization of estrogens and BPA was only slightly affected. Anisole enables a charge exchange reaction between the anisole radical and an analyte resulting in acquiring $[M]^{+\bullet}$ cation radicals instead of $[M+H]^{+}$ ions under reversed-phase liquid chromatography conditions [22].

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