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Enhanced detection sensitivity by large volume injection in reversed-phase micro-high-performance liquid chromatography

Mehdi Attaran Rezai, Giorgio Famiglini, Achille Cappiello*

Istituto di Scienze Chimiche, Università di Urbino, Piazza Rinascimento 6, 61029 Urbino, Italy

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Abstract

High-performance liquid chromatography (HPLC) packed capillary columns have gained a great deal of interest for their role in conjunction with other analytical techniques, particularly mass spectrometry. However, the advantages offered by the low mobile phase flow-rate are often compromised by modest method detection limits due to sub-microliter injection volumes. This is particularly significant with a particle beam interface where the benefits obtained at very low solvent flow-rate (1 μl) were outlined. In this paper, the effect of large injection volumes (up to 50 μl) on the chromatographic behaviour of a reversed-phase 250 μm I.D. packed capillary column was studied. Due to their differences in chemical properties and to their environmental impact, a selection of basic–neutral and acidic pesticides was found to be appropriate for this investigation. This study was focused on the peculiar sample–column relationship encountered in a micro-HPLC system. Loop shape and matrix composition were found to be the determinant factors for a correct solute displacement into the capillary column. Suitable conditions for the injection of large volumes of trace-level pesticides were described. Previously developed liquid chromatographic–mass spectrometric methods for the determination of several pesticides in water took great advantage from the larger sample availability.

Keywords: Large volume injection; Injection methods; Pesticides

1. Introduction

Scaled-down high-performance liquid chromatographic techniques (micro-HPLC) have gained worldwide diffusion and popularity and the number of applications increases every year. Micro-HPLC columns are usually classified on the basis of their internal dimension: microbore (1–2 mm I.D.), packed capillary (150–500 μm I.D.) and open tubular (<50 μm I.D.). Optimum flow-rate varies accordingly, from over 200 μl/min to under 1 μl/min. Following a similar trend already observed for the evolution of capillary gas chromatography (GC), micro-HPLC offers several advantages over conventional columns: increased efficiency in shorter time, significantly lower solvent consumption and enhanced signal-to-noise ratio of chromatographic peaks using concentration sensitive detectors [1–4]. However, the biggest incentive arises from the attractive possibility of coupling HPLC and mass spectrometry (MS). An increasing number of new ionization techniques allows and often requires direct HPLC interfacing but only at a mobile phase flow-rate compatible with the vacuum system capacity. Because of sample waste, post-column stream splitting cannot be considered a valid approach to sustain

*Corresponding author.

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acceptable solvent input, and thus low flow-rate HPLC columns must be used. Packed capillary columns are efficient and reliable and offer an operative flow-rate appropriate for liquid chromatography (LC)–MS coupling conditions. The evolution of specific chromatographic apparatus or devices designed to adapt preexisting conventional systems has followed the development of micro-columns, allowing the execution of complex gradient separations without loss of the tested efficiency of a conventional HPLC column. Our research group has recently designed a micro-flow-rate particle beam interface for capillary LC–MS [5,6] which allows greater sensitivity and much simpler operation procedure. The approach consisted in reducing the mobile phase flow-rate to as low as 1 μl/min in order to eliminate most of the drawbacks associated with a massive solvent intake. The new interface was successfully employed for the analysis of basic–neutral and acidic pesticides in water [7,8]. Unfortunately, the great benefit in terms of sensitivity shown by the new interface was often limited by the inherent constraint of the injection volume compatible with a micro-column. The strenght of a method for the analysis of trace level components can be easily impaired by the shortage of sample injected. Considering, for example, an instrument detection limit of 0.7 ng for 2,4-DB, a phenoxy acid herbicide analyzed by LC–MS, with a 10 000-fold extraction and preconcentration method, the injection of 60 nl of sample would provide a method detection limit of just 1 ppb. A slight increase of the injection volume (0.5 μl) would improve this limit by almost of a factor of ten as recently reported [8]. The sample transfer onto a column is a critical step in both GC and LC. Sample volume and the way such volume is transferred to the column heavily affects band dispersion and chromatographic resolution. The maximum injection volume can be theoretically predicted by the following equation [9,10]:

$$V_{inj}^2 = (\emptyset K \pi r^2 \varepsilon_T)^2 HL = K^2 \sigma_{inj}^2 \quad (1)$$

where ε_T is the total porosity, r is the column radius, H is the plate height, L is the column length, K is a constant relative to the injection profile, σ is the injection variance and $\emptyset$ is the column variance. The column radius is by far the factor which most affects the injection volume. The constant K^2 is relative to variations of the injection profiles and is equal to 12 for ideal sharp solute transfer, 1 for an exponential function of solute concentration. For a 25 cm × 250 μm I.D. column, packed with 5 μm C_{18} particles, the calculated injection volume must not exceed 100 nl. This value can be easily lowered by other factors relative to the injector design, type of connections, materials, etc. For all these reasons 60 nl is widely used and it is available as an internal loop.

Several attempts have been made to overcome the injection volume limitation [11–13]. This research effort has been mainly devoted to improve the injection procedure implemented with conventional and large microbore columns. Kuyken [3] made a comparative study using different injection techniques with a 1.35 mm I.D. microbore column. Guinebault and Broquaire [13] considered various matrices' behaviour in large volume injections with conventional analytical columns. As a general rule, from a finite volume of sample injected, a solute zone will be formed at the column head reflecting the degree of sample axial displacement during the injection process. The study reported in this paper is focused on a 250 μm I.D. packed capillary column and is mainly based on strong retardation and compression of the solutes, after the injection step, in a narrow initial band at the head of the column. The aim was to lower current method detection limits for trace level pollutants by improving the injection procedure implemented with capillary columns. The study was also focused on those injection techniques normally employed in micro-HPLC in order to highlight the specific behaviour of the capillary columns with respect to the conventional ones. Standard solutions of basic–neutral and acidic pesticides were injected using loops of different volumes and shapes. A significant application of this procedure was reported performing a LC–MS analysis. This example clearly showed that very low levels of pollutants can be detected by injecting large sample volumes.

2. Experimental

2.1. Mass spectrometry

All the experiments were carried out with a Hewlett-Packard 59980B particle beam unit, coupled

with a Hewlett-Packard 5989A mass spectrometer. The original nebulizer was replaced by a laboratory-made micro-flow nebulizer [5,6]. This device generates a mobile phase aerosol using flow-rates as low as 1 μl/min. A 50 μm I.D., 180 μm O.D. fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) was used as the nebulizer tip and to connect the liquid chromatograph. The nebulizing gas was helium 5.6 purity grade (>99.9996%) and was purchased from SOL (Milan, Italy). The helium pressure needed was 70 to 90 psi to supply 0.1 l/min. The desolvation chamber temperature was kept at 40°C. The operating pressures were 0.5 Torr in the desolvation chamber, 0.3 Torr in the second stage of the momentum separator and $8 \cdot 10^{-5}$ to $10 \cdot 10^{-5}$ Torr in the manifold of the ion source. Mass spectrometer tuning and calibration were performed automatically using perfluorotributylamine (PFTBA) as a reference compound and monitoring *m/z* 69, 219, 502. The repeller potential was adjusted manually. Mobile phase was allowed into the ion source during calibration. The dwell times during selected ion monitoring (SIM) analyses were adjusted in order to obtain 0.5 cycles/s and a mean of 10 acquisition samples for each HPLC peak. The ions used in SIM mode were selected on the basis of their intensity and were collected in a single ion program. The final transfer tube, prior to the ion source, was shifted to a fully retracted position after the tuning procedure. The ion source temperature was set at 250°C.

2.2. Liquid chromatography

LC was carried out with a Kontron Instrument 420 dual-pump, binary-gradient, conventional HPLC system (Kontron Instrument, Milan, Italy). Microliter flow-rates were obtained with a laboratory made splitter that was placed between the pumping system and the injector [14]. For sample injection, two Valco injectors were employed (Valco, Houston, TX, USA). One was equipped with internal loops (0.06 μl, 0.5 μl); the other was a six-port valve connected to laboratory-made external loops of different volume (Table 1). Fused-silica tubings of different internal and external diameter were used for the loop preparation and were purchased from Polymicro Technologies. Final loop volume was calculated from the length and the internal diameter value

Table 1
External loop dimensions

I.D. (mm)	Length (mm)	Volume (μl)
0.05	254	0.5
0.05	509	1
0.10	255	2
0.15	226	4
0.15	453	8
0.25	407	20
0.32	622	50

supplied by the manufacturer. A laboratory-made packed capillary column was used for the chromatographic separations [15]. These columns are routinely made in our laboratory from 1/16 in. O.D., 250 μm I.D. PEEK tubing (Alltech, Deerfield, IL, USA) and are packed with C_{18} reversed-phase 5 μm particle size purchased from Phase Sep (Queensferry, UK). A 25-cm long column has a mean of 20 000 theoretical plates at a 1 μl/min flow-rate. Acetonitrile was used as organic solvent in the mobile phase. Acetonitrile was preferred to methanol because of its lower viscosity. Relative solvent concentration in the mobile phase, gradient programs and buffer additions are described in detail in Section 3.

2.3. Extraction procedure

A liquid–solid extraction procedure for the isolation of pesticides from aqueous samples was employed [16]. This procedure is capable of collecting both basic–neutral and acidic pesticides by a stepwise extraction. A cartridge was filled with graphitized carbon black (Carbograph 1) and was capable of sampling up to 2 l of water. The extraction cartridge was made by using a polypropylene tube, 6.5×1.4 cm I.D., packed with 250 mg of Carbograph 1, 120–400 mesh (Alltech). Polyethylene frits, 20 μm pore size, were located above and below the sorbent bed. Before a water sample was extracted, the cartridge was washed with 5 ml of methylene chloride–methanol (80:20, v/v) followed by 2 ml of methanol and 15 ml of 10 g/l ascorbic acid in HCl-acidified water (pH 2). Water samples were forced through the trap at a flow-rate of 150–160 ml/min by using a vacuum apparatus placed below the cartridge. Distilled water (7 ml) was passed through the trap after all the sample was passed through. Basic–neutral pesticides were eluted by

passing 1 ml of methanol, drop by drop, through the trap followed by 6 ml of methylene chloride–methanol (80:20, v/v). The extract was brought to dryness and then 100 μl of an appropriate solvent were added to the vial.

2.4. Reagents

All solvents were HPLC grade from Farmitalia Carlo Erba (Milan, Italy) and were filtered and degassed before use.

Pesticides were purchased from Riedel-De Haen (Hannover, Germany). Trifluoroacetic acid (TFA) was purchased from Sigma Scientific (St. Louis, MO, USA). Reagent water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

3. Results and discussion

In reversed-phase liquid chromatography, the retention volume is a function of the elution strength of the mobile phase. When hydrophobicity processes are involved, the retention volume increases when the relative concentration of water in the mobile phase increases. During the injection process, the solute is dispersed into a zone formed at the column head. The width of this zone depends on the mutual interactions established by the solute with the stationary phase and the mobile phase. Solute band focusing can be promoted by creating locally the conditions for a very strong retention. In a HPLC column, the sample plug is momentarily inserted into the mobile phase stream and transferred into the column. The concentration profile is assumed to be a square wave function with a limited axial dispersion for an unaffected chromatographic response. In a packed capillary column, the injection is usually restricted to sub-microliter internal loops and they may not represent a good approximation of this model. At the very low flow-rates proper of a capillary column, loop shape may be critical for a correct displacement of the sample into the column and it may represent a distinctive aspect of micro-column separations. As discussed in a previous paper [17], loops with a definite volume but different shape delivered samples differently. External loops, ranging from 0.5 to 50 μl, were realized using fused-silica capillary tubing of different diameters as reported in Table 1. External loops smaller than 0.5 μl are not feasible because of a noticeable extra-volume added by internal and external valve connections. Two internal loops of 0.06 and 0.5 μl were also used with the appropriate injector.

A number of selected pesticides of different polarity were chosen for this study. Their behaviour in reversed-phase chromatography was found to be particularly suitable for our purpose.

3.1. Basic–neutral samples

The analysis of several pesticides with a micro-HPLC column coupled to a mass spectrometer was already reported in a previous paper [7]. In that case, a mobile phase gradient from 20% to 80% acetonitrile in water was found suitable for pesticide separation. For this study, we have selected ten basic–neutral and seven acidic pesticides particularly amenable to HPLC (Table 2). A 30 ng/l solution of chloridazon, bromacil and monuron was prepared in methanol. This standard solution was injected using 0.06- and 0.5-μl internal loops and 1, 2, 4 and 8 μl external loops. Chromatographic runs were carried out using a gradient elution from 0% to 80% acetonitrile in 40 min. The integrated peak areas obtained from the injected pesticides were plotted and reported in Fig. 1. The solute mass injected increased proportionally by switching from a loop to a bigger one and linear calibration plots were thus expected (straight line). It appears clearly in the figure that only the first two experimental points followed the expected trend while all the others are distributed in a nearly constant pattern. In our opinion, it is not coincidence that the data obtained from the internal loops followed the expected trend. This result could be explained considering the elution strength of methanol in which the pesticides were dissolved and the different shape between internal and external loops. Because of their peculiar shape, a groove on a plastic rotor, only internal loops can promote the mixing with the water in the mobile phase thus enhancing band focusing. Otherwise, the samples stored in the larger external loops were distributed in a much longer shape formed by the narrow capillary tubing. In this case, the solute is

Table 2
Selected pesticides and their solubility in water

	Class[a]	CAS-RN[b]	Solubility in H_2O mg/l (20°C)
Basic–neutral pesticides			
Chloridazon	pyridazinone(H)	1698-60-8	400
Bromacil	uracil(H)	314-40-9	815
Monuron	phenylurea(H)	150-68-5	–
Carbofuran	carbamate(I)	1563-66-2	320
Carbaryl	carbamate(I)	63-25-2	40
Diuron	phenylurea(H)	330-54-1	42
Propanil	propionalide(H)	709-98-8	130
Chlorbromuron	phenylurea(H)	13360-45-7	35
Azinphos-ethyl	phosphorothioate(I)	2642-71-9	5
Parathion-ethyl	phosphorothioate(I)	56-38-2	11
Acidic pesticides			
Dicamba	methoxybenzoic(H)	1918-00-9	6500
Bentazone	thiadiazinone(H)	25057-89-0	500
Bromoxynil	phenol(H)	1689-84-5	130
Mecoprop	phenoxy acid(H)	7085-19-0	620
2,4,5-T	phenoxy acid(H)	93-76-5	150
MCPB	phenoxy acid(H)	94-81-5	44
2,4,5-TP	phenoxy acid(H)	93-72-1	–

[a] Key: H, herbicide; I, insecticide.
[b] Chemical Abstract Service registry number.

entirely dissolved in methanol which, once pushed by the incoming water, works as 100% methanol mobile phase dragging the pesticides away from the column in the dead volume. It can be supposed that only a small aliquot, in the rear of the sample plug, mixed with water and was thus retained by the column. Because of the limited solvent exchange, unaffected by the loop size, the retained solute and the consequent signal response remained constant with any external loop. Very low diffusion velocity in liquids and the capillary dimension contribute to limit the extent of this exchange. To highlight the role played by the loop shape, the injection of the same pesticide mixture was performed twice via an internal and external loop of the same volume (0.5 μl). A gradient elution from 0% to 80% acetonitrile in 15 min was used. The chromatographic profile was recorded and is reported in Fig. 2. An evident alteration of the chromatographic profile is shown by the chromatogram (a) relative to the external loop. Unretained compounds are visible in the dead time disturbance of the external loop profile (a). Evident peak tailing is also observed, especially for the poorly retained bromacil, as a result of the sample methanol elution. It is clearly demonstrated that for slightly higher loop volumes the internal loop promotes solvent mixing and excellent sample focusing as shown by the higher response and better chromatographic performance (chromatogram b).

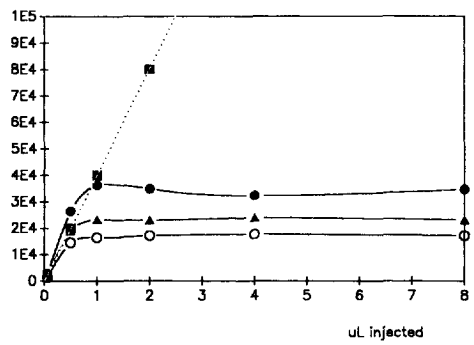

Fig. 1. Calibration plots obtained using methanol as sample matrix: (●) chloridazon, (△) monuron, (○) bromacil; (■) calculated linear calibration plot. Chromatographic conditions: water–acetonitrile, 100:0 to 20:80 in 40 min; flow-rate, 1 μl/min; sample concentration, 30 ng/μl.

In order to evaluate the role played by the solvent used in the sample matrix, a 30 ng/μl solution of

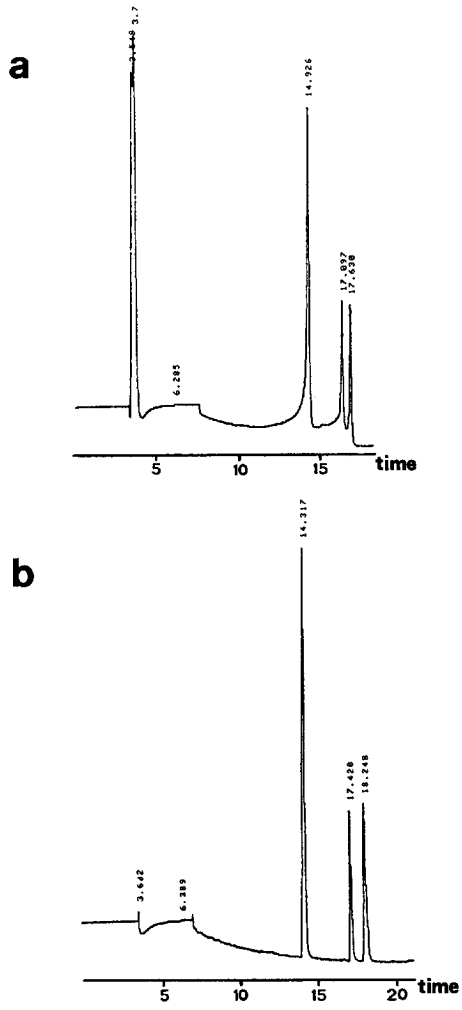

Fig. 2. Comparison of chromatographic separations of bromacil, monuron and chloridazon obtained on injecting 0.5 μl: (a) external loop, (b) internal loop. Chromatographic conditions: water–acetonitrile, 100:0 to 20:80 in 15 min; flow-rate, 1 μl/min; sample concentration, 1000 ng/μl.

chloridazon was prepared with different relative concentrations of methanol–water: (1) 100:0; (2) 50:50; (3) 25:75; (4) 10:90; (5) 3:97 and (6) 0:100. The samples were injected using three different loops: 0.06 μl; 0.5 μl and 4 μl. The influence of the matrix on the extent of the retained solute is clearly evident in Fig. 3. The highest signal among the loops is obtained with pure water. Instead of reporting the absolute peak areas on the y-axis, a relative scale is used with 100 corresponding to the highest signals.

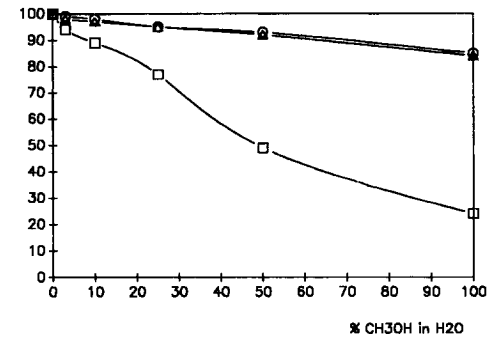

Fig. 3. Effect of sample matrix composition on relative signal response of chloridazon for different injection volumes: (○) 0.06 μl loop, (△) 0.5 μl loop, (□) 4 μl loop. Chromatographic conditions: water–acetonitrile, 100:0 to 20:80 in 40 min; flow-rate, 1 μl/min; sample concentration, 30 ng/μl.

As the relative concentration of methanol increased in the sample matrix, focusing efficiency decreased. As expected, the slope appears steeper with the larger external loop which showed more influence of the eluent properties of the matrix solvent. Both internal loops showed better mixing with the incoming mobile phase and showed only a modest solute loss at the highest concentration of methanol. At 100% methanol, using a 4-μl external loop, signal loss was almost 80%. Larger losses can be expected using bigger loops. It can be pointed out that the use of a high percentage of water in the sample matrix is recommended when external loops of any volume are employed. Solubility in water for the selected compounds is reported in Table 2 [18]. For all of them, appreciable concentration can be reached in pure water without precipitation of solid solute. It is evident that the use of large volume injection is required only when very dilute sample are available. At trace level concentration, almost every organic compound becomes water soluble and therefore can be conveniently injected by a larger loop. Addition of very small quantities of an organic solvent improves solubility without impairing significantly the chromatographic performance. The data so far obtained demonstrated that with an initial mobile phase composed of 100% water, the intensity of the signal depends only on the matrix composition. The retention volume V_R increased with the injected volume V_{inj} according to $V_R = V_{inj} + V_{R^\circ}$, where V_{R° is the retention volume obtained at standard conditions

(0.06 μl injected) [Eq. (1)]. The increase of the retention volume in respect of those observed at 0.06 μl depends only by the size of the loop, since programmed variations of the mobile phase composition in gradient analyses always follow the initial transfer of the loop content. Using water as solvent matrix may not necessarily require starting at 100% water in the mobile phase, since its focusing effect follows that produced by the matrix water. In order to cut down on analysis time, the effect of a different initial mobile phase composition in the gradient analysis was also evaluated. The separation of a large number of basic–neutral pesticides can be conveniently performed starting from 80% instead of 100% water and ending at 20% water in 40 min. A 100% water standard solution of 1.2 ng/μl of chloridazon, bromacil and monuron was injected with an external loop of 20 μl. Fig. 4 shows a comparison between the chromatographic profiles obtained with initial mobile phase compositions of (a) 100% and (b) 80% water. The column was conditioned for 15 min with 100% and 80% water respectively. The use of higher concentrations of organic solvent in the mobile phase can save a considerable amount of run time without affecting the quality of the results.

It can now be pointed out that when using external loops, only the elution strength of the matrix has an influence on the solute band focusing with this type of column. Otherwise, when 100% methanol is used as sample solvent, the combination of an internal loop and a 100% water initial mobile phase is sufficient to completely arrest the solute at the head of the column. Using aqueous samples, solute band focusing is ensured by the matrix. The incoming mobile phase, no matter of which composition, will start the chromatographic process as soon as the loop content is displaced onto the column. A model for different injection techniques is proposed and it is shown in Fig. 5. This model suggests a mechanism for the column–solute interactions inside the capillary system which has been previously described. The experiment shown in Fig. 1 was repeated replacing methanol with water as sample solvent for the three test compounds at a concentration of 12 ng/μl. The solution was injected using a 0.5-μl internal loop and 2-, 4- and 8-μl external loops. Linear calibration plots were now obtained and the

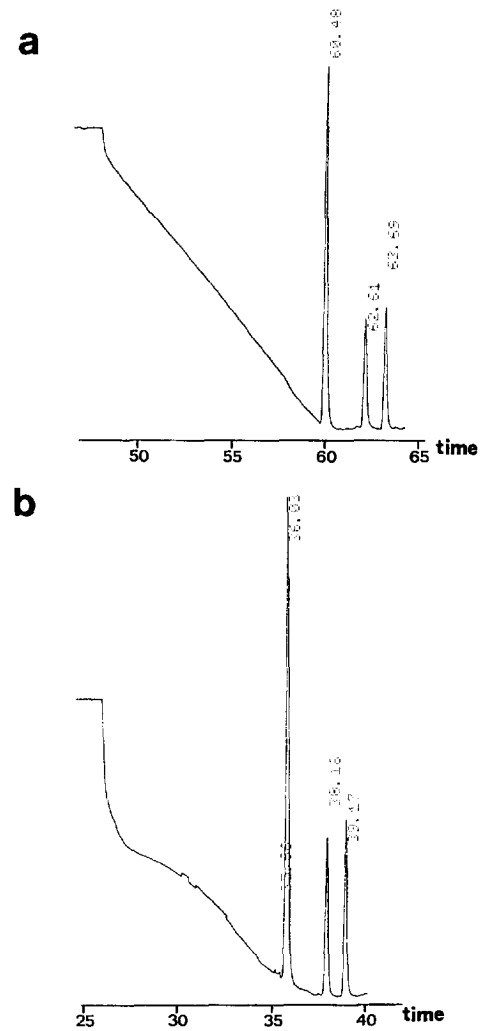

Fig. 4. Chromatographic separations of chloridazon, bromacil and monuron obtained using a 20-μl loop. Chromatographic conditions: (a) water–acetonitrile, 100:0 to 20:80 in 15 min; (b) water–acetonitrile, 80:20 to 20:80 in 15 min; flow-rate, 1 μl/min; sample concentration, 1.2 ng/μl.

relative equations are: chloridazon, $y=29340x-5294$ ($r=0.99670$); monuron, $y=14557x-4056$ ($r=0.99563$); bromacil, $y=8176x-2380$ ($r=0.99553$).

In order to evaluate the reproducibility of large volume injections into a packed capillary column, a 10 ng/l water mixture of bromacil, monuron and chloridazon was injected seven times at 1 μl/min using a 10 μl external loop. The separation of the analytes was performed using solvent gradient conditions starting from water–acetonitrile 20:80 to

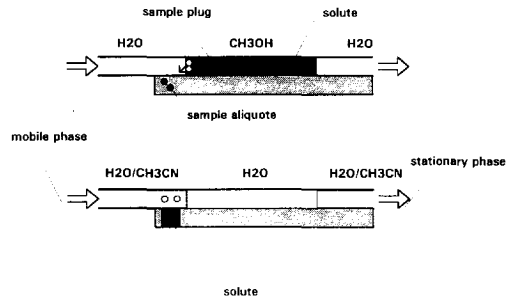

Fig. 5. Schematic representation of the injection process using methanol and water as sample matrix. For discussion see text.

80:20 in 15 min. The data, summarized in Table 3, show an excellent reproducibility in terms of retention time and integrated peak area.

3.2. Acidic samples

Acidic pesticides are advantageously analyzed by HPLC. Some of them are particularly water soluble and when dissolved in an aqueous matrix, loss of solute can be observed. This behaviour is similar to that observed for the neutral pesticides in methanol. Water solubility of these compounds depends largely on the acidic dissociation. Proton dissociation is usually suppressed adding TFA in the mobile phase. Nevertheless, pH modification of the mobile phase marginally affects water-soluble compounds in large volume injections. As illustrated in Fig. 5, retention is achieved only for a small aliquot of solute close to the interface at the rear of the sample plug. Addition of TFA directly to the sample will change solute behaviour toward the stationary phase contributing to solute focusing. Fig. 6 reports a comparison between the analysis of seven acidic pesticides (Table 2) at a concentration of 2 ng/μl using a 20-μl loop without and with addition of TFA in the aqueous sample matrix. TFA was added at a concentration of 0.05% in the sample and in the mobile phase. Acetonitrile was linearly changed from 20% to 80% in 15 min. Because of its higher solubility, dicamba was not retained without TFA and was not present in the first chromatogram. The addition of TFA allowed higher signal response and the correct separation of the seven pesticides.

3.3. Liquid chromatography–mass spectrometry

The ultimate aim of this study was to consistently lower the detection limit of several compounds found at ultra-trace level concentrations in different compartments. A micro-flow-rate particle beam has been shown to improve the overall interface performance, but the small volume injection, required by the micro-HPLC, has affected this method sensitivity. In order to prove the advantage offered by large volume injections, 1 l of tap water was spiked with a standard solution of ten basic–neutral pesticides reaching a concentration in water of 20 ppt. The water was extracted as described in Section 2. The extract was brought to dryness and 100 μl of water were added. The sample was put for a few minutes

Table 3
Reproducibility data obtained at 1 μl/min flow-rate using a 10-μl loop. Run conditions: water–acetonitrile, 20:80 to 80:20 in 15 min. Test mix: 10 ppm in water

	Retention time								
Compound	I	II	III	IV	V	VI	VII	$\bar{x}$	σ
Bromacil	13.84	13.86	13.99	13.94	13.75	13.91	13.77	13.87	0.09
Monuron	16.57	16.41	16.57	16.55	16.32	16.52	16.33	16.47	0.11
Chloridazon	17.44	17.23	17.36	17.35	17.12	17.34	17.13	17.28	0.12
	Peak area								
	I	II	III	IV	V	VI	VII	$\bar{x}$	σ
Bromacil	280.5	272.1	273.3	281.0	276.6	285.7	279.3	278.4	4.73
Monuron	93.9	90.4	90.3	90.6	94.5	97.2	96.5	93.3	2.94
Chloridazon	89.1	86.0	86.1	89.1	92.3	92.3	93.1	89.7	3.00

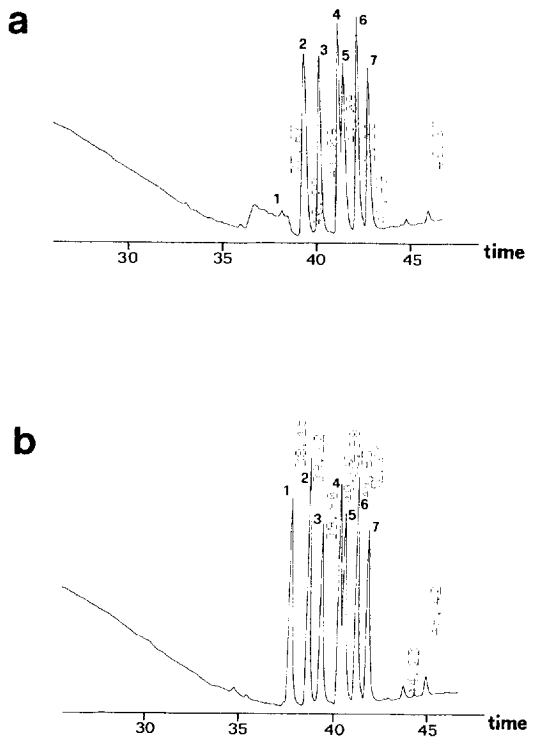

Fig. 6. Chromatographic separation of acidic pesticides using a 20-μl loop without TFA in the sample matrix (a) and with the addition of 0.05% TFA in the sample matrix (b). Peaks: 1 = dicamba; 2 = bentazone; 3 = bromoxynil; 4 = mecoprop; 5 = 2,4,5-T; 6 = MCPB; 7 = 2,4,5-TP. Chromatographic conditions: (water + 0.05% TFA)–acetonitrile, 80:20 to 20:80 in 15 min; flow-rate, 1 μl/min; sample concentration, 2 ng/μl.

into a ultrasonic bath. The sample was then transferred into a 50-μl loop and analyzed with the LC–MS system. The relative reconstructed ion chromatogram is shown in Fig. 7. The result confirmed two important aspects of this method: a good recovery for all the components ranging from about 70% to 90% at this level of concentration and the achievement of a new detection limit of 20 ppt for the selected compounds with a method based on a particle beam interface. It is interesting to note that using the same instrumentation and with the same signal-to-noise ratio, only 1.3 ppb of chlortoluron could be detected in a previous method [7].

In conclusion, this study clarified several aspects of the injection process when larger volumes are transferred in a packed capillary system. The results obtained, even if with a limited class of compounds,

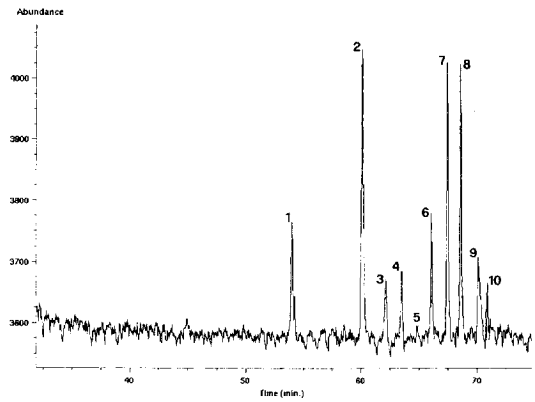

Fig. 7. Reconstructed ion chromatogram obtained from the analysis of tap water spiked with 20 ppt of selected basic–neutral pesticides. Peaks: 1 = chloridazon; 2 = bromacil; 3 = monuron; 4 = carbofuran; 5 = carbaryl; 6 = diuron; 7 = propanil; 8 = chlorbromuron; 9 = azinphos ethyl; 10 = parathion ethyl. Chromatographic conditions: water–acetonitrile, 80:20 to 20:80 in 20 min; flow-rate, 1 μl/min.

proved the validity of this approach for samples with very low concentrations.

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