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On the signal response of various pesticides in electrospray and atmospheric pressure chemical ionization depending on the flow-rate of eluent applied in liquid chromatography–tandem mass spectrometry

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Abstract

The API–MS signal response of several pesticides (atrazine, simazine, isoproturon, diuron, chlorfenvinphos, chlorpyrifos, alachlor, trifluralin) depending on the flow-rate of eluent entering the MS interface was investigated. The investigations were based on API–MS–MS analyses of standard pesticide mixtures in the flow injection mode (FIA) at systematically varied eluent flow-rates using both an ESI interface (Turboionspray) and a heated nebulizer type APCI source. In the result, the individual compounds included in this study showed significant differences in their signal response behaviour depending on the flow-rate of eluent applied. The most hydrophobic compounds among the investigated pesticides (chlorpyrifos and trifluralin) showed drastic losses of sensitivity with increasing eluent flow-rate in both ESI and APCI, while more hydrophilic compounds like atrazine, simazine and isoproturon showed the expected signal response (concentration-sensitive in ESI, mass-flow-sensitive in APCI) at least within a certain range of flow-rates (200–600 µl/min in ESI, 200–2000 µl/min in APCI). These findings lead to the conclusion that application of a programmed HPLC eluent flow-rate may be advantageous to achieve maximum sensitivity of API–MS detection for all pesticides of interest. This is exemplified by the implementation of a flow gradient into an online SPE–HPLC–APCI–MS/MS method for improved analysis of pesticides in drinking water. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Atmospheric pressure ionization (API) techniques like electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) have significantly improved the capabilities of liquid chromatography in terms of selectivity and sensitivity of the detection and have, therefore, enlarged the field of HPLC applications in environmental analysis [1–3].

ESI and APCI are rather complex processes [4–8]. There are several instrumental parameters which are of drastic influence to the ionization efficiency. Thus, careful optimization of the mass spectrometer in use

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is required to achieve maximum sensitivity. This includes adjustment of typical interface parameters like ionization voltage in ESI and discharge needle current in APCI, respectively, pressure of the spraying/nebulizing gases, interface temperature and de-clustering potential. When working in tandem MS mode, collision energy and pressure of the collision gas are further MS parameters to be optimized. Also, the composition of the LC eluent used and the nature and amount of ionic additives added to the mobile phase to support ion formation may be of influence to the yield of formed analyte ions [3,9,10].

The choice of an appropriate eluent flow-rate in LC applications depends on the dimensions of the LC column used mainly, since maximum column performance is explored at a well-defined optimum flow-rate. Flow rates between 20 and 1000 μl/min are commonly applied when working with packed LC columns of diameters ranging from 1 to 4.6 mm. A new type of LC column based on monolithic C_{18} modified silica stationary phases may be operated at even higher flow-rates than those mentioned above, enabling very fast LC separations [11–13]. Consequently, API interfaces have to tolerate rather large amounts of solvents when coupled to HPLC directly. To manage this, pneumatic, ultrasonic and thermal assistance of the aerosol formation and vaporization, respectively, have been implemented in API interfaces (ionspray, sonic spray, turboionspray), reducing the need of column effluent splitting, and, thus, leading to further improvement in API–MS performance [14–16].

While APCI proceeds in direct proportionality to the mass flow of an analyte, ESI (when performed at low flow-rates) has been claimed to be a concentration-dependent ionization technique [1,4,5,17–19]. Under these suppositions, flow injection (FIA) of constant volumes of a sample solution into an APCI source should lead to peaks of constant area, but increasing height and decreasing width as the eluent flow-rate is increased, while ESI–MS should theoretically produce FIA peaks of identical height, but of decreasing area due to decreasing peak width when increasing the eluent flow. In contrast to the latter, Abian et al. and Hopfgartner et al. described the application of a high-flow ionspray interface, which turned out to function as a mass-flow-dependent device [18–20].

However, the statements made above may be limited in their validity by the specific design of any individual interface and by several parameters influencing the nebulizing/vaporizing efficiency, for instance eluent composition, source temperature, pressure of spraying/nebulizing gases, temperature and flow-rate of drying gas etc. [21,22].

Recently, we have described the development of a rapid and versatile on-line SPE–HPLC–MS/MS method for the determination of various priority pesticides in surface and drinking water [23]. During the optimization of the MS conditions used in this method it had been observed that the individual pesticides showed significant differences in their MS sensitivity depending on the flow-rate of eluent applied. Thus, the paper presented here reports in detail on the API–MS signal response of various pesticides depending on the flow-rate entering the MS interface. The investigations described here are based on FIA–MS/MS experiments at systematically varied eluent flow-rate using both an ESI (Turboionspray®, a pressurized electrospray with thermal assistance of aerosol vaporization) and a heated nebulizer type APCI ion source.

As a conclusion derived from these systematic investigations, the column effluent flow-rate has to be considered a factor of importance to the sensitivity of HPLC–API–MS methods. Thus, application of a programmed flow-rate to HPLC–API–MS may be advantageous to achieve maximum sensitivity for all compounds to be determined in one and the same LC–MS run. This will be exemplified by the application of a programmed eluent flow-rate to the online SPE–HPLC–MS/MS analysis of pesticides in drinking water.

2. Experimental

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 (fractopur) was purchased from Merck. Eight pesticides, covering various com-

pound classes like triazines, phenyl ureas, organo-phosphorous and further species (see Fig. 1), were included in the systematic experiments on MS response described in the first part of this study.

The spectrum of compounds was then extended by terbutylazine, prometryne and chlortoluron when performing the experiments on flow-rate pro-grammed HPLC–MS/MS.

Pesticide standards were obtained from Dr. Ehrens-dorfer (Augsburg, Germany) and Promochem (Wesel, Germany), respectively. Methanolic stock solutions were prepared at a concentration of 500 μg/l. Spiked water samples and pesticide standard mixtures were prepared from these stock solutions.
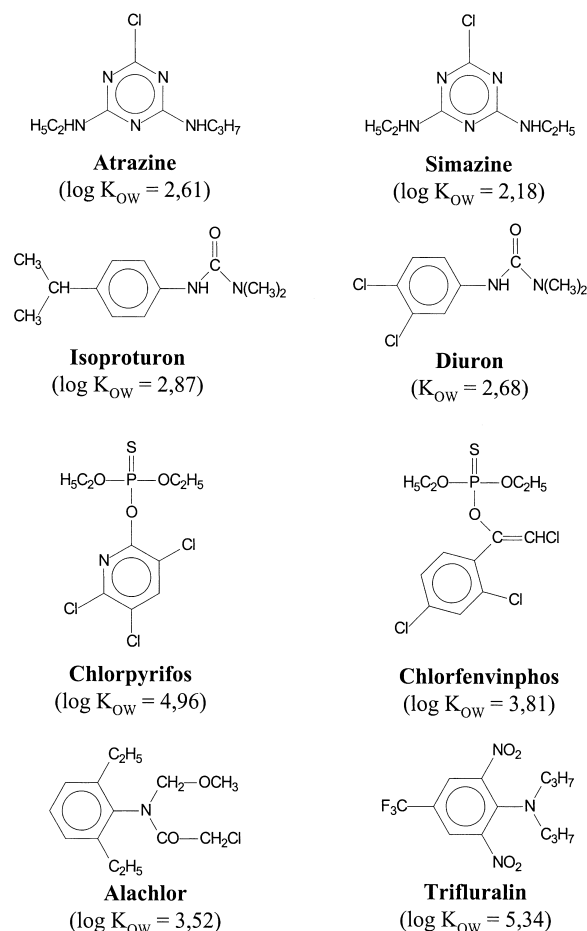
2.2. Water samples

Drinking water samples were taken from the water pipe in the laboratory where the experiments were carried out (Leipzig, Saxony, Germany). The sample pretreatment procedure applied has been described elsewhere [23].

2.3. Instrumental set-up

Experiments were performed on an LC–MS/MS system consisting of a HTLC 2300 liquid chromato-graph (Cohesive Technologies, Franklin, USA) and a triple-quadrupole mass spectrometer API 2000 (Applied Biosystems, MDS Sciex, Concord, Canada) equipped with both ESI (Turboionspray®) and APCI (Heated Nebulizer®) interface. Nitrogen was used as collision gas. All experiments were carried out in positive ion mode. Interface parameters used for ESI and APCI, respectively, and the collision gas setting are listed in Table 1. The values resulted from optimization by means of FIA–MS/MS of a metha-nolic standard pesticide mixture at different flow-rates (200 and 1200 μl/min) under systematically varied source parameters.

The mass spectrometer was run in selective re-action monitoring (SRM) mode detecting one SRM transition for each pesticide at a dwell time of 40 ms. SRM transitions used for detection are given in Table 2. All precursor ions are $[M+H]^+$ adducts, except alachlor in APCI, where $[M-CH_3OH+H]^+$ gave slightly higher ion intensity. For some of the compounds, additional experiments were performed in Q1 scanning mode, recording spectra in the range

Fig. 1. Pesticides included in the systematic investigations on API–MS signal response depending on eluent flow-rate.

Table 1
Optimized settings for general interface parameters and collision gas

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

Table 2
SRM transitions used for MS/MS detection

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

between $m/z = 100$ amu and $m/z = 500$ amu at a scanning time of 1 s.

Eluents applied for FIA and gradient HPLC were of the following composition: eluent A — methanol–water 97:3 v/v; eluent B — methanol–water 3:97 v/v. Both eluents contained ammonium acetate at a concentration of 1 mM. Experiments were performed using a 10 μl sample loop and a 10-port Valco valve.

Two sets of FIA–MS/MS analyses were carried out, one with pure eluent A and a second one with more aqueous eluent (A:B = 50:50 v/v).

A Chromolith SpeedROD RP-18e (50 mm×4.6 mm, provided by Merck, Darmstadt, Germany) was used for LC separations. Details of the gradient elution programs applied are given in the text and in the legends.

The device and the methodical parameters used for on-line SPE–HPLC coupling have recently been described in detail [23].

3. Results and discussion

The influence of the eluent flow-rate on the MS signal response of the individual pesticides in ESI (Turboionspray) and APCI, respectively, was investigated by means of FIA–MS/MS analyses of a standard mixture containing the compounds shown in Fig. 1. Since the design and operational parameters of any individual API ion source may lead to differing results, the data discussed in the following may be specific to the LC–MS system used here to a certain extent.

3.1. Signal response of various pesticides depending on eluent flow-rate in ESI and APCI

Fig. 2 contains the results obtained from FIA–ESI–MS/MS analyses in a range of flow-rates between 200 and 1200 μl/min. The eluent composition was 97:3 methanol–water (v/v). The upper diagram displays the FIA–MS/MS peak areas plotted versus flow-rate, the bottom diagram shows the corresponding peak heights plotted versus flow-rate.

As obvious from Fig. 2, the signal response of atrazine, simazine and isoproturon clearly follows a concentration-dependent trend when increasing the flow-rate up to 600 μl/min approximately. This means, the height of the FIA–ESI–MS/MS peaks remains nearly constant, while the peak width and, thus, the peak area decrease at approximately a factor of F_1/F_n, with F_1 being the lowest flow-rate investigated (200 μl/min) and F_n being the actual flow-rate. As an example, a flow-rate of 400 μl/min
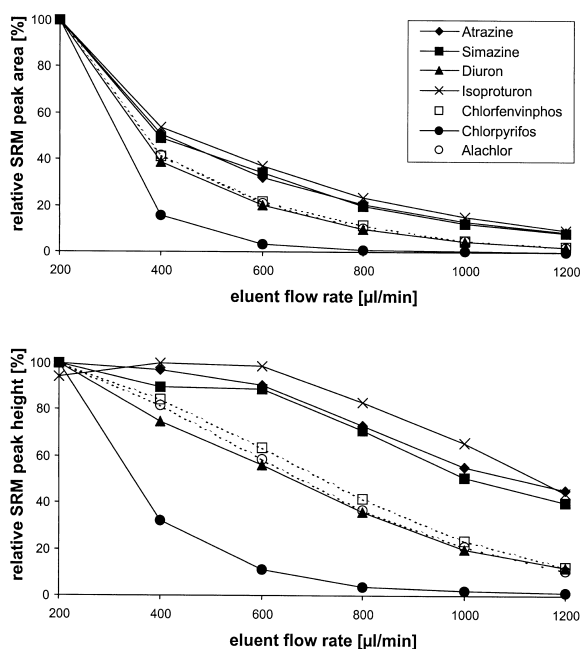

Fig. 2. FIA–ESI–MS/MS signal response of seven pesticides depending on the flow-rate of eluent (methanol–water = 97:3, v/v); standard pesticide mixture: $c = 100$ ng/ml. Top, peak area plotted versus flow-rate. Bottom, peak height plotted versus flow-rate.

results in an FIA peak covering ~50% of the area of the peak obtained at 200 μl/min. This is in contrast to results published by Abian et al. [18] and Hopfgartner et al. [20], who stated mass-flow dependent detector response of high-flow ionspray interfaces when working at flow-rates up to 2 ml/min.

According to Fig. 2, all compounds included in this study give decreasing FIA–ESI–MS/MS peak heights at flow-rates higher than 600 μl/min. This general trend may be due to decreasing spraying efficiency and vaporization of the sprayed aerosol, even at the actual source temperature of 450°C.

However, four of the investigated species (chlorpyrifos, chlorfenvinphos, alachlor, diuron) show more or less decreasing peak heights just from the beginning of the range of flow-rates investigated (200–1200 μl/min), see Fig. 2, bottom. This effect is most drastic for chlorpyrifos, which is detected three times less sensitively when increasing the flow-rate from 200 to 400 μl/min. At a flow-rate of 1200 μl/min, chlorpyrifos is detected with only 5% of its sensitivity achieved at 200 μl/min. Diuron, alachlor and chlorfenvinphos also show a drop in signal intensity at higher flow-rates, but the rate of decrease is not that drastic. The relative peak height at a flow-rate of 1200 μl/min is still around 20% compared to that at 200 μl/min.

Adequate experiments using the APCI interface lead to the diagrams given in Fig. 3. The range of flow-rates investigated was enlarged up to 2800 μl/min at the same eluent composition (methanol–water 97:3 (v/v)). Trifluralin, susceptible to APCI but not to ESI at reasonable intensity was additionally included in this series of experiments. The resulting data basically confirm the specific response behaviour of the individual pesticides in ESI, with the exception of chlorfenvinphos, which shows a different behaviour in APCI compared to ESI. Also in APCI, chlorpyrifos shows a drastic loss of sensitivity with increasing eluent flow. The relative peak height is nearly zero at high flow. Trifluralin follows the same trend. This means that both compounds are virtually non-detectable by means of APCI–MS under high-flow conditions. Similar to ESI, diuron and alachlor show a less drastic decrease of signal intensity with increasing eluent flow. At 1000 μl/min, there still remains ~50% of the initial peak height measured at a flow of 200 μl/min. Atrazine,

simazine, isoproturon and, in contrast to ESI, chlorfenvinphos give peaks of nearly constant area and, thus, of rising height in a wide flow range up to ~1600 μl/min as it would be expected, supposing ionization depending on mass-flow. However, there occurs a significant drop of detection sensitivity also for these compounds beginning at flow-rates higher than 2000 μl/min. The nebulizing capacity of the APCI interface (means spraying/vaporizing efficiency) has obviously been exceeded at such high amounts of eluent. This is indicated by noticeable FIA peak tailing due to condensation and delayed vaporization of the sprayed eluent before reaching the ionization region of the APCI source.

A second series of FIA–MS/MS experiments was performed, again using both ESI and APCI, but changing to a more aqueous eluent (methanol–water=50:50 v/v). At this eluent composition, the general decrease in signal response started occurring at lower flow-rates in comparison to the methanolic eluent applied first. Increasing amounts of water in the eluent obviously affect the spraying efficiency and the vaporization process. However, the specific response behaviour of the individual compounds was very similar to that described above for methanolic eluent.

For a better interpretation of the data measured in FIA–MS/MS mode, additional experiments were performed in single quadrupole scanning mode for those compounds, which showed most drastic sensitivity drops at higher flow-rates (chlorpyrifos in ESI and APCI, trifluralin in APCI). These analyses were carried out to prove if the look of the Q1 spectra showed significant changes depending on flow-rate. Special attention was paid to the relative intensity of adduct ions ($[M+NH_4]^+$, $[M+Na]^+$, $[M+K]^+$) other than those used as precursor ions in the detected SRM transitions and on the occurrence of further fragment ions, respectively. However, the signal patterns in the Q1 spectra did not show significant changes depending on flow. Consequently, the described SRM signal losses at high eluent flow-rates may not be attributed to an intensity shift to any other ion at the expense of the adduct ion used as SRM precursor. These findings lead to the conclusion that the losses in signal intensity observed for several compounds in FIA–MS/MS under high-flow conditions, most drastic for chlorpyrifos and tri-
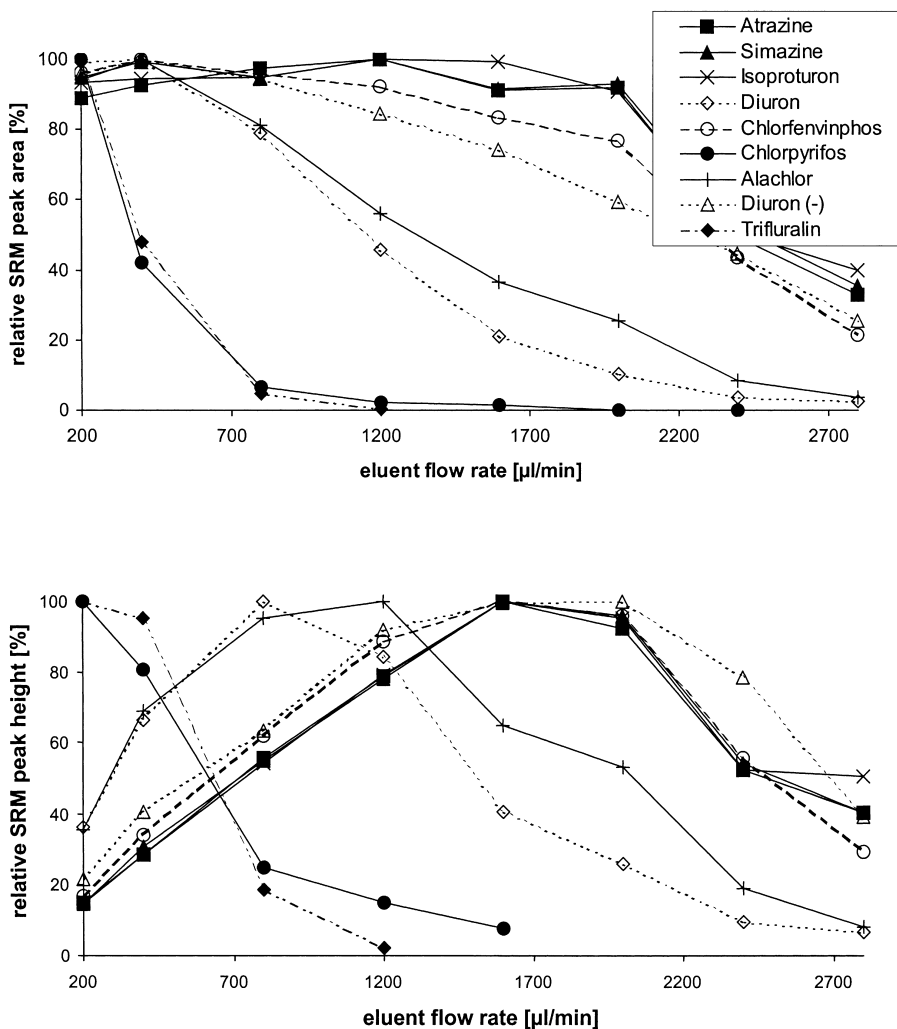
Fig. 3. FIA–APCI–MS/MS signal of eight pesticides depending on the flow-rate of eluent (methanol–water = 97:3, v/v); standard pesticide mixture: $c = 100$ ng/ml. Top, peak area plotted versus flow-rate. Bottom, peak height plotted versus flow-rate.

fluralin, are mainly due to decreasing efficiency of the ionization process with increasing amount of eluent sprayed into the ion source.

To explain the differing behaviour of the individual compounds included in this study, correlations with physical constants expressing hydrophilic/hydrophobic properties, e.g. partition coefficients octan-1-ol/water (log K_{OW}) were attempted. The results obtained are somewhat indifferent. A certain correlation may be found between increasingly hydrophobic character of the investigated pesticides (increasing log K_{OW}) and the extent of sensitivity

loss with increasing flow-rates. According to that, chlorpyrifos and trifluralin, which are the most hydrophobic compounds among the pesticides investigated, show the most drastic losses in signal intensity under high flow conditions. However, diuron and chlorfenvinphos do not match this correlation. Diuron has a relatively low log $K_{OW} = 2.68$, comparable to isoproturon (log $K_{OW} = 2.87$), but shows a significantly negative trend in sensitivity at high eluent flow-rates in both interfaces, similar to alachlor, which has a log $K_{OW} = 3.52$. Chlorfenvinphos shows an APCI response behaviour comparable

to that of atrazine and simazine, although it has a K_{OW} value being at least one order of magnitude higher than those of the two triazines. These comparisons indicate, that further factors are of specific importance to the efficiency of ionization under atmospheric pressure conditions.

3.2. Consequences to HPLC–API–MS/MS analysis

To explore maximum LC column performance, the choice of an appropriate linear velocity of the eluent and, thus, of an optimum flow-rate is essential. However, as shown above, the flow-rate may be of significant influence to the efficiency of the ionization process in the API interface too. Conse-
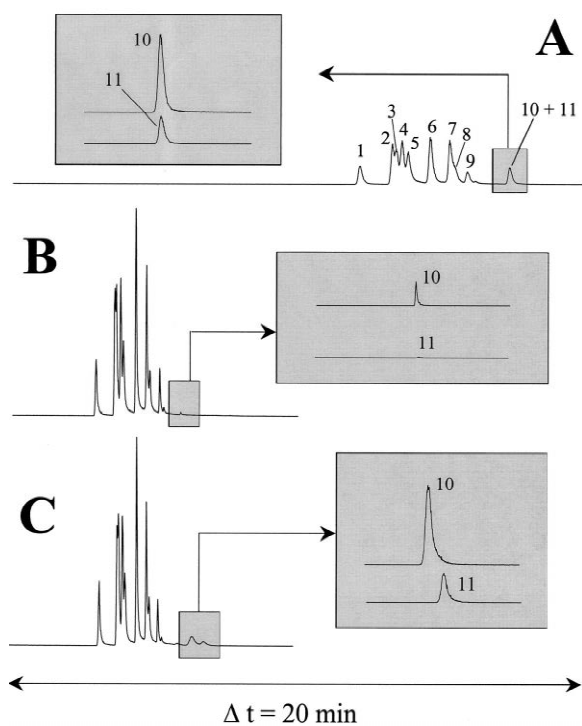

Fig. 4. Total ion chromatograms (sum of 11 SRM transitions) and extracted SRM traces of chlorpyrifos and trifluralin obtained from HPLC–APCI–MS/MS of a standard pesticide mixture ($c = 100$ ng/ml; for gradient HPLC conditions see text, Section 3.2) applying varied column flow-rates: (A) constant column flow, 200 μl/min; (B) constant column flow, 1200 μl/min; (C) programmed column flow, 0.0–5.1 min: 1200 μl/min, 5.1–10 min: 200 μl/min (1, simazine; 2, chlortoluron; 3, atrazine; 4, isoproturon; 5, diuron; 6, terbutylazine; 7, prometryne; 8, alachlor; 9, chlorfenvinphos; 10, chlorpyrifos; 11, trifluralin).

quently, both factors have to be considered to achieve best LC–MS sensitivity.

To underline the practical consequences arising from this, Fig. 4 shows a comparison of three HPLC–APCI–MS/MS chromatograms obtained from one and the same standard pesticide mixture using different eluent flow regimes. In all cases, a gradient from 20 to 100% of organic eluent A was run within 5 min. Chromatogram A was recorded at constant column flow of 200 μl/min, resulting in long retention times, broad peaks, but high peak intensities of the two lately eluting compounds chlorpyrifos and trifluralin. By means of increasing the flow-rate to 1200 μl/min (chromatogram B in Fig. 4), analysis time is significantly reduced, peak width and height of compounds no. 1–9 are considerably improved, but the last two pesticides are suppressed nearly to zero due to the above described ionization problems under high flow conditions. Concluding from chromatograms A and B, maximum gain of time and highest sensitivity for all compounds would be achieved by application of a programmed HPLC eluent flow-rate. This is demonstrated in the bottom chromatogram C in Fig. 4. The LC run is started at a flow-rate of 1200 μl/min. At $t = 5.1$ min, after elution of the first nine compounds, but directly prior to elution of the last two pesticides, chlorpyrifos and trifluralin, the column flow-rate is reduced to 200 μl/min. This way, increase of analysis time is negligible, while gain in sensitivity of more than a factor of 5 (chlorpyrifos) and a factor of 60 (trifluralin), respectively, is achieved at the same time.

Flow-rate programming as described above was then implemented in an on-line SPE–HPLC–APCI–MS/MS method developed for the rapid determination of various priority pesticides in drinking and surface water, which has been described by us recently [23]. Fig. 5 shows a comparison of chromatograms obtained from on-line SPE–HPLC–APCI–MS/MS of 10-ml samples of drinking water spiked with 50 ng/l of each pesticide. Chromatogram A was recorded under the LC conditions according to Ref. [23] (constant eluent flow-rate of 600 μl/min). Trace B is the result of programmed eluent flow, starting with 1200 μl/min and reducing the flow-rate to 200 μl/min prior to elution of the last two target compounds chlorpyrifos and tri-
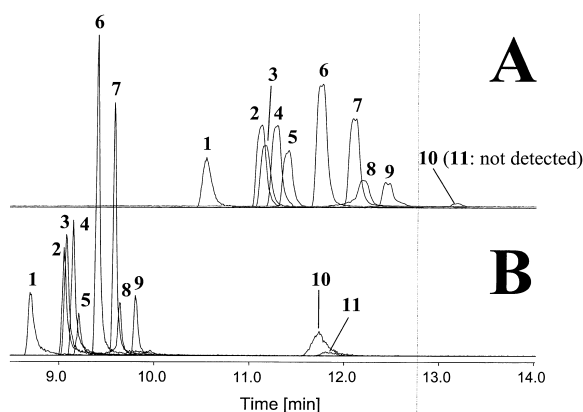
Fig. 5. SRM chromatograms obtained from on-line SPE–HPLC–APCI–MS/MS analysis of 10-ml drinking water samples spiked with ~50 ng/l of each pesticide. (A) Constant column flow, 600 µl/min (gradient elution: 33.3–100% eluent A within 5 min); (B) programmed column flow, 0.0 min–9.9 min: 1200 µl/min, 9.9 min–14.0 min: 200 µl/min (gradient elution: 33.3–100% eluent A within 2.5 min) (1, simazine; 2, chlortoluron; 3, atrazine; 4, isoproturon; 5, diuron; 6, terbutylazine; 7, prometryne; 8, alachlor; 9, chlorfenvinphos; 10, chlorpyrifos; 11, trifluralin).

fluralin. In the result, the modified method enabled further gain of analysis time and improvement of the detection limits for most compounds, most significantly for chlorpyrifos and trifluralin.

4. Conclusions

As the results show, the eluent flow-rate entering the MS interface has to be considered a factor of specific influence on the ionization efficiency of individual compounds under atmospheric pressure conditions. Thus, various pesticides differ significantly in their flow-dependent signal response in both ESI and APCI. Most drastic losses in API–MS sensitivity at high flow-rates were observed for fairly hydrophobic pesticides, like chlorpyrifos and trifluralin.

As has been demonstrated, implementation of a programmed eluent flow-rate into HPLC–API–MS methods may be advantageous to explore highest sensitivity for all compounds of interest at minimized analysis time.

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