Journal of Chromatography A, 654 (1993) 65–77
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 424

Coupling of ion-pair liquid chromatography and thermospray mass spectrometry via phase-system switching with a polymeric trapping column

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(First received April 15th, 1993; revised manuscript received July 12th, 1993)

ABSTRACT

A trapping column packed with polymeric material (PLRP-S) was used to couple ion-pair liquid chromatography on-line with thermospray mass spectrometry by phase-system switching. Phase-system switching was used to remove non-volatiles from the eluent before it entered the mass spectrometer. The total analytical system was optimized for chlorinated phenoxy acids, which were separated as ion pairs with cetyltrimethylammonium bromide as ion-pair reagent. Parameters such as percentage of modifier and concentrations of ion-pairing reagent and buffer affected the sorption of the ion-pair on the trapping column. Desorption was effected by protonation of the acids with, *e.g.*, trifluoroacetic acid or an ion-pair switch with, *e.g.*, ammonium formate. The influence of pH and modifier concentration during desorption was examined. In addition to target-compound analysis, group-selective analysis was also demonstrated. As an example, the system was used to identify chlorinated phenoxy acids in river Rhine water.

INTRODUCTION

In recent years, the on-line combination of liquid chromatography and mass spectrometry (LC–MS) has developed rapidly [1–3]. The currently popular thermospray (TSP) and particle beam (PB) interfaces are now available from several manufacturers. The choice of the interface depends strongly on the characteristics of the analytes and the information desired; it also limits the range of LC methods available, because all current interfaces have problems with non-volatiles, such as buffer salts, ion-pairing reagents and complexing agents.

So far, research in LC–MS has mainly focused on interface design and MS compatibility. Today, especially with LC–TSP-MS [3], much attention is devoted to the LC part of the system and to topics such as the introduction of on-line preconcentration techniques [4,5] or derivatization procedures [6,7]. The incompatibility of complex LC systems with MS can be solved by eliminating the non-volatiles from the LC eluent [8–25]. In addition to the substitution of volatile additives for non-volatiles [21,23–25], which may affect the selectivity of the LC system, post-column removal of the non-volatiles should be considered. Suppressor membranes [17–20] and postcolumn segmented ion-pair extraction, with subsequent phase separation [15,16,22], have been reported. Several workers have reported the use of valve-switching techniques, *i.e.*, so-called phase-system switching (PSS), to overcome some of the problems [8–14]. Via heart cutting, the analyte is transferred from the LC column to a trapping column (TC), placed at the

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0021-9673/93/$06.00

LC column outlet. Here the analyte is retained and, after removal of the non-volatile constituents, it is desorbed to the MS system using a solvent compatible with the interface and the mass spectrometer. PSS can also be used to change the flow-rate or the modifier concentration or to change the complete solvent system, *e.g.*, from reversed-phase to normal-phase [26]. Flow-rate and modifier concentration certainly affect peak shape after desorption [8,27]. Especially with a mass-flow sensitive detector, *i.e.*, a mass spectrometer, these parameters can improve analyte detectability.

So far, PSS using hydrophobic alkyl-bonded silica or polymeric packing materials as the stationary phase in the trapping column has been used to couple LC with mobile phases containing non-volatile buffers or using eluents with extremely high or low modifier contents to both TSP [10], moving-belt [8,9] and continuous-flow fast atom bombardment (CF-FAB) [11,14] interfaces. In previous work [27], we used an ion-exchange trapping column in LC–PSS–TSP-MS. A benzenesulphonic acid-type cation-exchange column was used to determine quaternary ammonium compounds separated from nasal drops by RPLC with a mobile phase containing 0.1 *M* phosphate.

Nowadays, there is distinct interest in using LC–MS for the determination of ionogenic compounds [1,2,28,29]. Ion-pair LC (IPLC) is a technique used to separate ionogenic compounds that has found many applications in recent years [30]. In IPLC, non-volatile buffers and ion-pairing agents are generally used, which makes on-line coupling with MS virtually impossible. However, on the basis of the above experience, there appears to be a reasonable chance that PSS can help solve this problem.

In this paper, the use of a trapping column packed with a hydrophobic polymer for the on-line coupling of an IPLC procedure involving the use of a non-volatile ion-pairing agent and a buffer with TSP-MS via PSS will be reported. Chlorinated phenoxy acids (pK_a 2.5–3.0) [31] were used as test compounds. They are normally determined by GC–MS after derivatization [32,33], but recently LC [31,34–36] and LC–MS methods [16,23,28,29,37] have been reported. At high pH and with a suitable counter ion, these acids can be separated by IPLC. The hydrophobicity of the trapping column packing should enable one to trap the ion pairs quantitatively and eliminate the non-volatiles present in the LC eluent. After flushing of the trapping column, the compounds of interest can be desorbed and, by using valve-switching techniques, directed to the MS system. The system was studied with regard to the LC modifier concentration and the nature and concentrations of the buffer and ion-pair reagent. The nature of the desorption solution, *i.e.*, the displacer and its concentration, modifier concentration and pH, was also studied.

EXPERIMENTAL

Chemicals

2-Methoxy-4-chlorophenoxyacetic acid (mecoprop), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4-dichlorophenoxypropionic acid (2,4-DP), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4,5-trichlorophenoxypropionic acid (Silvex), all of 98% purity, were obtained from Sandoz (Basle, Switzerland). Acetonitrile (gradient grade), ammonia solution (25%), formic acid (98%), potassium monohydrogenphosphate and potassium dihydrogenphosphate were obtained from J.T. Baker (Deventer, Netherlands). Ammonium formate (AmFo) solutions were prepared from concentrated formic acid, which was diluted with water and adjusted to the desired pH with ammonia solution. Doubly distilled, demineralized water was used throughout. Tetrahexylammonium bromide ($THxA^+B^-$) and tetraheptylammonium bromide ($THpA^+B^-$) were obtained from Aldrich (Milwaukee, WI, USA), tetramethylammonium bromide (TMA^+B^-) and cetyltrimethylammonium bromide (CTA^+B^-) from Baker and tetrabutylammonium bromide (TBA^+B^-) and iodide (TBA^+I^-) and tetrapentylammonium iodide (TPA^+I^-) from Eastman Kodak (Rochester, NY, USA). Trifluoroacetic acid was purchased from Merck (Darmstadt, Germany). All chemicals were of analytical-reagent grade and were used as received.

Water samples were collected at Lobith (river Rhine) and were obtained from RIZA (Lelystad, Netherlands). Prior to use, they were filtered

through a 0.45-μm BA membrane (Schleicher & Schüll, Dassel, Germany).

Liquid chromatography

The LC system (see Fig. 1) consisted of two Gilson (Villiers-le-Bel, France) Model 302 LC pumps (P1 and P2, flow-rates 0.5 and 1 ml/min, respectively) and one Applied Biosystems (Foster City, CA, USA) Model 400 LC pump (P3, flow-rate 1 ml/min), with laboratory-made pulse dampers. Four six-port valves (Model 7010) were obtained from Rheodyne (Berkeley, CA, USA). Valves 1 and 3 were equipped with a 20- and a 70-μl loop, respectively. A 150 mm × 4.6 mm I.D. analytical column (AC) packed with 5-μm, 90-Å cyano-bonded silica (Chemie Uetikon, Eke, Belgium) and a trapping column consisting of a preconcentration column holder (Chrompack, Middelburg, Netherlands) containing a 10 mm × 4.0 mm I.D. precolumn manually filled with 15–25-μm, 100-Å PLRP-S copolymer material (Polymer Labs, Church Stretton, Shropshire, UK) were used. A Perkin-Elmer (Norwalk, CT, USA) LC-75 UV detector operated at 280 nm was used together with a Kipp & Zonen (Delft, Netherlands) BD 40 recorder.

Mass spectrometry

A Finnigan Model 4500 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA) which was adapted for LC–TSP-MS with a Finnigan TSP interface was used. A typical source temperature was 200°C. The vaporizer temperature was set between 90 and 120°C. The discharge voltage was set at 1000 V; at higher voltages no gain in sensitivity was observed. The repeller voltage was optimized with every new eluent used and was set at a voltage where the background intensity was stable and not all of the clusters were yet dissociated. The voltage generally was in the range −100 to −150 V. All three modes of operation (filament-off, filament-on and discharge ionization) were used. Normally only the negative-ion (NI) mode was used. In addition to full-scan (100–550 u/s) data, selected ion monitoring (SIM) data were also acquired. SIM data were recorded on two ions per compound, *i.e.*, the $[M-H]^-$ and the $[M+HCOO]^-$ ions. The ions were scanned with a mass window of 4 u, *i.e.*, from $[X-1]^-$ up to $[X+2]^-$ (X being the $[M-H]^-$ or the $[M+HCOO]^-$ ion). This was done to determine the noise level ($[X-1]^-$ ion) and to check the isotope ratio ($[X+2]^-$ ion). Typical sampling times were 0.1 s per ion window (4 u wide).

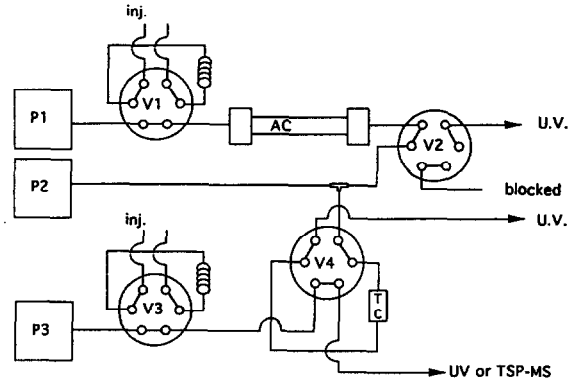

Fig. 1. Set-up of the analytical system used to study sorption, desorption and on-line IPLC–PSS–UV or IPLC–PSS–TSP-MS. P1, P2 and P3, LC pumps delivering a flow of 0.5 ml/min of mobile phase (P1) or 1 ml/min of water (P2 and P3); AC, analytical column; TC, trapping column; V1 and V3, six-port switching valves with 20- and 70-μl loops, respectively; V2 and V4, six-port switching valves.

PSS procedure

For PSS the analytical system depicted in Fig. 1 was used. During optimization of the PSS parameters (sorption, cleaning and desorption) the analytical column was removed. Table I shows the positions of the four valves during the various stages of the optimization procedure performed with flow-injection analysis (FIA). A brief explanation of the procedure is given below. With on-line LC–PSS–TSP-MS, *i.e.*, with the analytical column inserted, the time at which conditioning and sorption (steps 1 and 3, respectively) started depended on the retention time of the analyte; injection of the sample was at time $t = 0$.

In order to condition the trapping column with CTA^+B^- (step 1) after the previous run, valve V2 is switched at $t = 0$. After 2.5 min the analyte is injected into the carrier stream via valve V1 (step 2). After sorption of the analyte on the trapping column (step 3), valve V2 is switched in order to flush the capillaries and the trapping column with water (step 4). By switching valve

TABLE I

VALVE-SWITCHING SCHEDULE FOR THE PSS PROCEDURE

Step	Event	Time (min)	Valve position[a]			
			V1	V2	V3	V4
1	Conditioning of TC with LC eluent	0–2.5	B	B	B	B
2	Injection of analyte	2.5	A	B	B	B
3	Sorption of analyte on TC	2.5–5	A	B	B	B
4	Flushing of capillaries and TC with water	5–7.5	A	A	B	B
5	Changing flow direction of water through TC and making connection with UV or TSP-MS system	7.5–8	A	A	B	A
6	Desorption of analyte by triplicate injection of desorption solvent	8–10	A	A	A	A
7	Cleaning of TC by injection of several loop volumes of cleaning solvent	10–12	A	A	A	A

[a] A =  ; B = 

V4 (step 5) after 7.5 min, the direction of the flow through the trapping column is reversed, for backflush desorption, and the trapping column is switched on-line with the UV detector or the TSP-MS system. After another 30 s of flushing the trapping column in order to remove residual buffer, desorption is achieved by multiple injections of 70 μl of the desorption solvent, via valve V3, in the carrier stream, water (step 6). In order to clean the column completely, valve V3 is used for the injection of several loop volumes of cleaning solvent (step 7). The composition of the sorption, desorption and cleaning solvents will be discussed below.

RESULTS AND DISCUSSION

IPLC is not directly compatible with TSP-MS, because of the presence of non-volatiles in the eluent. PSS [3,8,9] seems to be a simple and straightforward solution to this problem, but designing an IPLC–PSS–TSP-MS system made us realise that many parameters are involved and that this optimization may well lead to mutually conflicting results (see Table II). For example, efficient trapping of the ion pairs on the trapping column requires narrow and well separated peaks eluting from the IPLC column, which have a high capacity factor on the trapping column. However, in IPLC such peaks are obtained after rapid analysis, *i.e.*, using a low concentration of counter ion, a high percentage of modifier and a low ionic strength. Adequate trapping on the trapping column, on the other hand, requires a solution with a low percentage of modifier, a high concentration of counter ion and a high ionic strength. Therefore, in order to optimize the total IPLC–PSS–TSP-MS system, the IPLC separation of the analytes and the PSS procedure using the trapping column were studied separately, taking into account the criteria set by the other steps in the procedure. Finally, the total system was assembled and its overall performance evaluated.

Ion-pair chromatography of phenoxy acids

Chlorinated phenoxy acids can be separated by means of IPLC with various counter ions such as TMA^+, TBA^+ and CTA^+ [31,34–36]. In our study, a reagent was desired that forms an ion pair with a high capacity factor on the trapping column (see Table II), but a much smaller capacity factor on the analytical column (see above). An analytical column containing a

TABLE II

REQUIREMENTS AND PARAMETERS INVOLVED IN DESIGNING AND OPTIMIZING AN IPLC–PSS–TSP-MS SYSTEM

Part of system	Requirements	Aspects
LC	Analyte in ionized form	Buffer mobile phase at $pH > pK_a + 2$
	Counter ion	Counter ion $R_4N^+X^-$ ($R = C_1$–C_{16})
	Stationary phase	RP-type material
Trapping	Narrow peaks (low k' for ion pairs in LC)	High % modifier, low counter ion concentration and low ionic strength
	Preconditioned TC	Loading time and concentration of counter ion
	High k' for ion pairs on TC	Low % modifier, high counter ion concentration, high ionic strength and hydrophobic stationary phase
Flushing	High k' for ion pairs on TC	No or low % modifier, hydrophobic stationary phase
Desorption	Low k' for analyte on TC	$pH \leq pK_a - 2$, high displacer concentration and high % modifier
	Extremely high k' for counter ion on TC	High displacer concentration and low % modifier
Detection with SP-MS	TSP-MS-compatible solvent	No counter ion and low % modifier
	Volatile additives	Low additives concentration

cyano-bonded silica and a trapping column packed with a polymer such as PLRP-S provide a large difference in hydrophobicity. With this combination, different tetraalkylammonium salts (concentration 1 m*M*), with alkyl groups from methyl up to hexyl, and CTA^+B^- were tested as ion-pair agents. As expected, the higher the hydrophobicity of the ion-pair agent and the lower the modifier percentage, the higher is the retention. CTA^+, a popular cationic counter ion in IPLC [38,39], appears to be a good choice in the present instance also. However, it will certainly clog the vaporizer of the TSP-MS unit unless it is completely removed beforehand.

With 10 m*M* sodium phosphate buffer (pH 7)–acetonitrile (90:10, v/v) as the LC eluent, the concentration of CTA^+B^- was varied from 0.1 to 10 m*M*. Although the retention times of the phenoxy acids increased rapidly with increasing CTA^+B^- concentration, *i.e.*, up to 65 min [29], the resolution increased only slightly. A compromise between analysis time and resolution was found at a CTA^+B^- concentration of 1 m*M*, resulting in two sets of peaks. Fig. 2 shows the IPLC–UV traces for each of the compounds with an aqueous 1 m*M* CTA^+B^-, 10 m*M* sodium phosphate (pH 7)–acetonitrile (70:30, v/v) eluent. One group consists of mecoprop, 2,4-D and 2,4-DP ($k' = 1.8$–2.2) and the other of 2,4,5-T and 2,4,5-TP ($k' = 3.3$–3.4). In fact, the in-

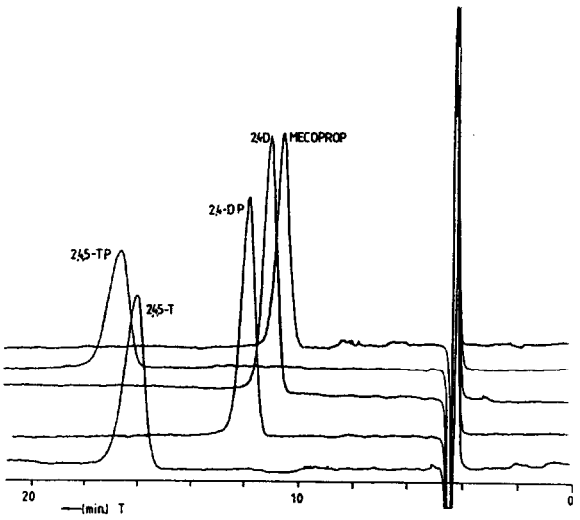

Fig. 2. IPLC–UV traces for the five test compounds (mecoprop, 2,4-D, 2,4-DP, 2,4,5-T and 2,4,5-TP; concentration 10^{-5} *M*) injected separately on a cyano-bonded silica (5-μm, 90-Å) column. Eluent, water (1 m*M* CTA^+B^-, 10 m*M* sodium phosphate buffer, pH 7)–acetonitrile (70:30, v/v); injection volume, 20 μl; flow-rate, 0.5 ml/min; UV detection at 280 nm.

complete separation is interesting, because simultaneous trapping and desorption of several compounds at the same time can now be studied.

PSS

Using a PSS procedure for on-line IPLC–TSP-MS is complicated (see Table II). For example, in order to have sufficient retention, the capacity factors of the test compounds on the trapping column should at least be equal to $(6\sigma_{v,AC}/V_{0,TC}) - 1$ ($\sigma_{v,AC}$ is the peak standard deviation of the analytical column and $V_{0,TC}$ the void volume of the trapping column [3,9]). Further, if instead of selective packing materials such as an anion or a cation exchanger [27] a non-selective stationary phase is used, one should be aware of the fact that, in addition to the counter ion–analyte ion pair, also the ion-pair reagent itself will be trapped. Third, a solvent must be found that effects rapid desorption of the phenoxy acids from the trapping column and is suitable for direct introduction into the TSP-MS system. Finally, a cleaning step will be necessary, especially after selective analyte desorption leaving the ion-pairing reagent on the trapping column.

Several parameters will influence the recovery of the analytes during each of the steps in the PSS procedure. Therefore, each step was optimized separately. The analyte recovery was determined as the ratio of the summed peak areas (TSP-MS) or peak heights (UV) of the desorbed compound after multiple desorptions (see below) and the peak area or height in FIA.

Sorption. In order to check the capacity of the 10 mm × 3.0 mm I.D. trapping column packed with 15–25-μm, 100-Å PLRP-S, breakthrough volumes (V_b) of 2,4-D and 2,4,5-T (concentration 10^{-4} *M*), were determined using various eluents. Without CTA^+B^- added to the eluent (water), the phenoxy acids showed breakthrough volumes of less than 1 ml. Adding CTA^+B^- and adjusting the pH to 7, to ensure dissociation of the acids, gave a marked increase to values of over 100 ml (Table III). The addition of acetonitrile, of course, caused the breakthrough volumes to decrease rapidly. Quantitative trapping was still possible, however, with an eluent containing *ca.* 20% of acetonitrile. Although, theoretically, breakthrough volumes of 1.5–2.5 ml are large enough to trap the phenoxy acids, in practice they are too small, especially when flushing of the trapping column, to remove non-volatiles, is considered. In other words, the acetonitrile concentration should be lower than 15%.

TABLE III

BREAKTHROUGH VOLUMES OF 2,4-D AND 2,4,5-T (10^{-4} *M*) ON A TC (PLRP-S) IN WATER (1 m*M* CTA^+B^-, 10 m*M* PHOSPHATE BUFFER, pH 7) CONTAINING 0–30% OF ACETONITRILE

Concentration of acetonitrile (%, v/v)	Breakthrough volume (ml)	
	2,4-D	2,4,5-T
0	>100	>100
10	50	75
15	4	5
20	1.5	2.5
25	0.5	1.5
30	–	0.5

Subsequently, preloading of the trapping column with CTA^+ was examined to achieve higher breakthrough volumes, which could result in higher allowable modifier concentrations during IPLC. Loading the trapping column with CTA^+B^- by passing 1 ml of water (1 m*M* CTA^+B^-, 10 m*M* phosphate buffer, pH 7)–acetonitrile (75:25, v/v) over it at 1 ml/min caused a 2–3-fold increase of the breakthrough volumes of the test compounds compared with a non-loaded trapping column. No further gain was observed with longer preconditioning times.

In summary, when using water (1 m*M* CTA^+B^-, 10 m*M* phosphate buffer pH 7)–acetonitrile (85:15, v/v) as eluent and a CTA^+B^--loaded trapping column, the breakthrough volumes will be sufficiently large to prevent breakthrough of the phenoxy acids during sorption and flushing (see below).

Finally, the ionic strength of the sorption eluent was examined. Changing the concentration of the sodium phosphate buffer (pH 7) from 1 to 100 m*M* in an aqueous 1 m*M* CTA^+B^-–acetonitrile (85:15, v/v) solution gave no signifi-

cant changes in the breakthrough volumes. A 10 m*M* buffer was used in all further experiments.

Flushing. Prior to desorption of the analytes, the PSS unit, *i.e.*, the connecting capillaries and the trapping column, must be flushed to remove the ion-pairing reagent and the phosphate buffer in order to avoid contamination of the mass spectrometer. Avoiding contamination of the MS, the system was flushed with 2.5 ml of water at 1 ml/min, *i.e.*, 50 times the void volume. With UV detection no breakthrough of the test compounds was observed during this washing step.

Desorption. Once the counter ion–analyte ion pair has been trapped, desorption can be performed, either by desorption of the ion pair or by selective desorption of the analyte itself. If the ion pair is sufficiently volatile not to block the vaporizer, *e.g.*, with tetramethyl- and tetraethylammonium ion pairs [40], the intact ion pair can easily be desorbed to the mass spectrometer by increasing the modifier concentration. However, in the case of a non-volatile ion pair, *e.g.*, CTA^+ ion pairs, selective desorption should be used.

In this study, *i.e.*, using CTA^+–phenoxy acid ion pairs, desorption can be accomplished by breaking up the ion-pair via protonation of the acid or by forming a volatile ion pair with a volatile counter ion via an ion-pair switch.

First, the effect of the modifier concentration (range tested 0–80%) on the desorption of the ion-pairing reagent and analyte was studied to determine the maximum allowable modifier percentage that still retains the ion-pairing reagent on the trapping column. Quantitative desorption of the analyte could be accomplished with an aqueous desorption solvent containing at least 40% of acetonitrile. However, as was confirmed by the decrease in breakthrough volumes after desorption, the analyte is then desorbed as a CTA^+ ion pair. In fact, more than 90% of the CTA^+X^- (X being the phenoxy acid or bromide) is desorbed. In order to prevent CTA^+ desorption completely, the acetonitrile content of the desorption solvent should be less than 25%.

Desorption by protonation. Protonation of the phenoxy acids at low pH was studied by adding trifluoroacetic acid (TFA) to the desorption solvent. As the phenoxy acids have pK_a values of 2.5–3.0, quantitative protonation only occurs at $pH < 1$. However, at these pH values the PLRP-S material in the trapping column slowly dissolves. When, on the other hand, desorption is carried out by plugs of a strongly acidic solution, injected via a loop mounted on valve V3 (see Fig. 1), into the carrier stream, the polymer material will be less affected. In most instances, 3–5 plugs of aqueous TFA solution were injected. Varying the TFA concentration (range tested 0.06–6 *M*) showed a shallow optimum at 3 *M* TFA (pH 0.1; 80–90% recovery for 2,4-D and 2,4,5-T). However, even using small loop volumes (10–15-μl plugs), the trapping column deteriorated rapidly. After fifteen analyses the breakthrough volumes had dramatically decreased and the trapping column had to be repacked. Therefore, as an alternative, the TFA concentration was decreased (to 0.25 *M*) and simultaneously acetonitrile was added to the solution (25%, v/v is allowed; see above). Further, the loop volume was increased to 70 μl (no deterioration of the trapping column was observed), because this will decrease the number of plugs necessary for complete desorption. Three plugs of aqueous 0.25 *M* TFA (pH 0.85)–acetonitrile (75:25, v/v) resulted in 80–85% recoveries of the phenoxy acids without any desorption of CTA^+.

TFA also served as a displacer for the phenoxy acids from the trapped ion pair. This was concluded from the fact that the first cleaning step with water–acetonitrile (17:83, v/v) (see *Cleaning*) showed an intense peak (not observed when no TFA was used) when UV detection was used. This signal is obviously caused by desorption of the CTA^+–TFA^- ion pair.

Desorption by ion-pair switching. Several quaternary ammonium compounds [ammonium acetate, formate (AmFo) and oxalate, tetramethyl- and tetrabutylammonium salts such as iodides, fluorides, bromides, hydroxides and nitrates] dissolved in water and in water–acetonitrile were tested with regard to their desorption efficiency. Using 70-μl plugs of 0.1 *M* salt solutions in water–acetonitrile (75:25, v/v), AmFo and the ammonium bromide and iodide salts effected desorption of the phenoxy acids from the trapping column (30–60% for 2,4-D

with a single 70-μl plug, compared with less than 15% with 0.1 *M* salt solutions in pure water). The desorption efficiencies of the iodide salts were higher than those obtained with the corresponding bromides. This is due to the larger ionic radius of the iodides [41], resulting in better ion-pair formation.

As tetramethylammonium iodide is of low volatility and will clog the vaporizer, AmFo is the only compound suitable for desorption. Optimization of the AmFo concentration (range tested 0.05–2 *M*) in water–acetonitrile (75:25, v/v) resulted in desorption efficiencies of 80–90% for 2,4-D and 2,4,5-T (three 70-μl injections; 60% after the first injection) with 0.5 *M* AmFo. At higher AmFo concentrations, the analyte recovery did not increase any further; in fact, it even decreased slightly, possibly because of salting-out of the analyte.

These first experiments were carried out at pH 5. Optimization with respect to the pH was performed with 2,4-D as test solute. Fig. 3 indicates that analyte recovery is essentially quantitative at pH 5.7–7.6 with one injection of 70 μl (the relatively good result at pH 2.6 can be attributed to protonation of the phenoxy acid). This can be explained by the formation of a volatile ion pair between the ammonium ions, present in large excess, and the deprotonated phenoxy acid, with the formate ion occupying the vacant position on the CTA^+-loaded stationary phase. Similar high recoveries (90–100%) were obtained for all other test solutes. Measuring the breakthrough volume of 2,4-D directly after desorption, with water (0.5 *M* AmFo, pH 7.6)–acetonitrile (75:25, v/v), confirmed that no CTA^+X^- (X being the phenoxy acid or bromide) is desorbed.

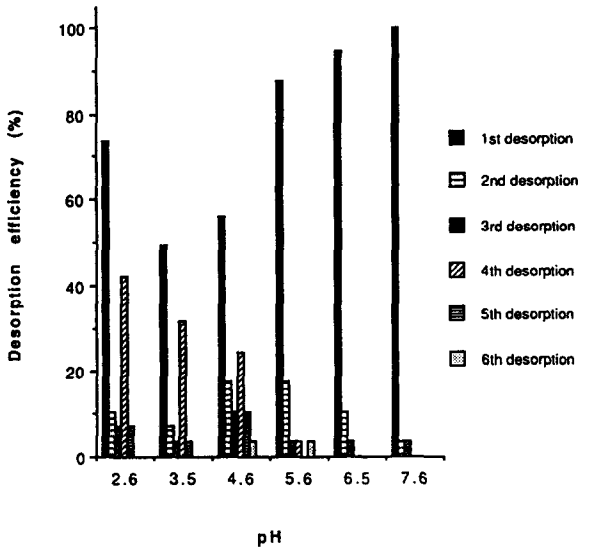

Fig. 3. Influence of the pH of the desorption solvent [70-μl plugs of aqueous 0.5 *M* AmFo–acetonitrile (75:25, v/v)] injected into the carrier stream (water) on the desorption efficiency of 2,4-D ($n = 5$, R.S.D. 10%). Analyte concentration, $5 \cdot 10^{-6}$ *M*; injection volume, 20 μl; flow-rate, 1 ml/min; UV detection at 280 nm.

Cleaning. After each run, the trapping column was cleaned with several loop volumes (70 μl) of water–acetonitrile (17:83, v/v). It became clear, by measuring the breakthrough volumes of 2,4-D on the trapping column directly after this cleaning step, that five loop volumes were necessary to clean the trapping column effectively (2–3-fold reduction of the breakthrough volume; see above).

IPLC–PSS–UV coupling

Separation and trapping of the phenoxy acids require different percentages of acetonitrile in the LC eluent, *viz.*, *ca.* 30% *versus* 10–15%. By using a flow-rate of 0.5 ml/min for the LC eluent and postcolumn addition of water at 1 ml/min, both criteria can be met. Although the reduced flow-rate increased the retention times, the separation of the phenoxy acids was still achieved within 20 min (see Fig. 2). In addition, the preloading time for the trapping column had to be adjusted, *i.e.*, up to 2.5 min, because of the lower concentration of CTA^+B^- (0.33 m*M* instead of 1 m*M*) in the mobile phase entering the trapping column.

Desorption was carried out with both water (0.25 *M* TFA, pH 0.85)–acetonitrile (75:25, v/v), and water (0.5 *M* AmFo, pH 7.6)–acetonitrile (75:25, v/v). After performing the whole procedure, *i.e.*, separation, trapping, flushing and desorption, the summed peak areas of three desorptions were compared with the peak areas measured by the UV detector at the outlet of the analytical column. The recoveries were 80–95% for all analytes with both desorption solutions. The method showed linear calibration graphs,

i.e., $r^2 = 0.981–0.993$, over two orders of magnitude of concentration (range 10^{-4}–10^{-6} *M*). For higher concentrations, the recovery decreased because of incomplete desorption, *i.e.*, a too low concentration of TFA or AmFo, or breakthrough of the compounds. Increasing the concentration of TFA or AmFo will cause problems with the PLRP-S material (TFA) or faster contamination of the ion source (AmFo). Under the present conditions, the limits of detection (LODs) of the phenoxy acids ranged from $5 \cdot 10^{-7}$ to $1 \cdot 10^{-6}$ *M* (20 μl injected).

During desorption of the test compounds from the trapping column, peak compression can be obtained or, in other words, the concentration of the analyte in the peak maximum, C_{max}, can be increased [9,13]. Increasing the flow-rate has the same effect (square root [13]), but the main gain stems from a proper choice of the trapping column material and the solvents used during sorption and desorption. They determine the capacity factors during trapping (k'_{in}) and desorption (k'_{out}). The above can be expressed by the following equation, which was adapted from Verhey [13]:

$$C_{max} = \frac{m}{(1 + k'_{out})} \sqrt{\frac{(1 + k'_{in})}{(2\pi A_{TC} H_{TC} 3\sigma_{AC})}}$$

where m is the mass of analyte injected, A_{TC} and H_{TC} the column area and plate height of the trapping column, respectively, and σ_{AC} the peak standard deviation.

The capacity factors, k'_{in}, can easily be increased by adding water to the column effluent. As the breakthrough volume is directly related to the capacity factor, the attainable peak compression can be roughly estimated from the data given in Table III. Decreasing the acetonitrile content from 30 to 10% causes an increase in C_{max} of $50^{0.5}$ and $75^{0.5}$ for 2,4-D and 2,4,5-T, respectively. Peak compression factors, determined by comparing peak widths directly after elution from the analytical column and after desorption, were 6.5 for mecoprop, 2,4-D and 2,4-DP and 8.5 for the later eluting 2,4,5-T and 2,4,5-TP. These data agree well with the predicted values, especially when the decrease in flow-rate (see above) and the extra band broadening in the connecting capillaries and valves are considered.

IPLC–PSS–TSP-MS coupling

Analyte detectability in LC–TSP-MS is influenced by factors such as ionization technique, percentage of modifier, vaporizer temperature and flow-rate stability. As regards the last aspect, Walhagen *et al.* [10] observed a background signal at all masses during desorption of the analyte which originated from the valve switching necessary to resume liquid introduction into the interface. We circumvented this problem by using an extra valve (valve V3 in Fig. 1). The TSP-MS system was now operated at a continuous flow of 1 ml/min of water, which led to a stable signal. Because water is used, desorption will not take place after on-line switching of the trapping column and the TSP-MS system. Desorption is carried out by injection(s) of (a) desorption plug(s) into the carrier stream.

When aqueous 0.25 *M* TFA (pH 0.85)–acetonitrile (75:25, v/v) was used for desorption of the analytes, many large signals of TFA-containing clusters (up to m/z 500) were observed in the mass spectrum. Because of the high electron-capturing ability of TFA, the ionization efficiency of the analytes was negatively influenced, and clusters between TFA and the phenoxy acids, expected on the basis of the gas-phase acidities [42], were not observed. Further experiments with this desorbent were considered to be superfluous.

With aqueous 0.5 *M* AmFo (pH 7.6)–acetonitrile (75:25, v/v) as desorbent, a high background signal was also observed during the first desorption. The mass spectrum was dominated by bromide ions and clusters containing bromide ions which originate from the CTA^+B^- used as ion-pairing agent. As these ions also influence the ionization efficiency, they must be removed before desorption of the analyte. A single clean-up desorption with aqueous 0.25 *M* AmFo (pH 5.2) was found to be sufficient to remove the bromide-containing ions from the trapping column. This is shown in Fig. 4, where the ion current traces (full-scan acquisition) of the deprotonated molecular ion and the formate adduct of mecoprop and one of the bromide-

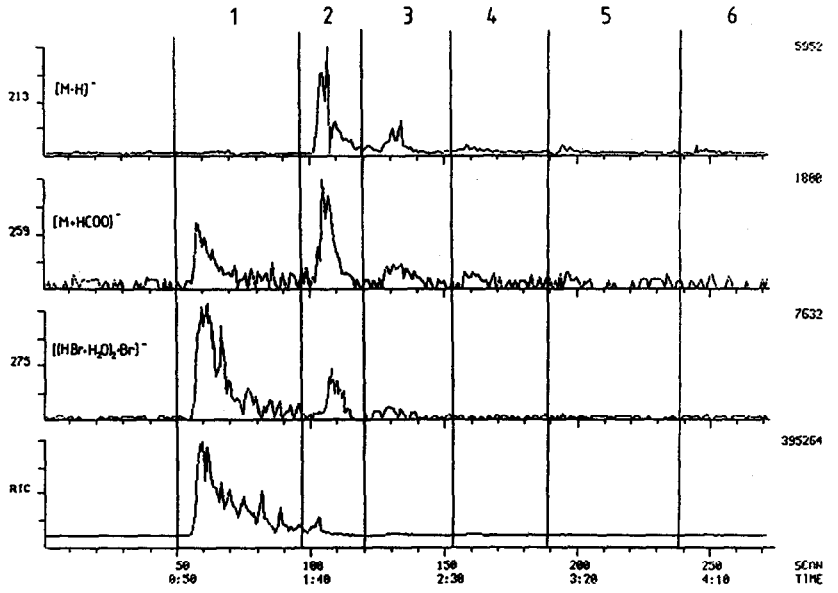

Fig. 4. Reconstructed ion current of the IPLC–PSS–TSP-MS (full-scan) analysis of mecoprop (concentration 10^{-5} *M*). Ions at *m/z* 213, 259 and 275 correspond to $[M-H]^-$, $[M+HCOO]^-$ and $[(HBr \cdot H_2O)_2 \cdot Br]^-$, respectively. Desorption No. 1: cleaning of the TC to remove the bromide with 70 μl of 250 m*M* AmFo (pH 5.2) in water. Analyte desorptions 2–6: five injections of 70 μl of aqueous 0.5 *M* AmFo (pH 7.6)–acetonitrile (75:25, v/v) into a carrier stream of water (flow-rate 1 ml/min). Time in min:s.

containing ions at *m/z* 213, 259 and 275, respectively, are shown. The clean-up desorption (desorption No. 1) clearly releases most of the bromide-containing ions. Next, the analyte is desorbed using injections of water (0.5 *M* AmFo, pH 7.6)–acetonitrile (75:25, v/v) into the carrier stream (water) (desorptions 2–6), as was described in the previous section. It is clear that analyte desorption is complete (>95%) after three desorptions (desorptions 2–4). A further optimization of the loop volume, *i.e.*, 70 μl (valve 3; Fig. 1), to reduce the number of desorptions was not carried out because otherwise it would have been necessary to optimize the AmFo concentration and the percentage of acetonitrile again.

Finally, the signal at *m/z* 259 suggests analyte release during the clean-up desorption (desorption 1); however, full-scan mass spectra showed that this signal can be attributed to the isotope peak of the $[(HBr)_2 \cdot H_2O \cdot Br]^-$ ion at *m/z* 257. Using the positive-ion mode it was confirmed that no CTA^+ desorption took place during the above sequence. This is in agreement with the results obtained above.

Fig. 5 shows the full-scan mass spectra of 10^{-5} *M* solutions of mecoprop and 2,4,5-TP after on-line IPLC–PSS–TSP-MS, using discharge ionization in the negative-ion mode. The $[M-H]^-$ ion was the base peak for all test compounds. The $[M+HCOO]^-$ ion was present in all instances with intensities up to 100%. Although the $[M+HCOO]^-$ ion was more abundant with filament-off and filament-on ionization, higher signal-to-noise ratios were obtained with discharge ionization. Therefore, discharge ionization was used in all further experiments.

Using the complete on-line system, good-quality full-scan mass spectra were obtained for the test compounds at levels down to 10 ng injected into the system, *i.e.*, 20 μl of $2 \cdot 10^{-6}$ *M* solutions. With SIM, on two ions per compound, the LODs were 0.1–1 ng for all five test compounds. Linear calibration graphs ($r^2 = 0.925$–0.973, seven data points, $n = 5$) were obtained over three orders of magnitude of concentration (range 10^{-7}–10^{-4} *M*) with the complete system.

As an example of group-selective analysis, mecoprop, 2,4-D and 2,4-DP were trapped simultaneously (elution window 9–13 min). The results were identical with those found for the individual compounds. However, at high concen-

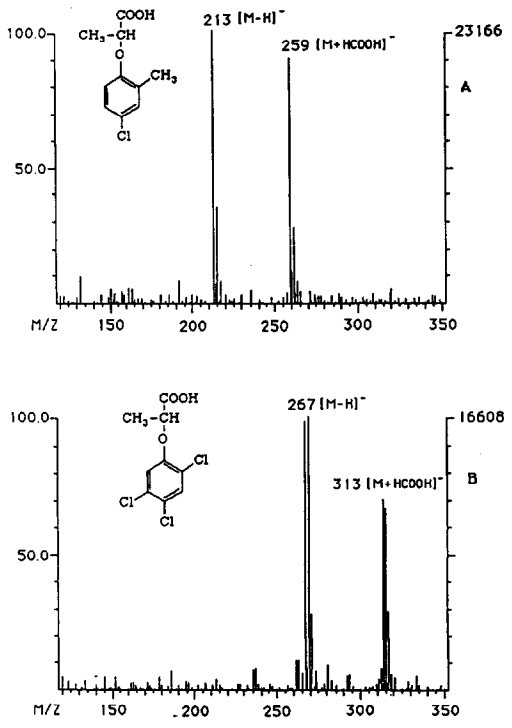

Fig. 5. Full-scan mass spectra of (A) mecoprop and (B) 2,4,5-TP after on-line IPLC–PSS–TSP-MS, using discharge ionization. Desorption solvent, 70 μl of water (0.5 *M* AmFo, PH 7.6)–acetonitrile (75:25, v/v) injected into the carrier stream (water, flow-rate 1 ml/min). Analyte concentration, 10^{-5} *M*.

trations, *i.e.*, above *ca.* $5 \cdot 10^{-5}$ *M*, the analyte recovery decreased because of early breakthrough.

In a first attempt to study a real sample, 100 μl of river Rhine water were analysed without any trace enrichment or sample clean-up (apart from membrane filtration). The test compounds (spiking level 20 μg/l) were separated, trapped on the PLRP-S column, desorbed and subsequently detected by TSP-MS. Although at this level, which was close to the LODs (amount injected 2 ng per analyte), reliable quantification could not be achieved, the SIM signals confirmed the presence of the phenoxy acids in the sample by their correct isotope ratios. In other words, even a modest degree of analyte trace enrichment (*e.g.*, *ca.* 50-ml volumes [5]) will be sufficient for the analysis of surface water samples containing phenoxy acids and related contaminants at or below the so-called alert level of 1 μg/l (amount then injected *ca.* 50 ng of each analyte).

CONCLUSIONS

The on-line coupling of IPLC and TSP-MS via PSS has been demonstrated for the first time. A non-selective hydrophobic polymer was used as the stationary phase in the trapping column. Designing an IPLC–PSS–TSP-MS system turned out to be very complicated because of the many experimental aspects involved (see Table II). The system described here, which uses CTA^+ as the counter ion, is in principle applicable to a wide variety of acidic compounds. In principle, although with another counter ion, it is also applicable to basic compounds, *e.g.*, secondary and tertiary amines, which can be separated by IPLC with sulphonates or sulphates as counter ions.

Optimum sorption of the phenoxy acids was achieved, after preloading the trapping column with CTA^+B^-, using water (1 m*M* CTA^+B^-, 10 m*M* phosphate buffer, pH 7)–acetonitrile (90:10, v/v). Desorption of the analytes was accomplished by three 70-μl injections of water (0.5 *M* AmFo, pH 7.6)–acetonitrile (75:25, v/v) into the carrier stream (water). Postcolumn addition of water proved to be a simple way to increase the capacity factor of the ion pair on the trapping column, *i.e.*, obtaining peak compression.

Desorption by means of plugs injected into a carrier stream, instead of desorption by flushing with an eluent, proved advantageous as many eluents suitable for desorption could be screened in a relatively short time. Further, the desorption solvent need not be the optimum solvent for the interface used. Nevertheless, desorption with high concentrations of the electronegative TFA is not advisable in this instance because of its adverse effect on the ionization.

On injecting standard solutions of the phenoxy acids into the IPLC–PSS–TSP-MS system, the recoveries were at least 90% for all five analytes tested. Recording full-scan mass spectra required an injected mass of at least 10 ng; the LODs using SIM were between 0.1 and 1 ng. First experiments with surface water samples showed

distinctly higher background signals and increased noise. However, even under these conditions, the phenoxy acids could be detected at the low-nanogram level. Therefore, using on-line trace enrichment of a sample volume of about 50 ml (see refs. 5, 43, 44 and 45 for its successful use in LC–UV and LC–MS), application to environmental analysis at contamination levels of, typically, 0.5 μg/l should pose no problems.

ACKNOWLEDGEMENTS

We thank G. Bakker, J. Brakenhoff and W.D. van Dongen for their experimental contributions to this work. The investigations were supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Scientific Research (NWO), grant No. 700.344.006.

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