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Trace determination of priority pesticides in water by means of high-speed on-line solid-phase extraction–liquid chromatography–tandem mass spectrometry using turbulent-flow chromatography columns for enrichment and a short monolithic column for fast liquid chromatographic separation

Arndt Asperger*, Jürgen Efer, Therese Koal, Werner Engewald

Leipzig University, Institute of Analytical Chemistry, Linnéstrasse 3, D-04103 Leipzig, Germany

Abstract

An integrated on-line SPE–HPLC–MS/MS system has been developed for the rapid analysis of various trace level priority pesticides in surface and drinking water. Eleven pesticides were included in this study, with various phenylureas, triazines and organophosphorous species among them. Use of turbulent-flow chromatography columns (TFC, 50×1 mm, 30–50 μm particle size) as extraction cartridges enables fast on-line SPE at high sampling flow-rate (5 ml/min). Polymeric and carbon based TFC columns (Oasis HLB, Cyclone, Hypercarb) allow complete extraction with good recoveries from water volumes up to 50 ml. On-line coupling to HPLC is performed with re-mixing of the organic TFC eluate with water in front of the analytical column to ensure efficient band focussing. For fast HPLC analysis, a short monolithic column is applied in combination with highly selective API–MS/MS detection. Matrix effects on the APCI–MS/MS signal were found to be reduced by the system to an acceptable minimum. Limits of detection, determined for 10-ml samples of river water were in the range between 0.4 and 13 ng/l typically, except trifluralin (approximately 280 ng/l), which is less susceptible to ionization under atmospheric pressure conditions. At an enriched water volume of 10 ml, the whole SPE–HPLC–MS/MS procedure requires less than 14 min. The method was successfully applied to the analysis of drinking and surface water samples taken from several sampling sites around the city of Leipzig, Germany. Concentrations measured (maximum: 16 ng/l simazine in river water) were far below the concentration limits scheduled by law.

Keywords: Water analysis; On-line solid-phase extraction; Turbulent-flow chromatography; Monolithic column; Pesticides

1. Introduction

Pesticides represent a serious problem to the natural environment, especially to marine eco-systems. Due to their intense use in todays agriculture and to their persistence as well, many of these compounds end-up in surface and ground water and have to be considered a potential risk for marine life as well as for drinking water quality.

In this context, high-performance analytical methods are of essential importance for the precise monitoring of trace level pesticides in the aquatic environment. In most cases, an enrichment step, for

*Corresponding author. Tel.: +49-341-973-6068; fax: +49-341-973-6115.

E-mail address: asperger@sonne.tachemie.uni-leipzig.de (A. Asperger).

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instance solid-phase extraction (SPE), has to be performed with respect to the low concentration level of pesticides in natural waters. Nowadays, a large number of SPE materials (e.g. modified silica based materials, polymeric phases, carbon based materials) are available, covering a wide range of selectivity and, thus, a large field of applications [1–9].

Analysis of SPE extracts obtained from water samples has commonly been performed by HPLC with UV detection, GC combined with FID, ECD or MS [3,10–12]. Mass spectrometric detection has been performed routinely in combination with GC for many years. However, the introduction of atmospheric pressure ionization techniques (API), like electrospray (ESI) and atmospheric pressure chemical ionization (APCI), opened the door to LC–MS as a routinely used coupling technique, and drastically improved the possibilities of HPLC for the analysis of polar and ionic analytes [13,14]. This is reflected by numerous publications reporting on the HPLC–MS analysis of a broad variety of pesticides, like triazines, phenylureas and organophosphorous pesticides [15–22]. Tandem-mass spectrometric detection results in further improved performance, providing additional selectivity and, consequently, enhanced sensitivity [23–25].

Steadily growing numbers of samples to be analyzed cause the need of higher sample throughput. This requirement may be met to a certain extent by automatization of all parts of analytical methods, including sample preparation. However, on-line SPE–HPLC coupling techniques may provide a further significant time benefit, accompanied by increased sensitivity and decreased amounts of sample and solvent required. Several fully automated on-line SPE approaches are commercially available (Prospekt, Spark Holland, Emmen, The Netherlands; OSP-2, Merck, Darmstadt, Germany) and have been successfully applied to the analysis of pesticides in water [26–34]. Limits of detection that have been achieved by automated on-line SPE–LC–MS (/MS) methods are in the range 0.2–30 ng/l typically, at extracted water volumes varying between 20 and 200 ml [26–28,30,32,34].

To further reduce the analysis time, integration of high-speed LC columns into on-line SPE–HPLC systems is recommended. Hoogenboom and coworkers [35–40] described in detail the application of short LC columns with and without on-line coupled SPE to the target analysis of water samples. Due to limited separation power of such short LC columns (typically 20×4.6 mm), highly selective detection, for instance tandem-mass spectrometry, is required, especially in on-line SPE–short column LC.

In the paper presented here, a new on-line SPE–HPLC–MS/MS system for the rapid multicomponent analysis of trace level pesticides is described. Eleven compounds have been investigated in this study, including four triazines, three phenylureas and two organophosphorous pesticides, all being part of a monitoring based priority list recently scheduled by the European Union [41]. The system described here employs turbulent-flow chromatography (TFC) columns, a recently introduced tool for high-speed separation of drugs and metabolites from biological matrices like blood or urine [42–44], as enrichment cartridges for fast on-line SPE. The capabilities of different TFC columns (modified silica-based, polymeric, carbon based) are investigated. Trace enrichment on TFC columns is combined with subsequent fast LC separation on a short monolithic column [45–47] coupled to tandem-MS detection. SPE–HPLC on-line coupling is performed with re-mixing of the organic TFC eluate with aqueous eluent for efficient band focussing on the analytical column. Performance of the method is evaluated and results obtained from first application to drinking and surface water samples taken from sites around the city of Leipzig (Saxony, Germany) are reported.

2. Experimental

The following compounds were included in this study: atrazine, simazine, terbutylazine, prometryne, isoproturon, diuron, chlortoluron, chlorfenvinphos, chlorpyrifos, alachlor, trifluralin.

2.1. Chemicals

Methanol (Lichrosolv) was obtained from Merck (Darmstadt, Germany). Ultraclean water was prepared in the lab using a water treatment device ‘‘Ultra-Clear’’ (SG Wasseraufbereitungsanlagen, Barsbüttel, Germany). Ammonium acetate (frac-

topur), added as an ionic additive to all eluents, was purchased from Merck. Standard pesticides were purchased from Dr Ehrensdorfer (Augsburg, Germany) and Promochem (Wesel, Germany), respectively. Methanolic stock solutions were prepared at a concentration of 500 μg/l. Ultraclean and surface water samples were spiked with aliquots of these solutions to achieve final concentrations in the range of 5–500 ng/l.

2.2. Sample materials and pre-treatment

Water samples were taken at the following sites: Institute of Analytical Chemistry of Leipzig University, Saxony, Germany (drinking water), River Parthe (area of the city of Leipzig), and Lake Cospuden (situated in the south of Leipzig).

Surface water samples were collected in 2.5-l brown glass bottles. Immediately after arrival in the lab, the samples were filtered through 1-μm glass fiber filters and 0.45-μm cellulose acetate filters, respectively. In case of higher content of suspended matter (River Parthe), both filters were applied in sequence. Drinking water was analyzed without filtration.

Samples collected for determination of real pesticide concentrations were analyzed not later than 48 h after sampling. All samples were stored in brown glass bottles at 4°C. Prior to analysis, 10% (v/v) of methanol were added to the water samples to prevent analyte losses due to adsorption to system.

2.3. TFC columns

Five TFC columns (1×50 mm) filled with different sorbents were tested in this study — silica-based phases: Turbo C_{18}; Turbo Phenyl (50-μm particles; Cohesive Technologies, Franklin, USA); polymeric phases: Oasis HLB (35-μm particles; Waters, Milford, USA); Cyclone (50-μm particles; Cohesive Technologies); porous graphitized carbon–Hypercarb (30-μm particles, ThermoHypersil, Runcorn, Cheshire, UK). The first four columns are commercially available products. Hypercarb was taken from Hypersep SPE cartridges. It was filled into an empty TFC column self-made from stainless steel. After a first packing step, the column was flushed with methanol. Subsequently, the column was opened again and the remaining empty space was filled with phase material. This procedure was repeated until the inner volume of the column was completely filled.

2.4. On-line SPE–HPLC coupling

A high-throughput liquid chromatograph HTLC 2300 (Cohesive Technologies) was used equipped with isocratic and binary pump and an integrated six port valve. Commonly, problems arise from the fact that optimum conditions of SPE elution are in conflict with the requirements of subsequent HPLC gradient analysis. High organic content of the SPE eluent is desirable to achieve a narrow elution profile, while the contrary is optimum for starting the gradient HPLC. To manage this conflict, our approach includes re-mixing of the organic SPE eluate with water before entering the analytical LC column. Therefore, the HTLC system was slightly modified as schematically shown in Fig. 1. The TFC column was placed in-line between pump head A of the binary pump delivering the organic eluent and the mixing chamber of the binary pump (see Fig. 1). The organic TFC eluate is mixed with aqueous eluent B in the mixing chamber just before entering the HPLC column. This results in efficient analyte trapping on the HPLC column and, thus, allows to start the subsequent gradient LC analysis under quasi-optimum conditions. The HPLC column used for separation was a short monolithic reversed-phase column (Chromolith SpeedROD RP-18e, 50×4.6 mm; Merck).

2.5. API–MS/MS conditions

The mass spectrometer used was an API 2000 triple quadrupole (PE Sciex, Concord, Canada) equipped with both ESI (Turboionspray®) and APCI source (Heated Nebulizer®). Interface settings and collision gas pressure were manually optimized (Table 1). Parameter tuning for maximum sensitivity of MRM detection in positive ion mode was carried out by means of the optimization algorithm supported by the Analyst 1.1 software® using the Turboionspray interface and a syringe pump continuously supplying a pesticide standard mixture (16 μl/min, c=100 μg/l each compound). The resulting instrumental values were then cross-checked for their

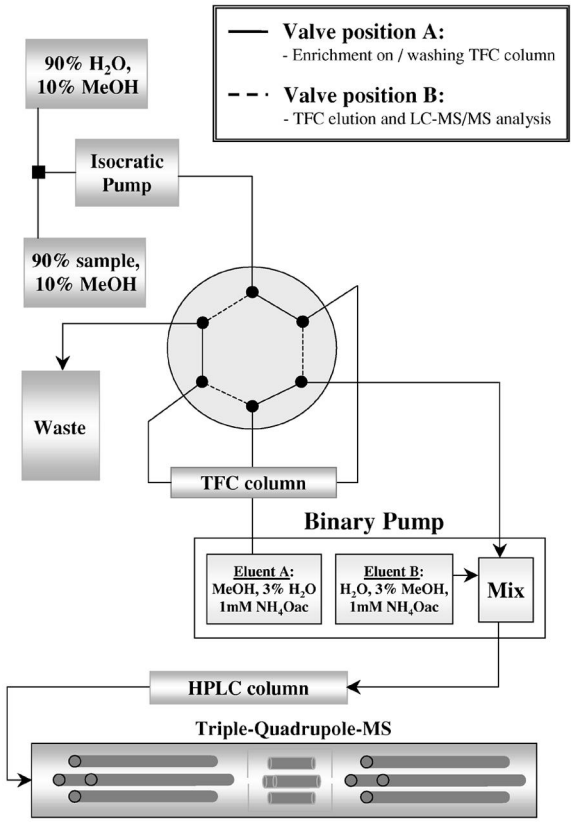

Fig. 1. Schematic view of the described high-speed on-line SPE–HPLC–MS/MS system.

validity under APCI conditions by flow-injection MS/MS analysis (FIA–MSMS) of the standard mixture and manual variation of the settings.

Table 2 contains the MRMs finally used for detection of 11 pesticides included in this study. For all pesticides, $[M+H]^+$ was found to be the most abundant precursor ion, except for alachlor, which showed highest intensity for $[M-CH_3OH+H]^+$ at $m/z=238$ a.m.u. when using the APCI interface. The dwell time was set to 40 ms for each MRM transition. The resulting MS cycle time was 492 ms, including dwell and pause times.

Table 1
Optimized interface settings for ionization in Turboionspray® and APCI

	Turboionspray	APCI
IS voltage (V)	5000	–
Nebulizer current (μA)	–	2
Temperature (°C)	450	450
IS gas 1 (p.s.i.)	45	70
IS gas 2 (p.s.i.)	75	45
Curtain gas (p.s.i.)	25	25
Collision gas (N_2)	6	6

Ammonium acetate at a concentration of 1 m*M* was added to all eluents diverted to the mass spectrometer.

Experiments on MS/MS optimization, characterization of TFC columns and optimization of on-line SPE conditions, respectively, were carried out using either Turboionspray or APCI. All on-line SPE–HPLC–MS/MS experiments and real sample analyses were performed using the APCI interface.

3. Results and discussion

3.1. Characterization of different TFC columns

3.1.1. Breakthrough experiments

A simplified instrumental setup was used for these investigations leaving out HPLC and coupling the TFC column to the MS interface directly.

First, breakthrough volumes (BTV) were approximated by means of elution chromatographic analyses injecting 10 μl of a standard pesticide mixture on the TFC column at various eluent compositions (methanol–water). The logarithm of the measured BTV was plotted versus the percentage of eluent A (methanol with 3% water). From the resulting linear graphs, BTV values valid for pure eluent B (water with 3% methanol) were extrapolated. These results are listed in Table 3. As can be derived from the data, the polymer packed TFC columns Oasis HLB and Cyclone give by far the highest BTV for all pesticides investigated here. In contrast, the modified silica based columns Turbo C_{18} and Turbo Phenyl do not provide enough retention for trace enrichment of several species (simazine, atrazine, chlortoluron, isoproturon, diuron) from water volumes in the range 10–50 ml. The self-packed Hypercarb column gives satisfying BTV for most of the pesticides. However, the data obtained from this approximation suggest a certain risk of breakthrough especially for three compounds (simazine, atrazine and isoproturon) at sampling volumes higher than 30 ml. As an interesting side effect, the completely different order of

Table 2
MRM transitions used for MS/MS detection

Compound	MRM (precursor/product ion)	Compound	MRM (precursor/product ion)
Isoproturon	207/72	Prometryne	242/158
Diuron	233/72	Chlorfenvinphos	359/99
Chlortoluron	213/72	Chlorpyrifos	350/198
Atrazine	216/174	Alachlor	270/162 (Turboionspray)
Simazine	201/132		238/162 (APCI)
Terbutylazine	230/174	Trifluralin	336/202

retention (elution) on Hypercarb should be noted, with trifluralin and alachlor showing drastically decreased retention in comparison to the polymeric and silica-based TFC columns. This reflects the differing retention mechanism present on carbon-based stationary phases [8].

To confirm the approximative data given above, more precise frontal chromatographic tests were performed using ultraclean water spiked with 50 ng/l of each compound. Rising water volumes (2–50 ml, flow-rate 5 ml/min) were enriched on each of the TFC columns, and the peak areas resulting from subsequent elution (methanol–water 97:3, v/v; 400 μl/min) were recorded. The graphs resulting from plotting the peak area versus the enriched water volume were then checked regarding their range of linearity. The obtained data confirm the BTV values determined by elution chromatography. On both silica based columns (Turbo C_{18} and Phenyl), significant breakthrough of some of the compounds (most drastic: simazine) is observed beginning at approximately 5–10 ml. Other three columns (Oasis HLB, Cyclone, Hypercarb) allow complete enrichment of all pesticides included here from water volumes up to 50 ml, the largest volume investigated.

3.1.2. TFC elution profiles and elution efficiencies

To evaluate the shape of elution profiles obtained from different TFC columns, 10 ml samples of ultraclean water spiked with a pesticide standard mixture ($c = 100$ ng/l each compound; trifluralin 500 ng/l) were enriched at a flow-rate of 5 ml/min and subsequently eluted (methanol–water 97:3, v/v) into the APCI interface at a flow-rate of 200 μl/min. Fig. 2 contains elution peaks obtained from the TFC columns Oasis HLB, Cyclone and Hypercarb. The profiles are based on the total ion current of 11 MRMs detected. The peaks obtained from Oasis HLB and Cyclone are virtually identical in shape and peak with. A volume of approximately 200 μl of methanolic eluent is sufficient to remove all com-

Table 3
Breakthrough volumes approximated by elution chromatography/extrapolation (valid for aqueous eluent H_2O–MeOH 97:3, v/v)

Compound	Breakthrough volume (BTV) [ml]				
	Oasis HLB	Cyclone	Turbo C_{18}	Turbo Phenyl	Hypercarb
Isoproturon	5.3*E* + 03	3.1*E* + 03	30	40	50
Diuron	6.1*E* + 03	1.3*E* + 03	30	20	290
Chlortoluron	2.1*E* + 03	1000	30	9	170
Atrazine	1.8*E* + 03	750	20	30	69
Simazine	200	160	8	5	30
Prometryne	2.7*E* + 04	7.6*E* + 06	360	410	190
Terbutylazine	1.8*E* + 04	1.4*E* + 06	100	80	100
Chlorfenvinphos	1.4*E* + 04	1.6*E* + 06	5.3*E* + 03	3.5*E* + 03	3.3*E* + 03
Chlorpyrifos	8.7*E* + 04	4.3*E* + 06	1.4*E* + 04	4.8*E* + 04	2.8*E* + 03
Alachlor	5.6*E* + 03	4.5*E* + 06	2.0*E* + 03	720	80
Trifluralin	1.7*E* + 05	1.3*E* + 08	n.d.	n.d.	350

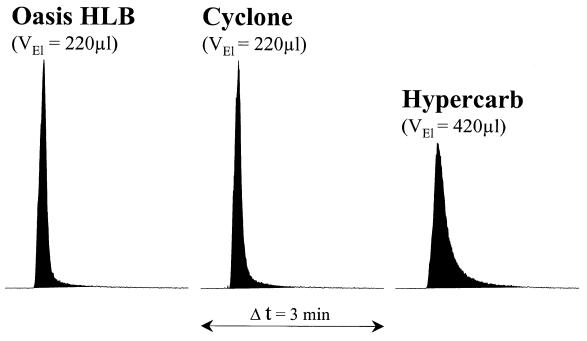

Fig. 2. Elution profiles (total ion current of 11 MRMs) obtained from different TFC columns at an elution flow-rate of 200 µl/min (spiked ultraclean water, c=60 ng/l, V=10 ml, sampling flow-rate: 5 ml/min; eluent: methanol with 3% water, 1 mM NH_4Oac).

pounds completely. Elution from the Hypercarb column requires a higher eluent volume of approximately 400 µl resulting in a broader substance band. However, this is still an acceptable peak width, even for on-line SPE applications.

Elution efficiencies were determined for Oasis HLB, Cyclone and Hypercarb. Experiments were carried out using a similar on-line SPE–HPLC–MS/MS setup as given in Fig. 1, injecting on the TFC column a 10-µl aliquot of an aqueous pesticide standard mixture (c=100 µg/l each compound, 500 µg/l trifluralin) and subsequently purging 10 ml of ultraclean water (with 10% methanol added) by means of the isocratic pump. The resulting peak areas were compared to those obtained from an identical injection directly on the HPLC column. The results are listed in Table 4. All pesticides are removed from the TFC columns with satisfying yield.

Summarizing the sorption/elution characteristics of the TFC columns tested here, the polymeric columns Oasis HLB and Cyclone perform best regarding retention capacity (BTV), elution efficiency and elution volume. The Hypercarb column requires a higher volume of methanol for complete elution, but is applicable to on-line SPE though. Due to insufficient retention capacity, the silica based TFC columns Turbo C_{18} and Phenyl are not recommended for trace enrichment from water volumes higher than 5 ml. All investigations described in the following were carried out using the Oasis HLB column.

Table 4
Elution efficiencies of 11 priority pesticides from various TFC columns

Compound	Elution efficiency [%]		
	Oasis HLB	Cyclone	Hypercarb (self packed)
Isoproturon	109	96	106
Diuron	99	90	102
Chlortoluron	104	100	108
Simazine	104	97	112
Atrazine	106	100	105
Terbutylazine	101	97	101
Prometryne	103	89	95
Chlorfenvinphos	99	95	105
Chlorpyrifos	84	84	85
Alachlor	104	93	101
Trifluralin	89	74	88

3.2. Performance of the on-line SPE–HPLC–MS/MS system

In the result of optimization of all parts of the method, a timetable was scheduled as given in Table 5. It is valid for the enrichment of a 10-ml sample.

On-line enrichment on TFC is performed at 5 ml/min. Applying the Oasis HLB column, this flow-rate results in a TFC head pressure of approximately 60 bar. At an inner column diameter of 1 mm, a flow-rate of 5 ml/min corresponds to a linear velocity of 10.6 cm/s and, thus, a contact time of 0.5 s between sample and TFC stationary phase. Completeness of enrichment at such an enormous velocity of the sample passed through the TFC was verified by means of analysis of 10 ml of spiked ultraclean water, c=60 ng/l, at varying flow-rates (1, 3 and 5 ml/min). As the resulting peak areas did not show any significant differences, a negative impact of the high velocity on the extraction efficiency may be excluded.

A ''washing'' step subsequently to the extraction was introduced to remove matrix compounds from the TFC surface, for instance salts and humic substances. Furthermore, this purging step was required to remobilize analytes retained in the system (sample delivery pump, tubings, valves etc.). Especially two most lipophilic species among the compounds investigated here (chlorpyrifos, trifluralin) showed serious affinity to inner surfaces of the system.

Table 5
Optimized on-line SPE–HPLC–MS/MS time schedule (sampling volume: 10 ml)

Step	Action	Time [min]	Valve position	Isocratic pump	Binary group
0	Pre-run	0.00	A	H_2O–MeOH (90:10, v/v; 5 ml/min)	Eluent A–B (33:67, v/v; 0.6 ml/min)
1	Sampling onto TFC	0.17	A	Sample–MeOH (90:10, v/v; 5 ml/min)	Eluent A–B (33:67, v/v; 0.6 ml/min)
2	Washing	2.17	A	H_2O–MeOH (90:10, v/v; 5 ml/min)	Eluent A–B (33:67, v/v; 0.6 ml/min)
3	TFC elution to HPLC	5.67	B	H_2O–MeOH (90:10, v/v; 0.5 ml/min)	Eluent A–B (33:67, v/v; 0.6 ml/min)[a]
4	Gradient HPLC	7.17	B	H_2O–MeOH (90:10, v/v; 0.5 ml/min)	Eluent A–B (33:67, v/v; 0.6 ml/min)[a]
6	Isocratic HPLC	12.17	B	H_2O–MeOH (90:10, v/v; 0.5 ml/min)	Eluent A–B (100:0, v/v; 0.6 ml/min)[a]
7	Re-conditioning	14.00	A	H_2O–MeOH (90:10, v/v; 5 ml/min)	Eluent A–B (33:67, v/v; 0.6 ml/min)

[a] Eluent A flows to mixing chamber via TFC column; eluent B flows to mixing chamber directly.

TFC elution in backflush mode is performed at a flow-rate of 200 μl/min of methanolic eluent A, which is then mixed with 400 μl/min of aqueous eluent B. This flow regime provides a narrow elution profile and efficient trapping of the analytes on the analytical LC column. Furthermore, the resulting LC effluent flow-rate (600 μl/min) is in good compatibility to the nebulizing capacity of the APCI source used. To demonstrate the final performance of the system, Fig. 3 contains a direct comparison of total ion chromatograms (11 MRM for 11 compounds) obtained from on-line SPE–HPLC–MS/MS (V_{sample} = 10 ml spiked ultraclean water, c = 125 ng/l) and HPLC–MS/MS ($V_{injection}$ = 10 μl spiked ultraclean water, c = 125 μg/l), respectively. Differences between the resulting traces are very minor confirming the efficiency of analyte trapping on the analytical column. The separation quality is good enough to have the retention times as another identification criterion, supplementing the specificity of the precursor and product ion masses. Incomplete chromatographic resolution of several compounds does not affect quantitation because of the selectivity of detection in MRM mode. At a sample volume of 10 ml, the on-line SPE–HPLC–MS/MS method requires less than 14 min.

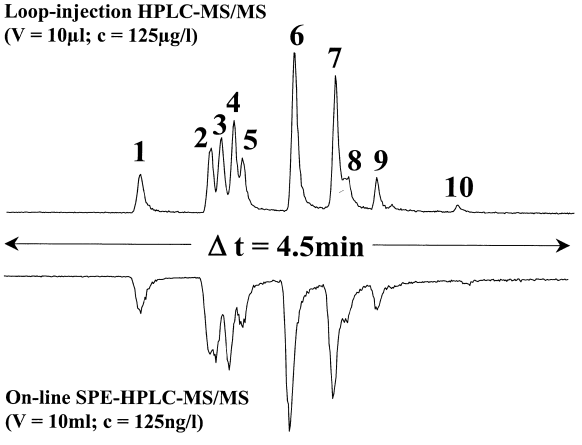

Fig. 3. Comparison of chromatograms obtained from loop-injection HPLC–MS/MS (top) and on-line SPE–HPLC–MS/MS (bottom). 1, simazine; 2, chlortoluron; 3, atrazine; 4, isoproturon; 5, diuron; 6, terbutylazine; 7, prometryne; 8, alachlor; 9, chlorfenvinphos; 10, chlorpyrifos/trifluralin.

Table 6 contains validation data of the on-line SPE–HPLC–APCI–MS/MS method, including recoveries, RSD values (c = 50 ng/l; n = 3), squared correlation coefficients of linear calibration plots and limits of detection. All data were determined for 10-ml samples of spiked river water. While nine of 11 pesticides included in this study give satisfying recoveries (83–94%), two more lipophilic pesticides are recovered at significantly lowered rates (chlorpyrifos 56%; trifluralin 42%) and give significantly higher standard deviations. This is due to the fact, that these two species tend to be retained on inner surfaces of the instrumental system, mainly inside the housing of the isocratic pump, which delivers the water sample to the TFC column.

Except for trifluralin, LODs are far below 100 ng/l, the concentration limit scheduled for single pollutants in the German Drinking Water Regulation (TVO) [48]. LODs typically vary from 0.4 ng/l (terbutylazine) to 13 ng/ml (chlorpyrifos). These differences are mainly due to differing ionization efficiencies of the individual pesticides. Trifluralin is by far the compound with the lowest ionization yield

Table 6
Validation data of the on-line SPE–HPLC–APCI–MS/MS method (10-ml samples of river water)

	Recovery (%) (c = 100 ng/l)	$RSD_{n=3}$ (%) (c = 50 ng/l)	Linearity (R^2) (c = 5–500 ng/l)	Limit of detection (LOD) [ng/l]
Isoproturon	89	1.5	0.9961	0.7
Diuron	93	2.8	0.9971	1.4
Chlortoluron	94	1.6	0.9976	0.8
Simazine	85	2.7	0.9994	1.6
Atrazine	84	0.9	0.9986	1.0
Terbutylazine	84	0.7	0.9990	0.4
Prometryne	83	2.4	0.9990	0.5
Chlorfenvinphos	83	3.4	0.9990	2.0
Chlorpyrifos	56	7.0	0.9997	13
Alachlor	88	1.0	0.9997	2.0
Trifluralin[a]	42	18	0.9995	283

[a] Five times of the concentration given in the table caption.

under both APCI and Turboionspray conditions, resulting in significantly higher LOD (280 ng/l).

3.3. Investigation of matrix effects

A set of experiments was performed to evaluate the robustness of the instrumental setup against matrix effects. Special attention was paid to the influence of the water matrix to the MS signal intensity, which is known to be a serious problem in API–MS [23,49,50]. The experiments were based on comparative analyses of ultraclean and real water samples spiked with identical amounts of pesticide standards. The slopes of calibration functions obtained from 10 ml of two types of surface water (river water, lake water) were compared to those resulting from ultraclean water (set to 100%) (Table 7). For both surface water samples, slopes of calibration functions were found to be in the range between 100 and 120% compared to ultraclean water. As an exception, chlorpyrifos showed more significant signal amplification in river water (145%). The extent of matrix effects to the MS signal intensity of the individual compounds varies depending on the type of surface water investigated (for instance: isoproturon 120% in river water, but 103% in lake water). These variations may be due to differing amounts and chemical nature of typical matrix compounds present in individual surface water sources, for instance humic substances. However, the on-line SPE–HPLC system described here obviously provides selectivity enough to reduce these effects to an acceptable minimum. Thus, external calibration using spiked ultraclean water should be applicable to the analysis of drinking and surface water without significant loss of accuracy of the results.

3.4. Application of the method to real water samples

The newly developed system was applied to the analysis of several drinking and surface water samples taken in and around the area of the city of Leipzig (Saxony, Germany). The measuring results are given in Table 8. A typical chromatogram obtained from on-line SPE–HPLC–APCI–MS/MS of a 10-ml sample of River Parthe (Leipzig, Saxony,

Table 7
Relative slopes (compared to ultraclean water, 100%) of calibration functions obtained from spiked river and lake water (enriched water volume: 10 ml)

Compound	Relative slope of calibration functions (compared to ultraclean water, 100%)	
	River water	Lake water
Isoproturon	120	103
Diuron	121	108
Chlortoluron	117	106
Simazine	103	102
Atrazine	105	102
Terbutylazine	110	101
Prometryne	109	107
Chlorfenvinphos	120	104
Chlorpyrifos	145	103
Alachlor	108	102
Trifluralin	n.d.	118

Table 8
Concentrations of pesticides determined in drinking and surface water samples taken at various sites in and around the city of Leipzig

	Concentration [ng/l]					
	Drinking water (Feb. 13, 2001) Standard addition	Drinking water (Feb. 15, 2001) Standard addition	River Parthe (Feb. 10, 2001)		Lake Cospuden (Feb. 21, 2001)	
			External calibration	Standard addition	External calibration	Standard addition
Isoproturon	0.9	0.9	3.9	3.9	1.7	1.7
Diuron	1.9	2.8	8.7	8.8	1.2	1.1
Chlortoluron	n.d.[a]	<0.5[b]	0.8	0.8	<0.8[b]	<0.8[b]
Simazine	10.5	15.9	10.8	11.6	2.9	2.9
Atrazine	4	8.9	4.7	5.1	0.9	0.9
Terbutylazine	n.d.[a]	0.7	0.8	0.9	1.7	1.6
Prometryne	n.d.[a]	<0.3[b]	3.7	3.6	<0.5[b]	<0.5[b]
Chlorfenvinphos	<1.1[b]	<1.1[b]	2.2	1.8	<2.0[b]	<2.0[b]

[a] n.d.: not determined.
[b] Below LOD.

Germany) is shown in Fig. 4 (TIC and extracted MRM traces of eight pesticides detected). For two of the samples (River Parthe and Lake Cospuden), both external calibration and standard addition were employed. The results are in very good correspondence with each other, which is a further confirmation of minimized matrix effects. For three compounds (chlorpyrifos, alachlor, trifluralin), the concentrations were below LOD in all four samples analyzed. All other pesticides were present at a concentration level far below the limit scheduled in the German Drinking Water Regulation for single pesticides (100 ng/l) [48]. Thus, the method is able to monitor the compliance of such limits, at least for ten of the pesticides investigated in this study. Due to its significantly higher LOD, monitoring of trifluralin requires a significantly higher sampling volume, increased by a factor of three or four.

4. Conclusions

We have introduced here a new integrated on-line SPE–HPLC–MS/MS system for rapid trace determination of priority pesticides in drinking and surface water. The system implies TFC columns for use as SPE cartridges for fast on-line enrichment. TFC columns packed with organic polymers (Oasis HLB, Cyclone) or graphitized carbon (Hypercarb) turned out to be highly capable for enrichment of trace pesticides from water volumes up to 50 ml. Complete removal of the enriched pesticides from the TFC columns requires little volumes of organic eluent (200–400 μl methanol at 200 μl/min) underlining the capabilities of TFC columns for on-line SPE applications. Re-mixing of the SPE eluate allows efficient band focussing and, thus, optimum exploitation of the separation power provided by a short monolithic high-speed LC column used in combination with highly selective tandem-MS detection. In the result, the system described here enables on-line SPE–HPLC–MS/MS multicomponent analyses in less than 14 min. Typical LODs determined for 10 ml samples of surface water are in the range between 0.4 ng/l (terbutylazine) and 13 ng/l (chlorpyrifos). Trifluralin gives significantly higher LOD (280 ng/l) due to less efficient ionization in both ESI and APCI. Negative effects of the water matrix to the method performance are reduced to an acceptable minimum. First application of the method to the analysis of drinking and surface water samples illustrated its suitability for routine pesticide monitoring, for instance with regard to the compliance of concentration limits regulated by law.

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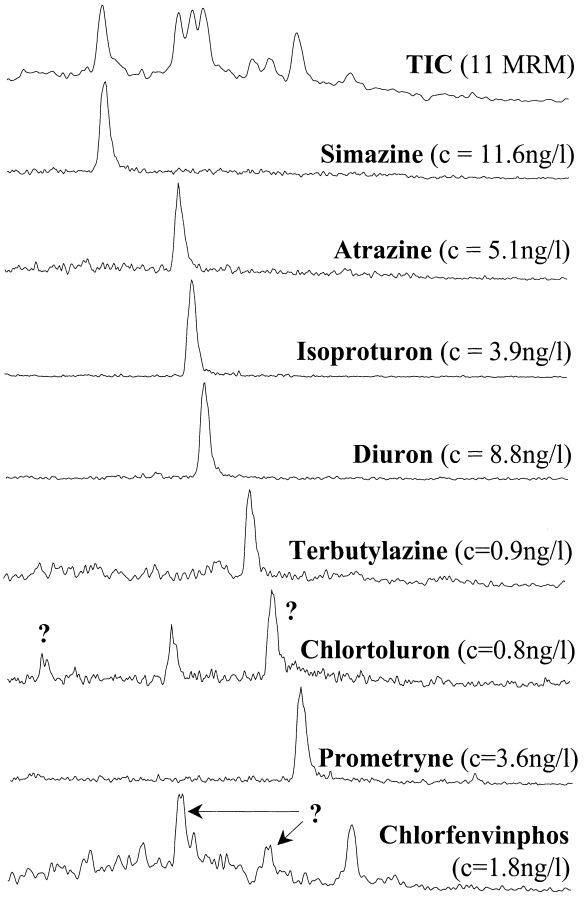

Fig. 4. Total ion chromatogram and extracted MRM traces of eight pesticides obtained from on-line SPE–HPLC–APCI–MS/MS analysis of a 10-ml sample of river water (River Parthe, February 10, 2001, Leipzig, Saxony, Germany).

for providing Cohesive TFC columns Turbo C_{18}, Phenyl and Cyclone. We are grateful to Merck (Darmstadt, Germany) for providing a Chromolith SpeedROD RP-18e column.

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