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Packed-Capillary Supercritical Fluid Chromatography of Pesticides Using Phosphorus-Selective Detection

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Key Words

Supercritical fluid chromatography
Packed-capillary columns
Modified mobile phases
Thermionic detection
Organophosphorus pesticides

Summary

Packed-capillary, supercritical fluid chromatography using carbon dioxide modified with methanol or 2-propanol as mobile phase, was coupled with a thermionic detector for phosphorus-selective detection. Optimum conditions for thermionic detection were established for a number of hydrogen flow rates by adjusting the air flow rate and bead position. Using the mentioned modifiers, a series of organophosphorus pesticides of varying polarity could be separated within 8 min. An optimum sensitivity of 55–128 fg P/s and linearity over four orders of magnitude were obtained. The detector showed good selectivity for phosphorus over carbon and nitrogen, viz., $9 \cdot 10^4$ g C/g P and 75 g N/g P, respectively. This was demonstrated by determining pesticides such as phoxim, dimethoate and azinphos-methyl in onion and tomato extracts.

Introduction

Supercritical fluid chromatography (SFC) is an increasingly valuable separation technique along with gas chromatography (GC) and column liquid chromatography (LC). The main advantages of SFC are the versatility during both separation and detection [4, 5, 6]. Separations can be optimized by proper choice of supercritical fluid – with or without modifier – and the stationary phase, and by density programming achieved via pressure or temperature regulation. This separation technique uses both open tubular columns with diameters between 50 and 100 μ m and packed columns diameters from 0.25 to 4.6 mm. For detection either LC (e.g., UV [1], fluorescence [2]) or GC (e.g., flame ionisation, electron capture, flame photometric or thermionic [3]) detectors can be used.

Nowadays there is a growing interest in packed-column SFC, especially in microbore and packed-capillary SFC, because of the low volumetric flow rates needed. As a result coupling with GC detectors is possible without flow splitting at the column outlet [7]. Compared with open tubular capillary SFC (OTC-SFC), packed-column SFC offers shorter analysis times and larger samples [6]. A disadvantage is a reduction in the number of theoretical plates that can be achieved, due to back pressure and pressure limitations caused by the packing. Another disadvantage with packed SFC is the strong surface activity of the silica-based packing materials. In the case of low solute solubility – e.g. with polar compounds in supercritical carbon dioxide – this will result in high retention and asymmetrical peaks. This effect can be reduced, by (i) the use of polar supercritical fluids such as ammonia or sulphur dioxide which, unfortunately, have rather unfavourable physical and chemical characteristics [6]; (ii) derivatization of the analyte, which also can be used to improve selectivity or sensitivity [8]; (iii) the use of polar modifiers, e.g., methanol, 2-propanol, dioxane or formic acid [9, 10] and (iv) the use of less active packing materials [4, 7].

An attractive characteristic of SFC is that it can be coupled relatively easily to selective GC detectors like the thermionic detector. Such detectors allow the use of organic modifiers. In the case pesticide analysis the sensitive and selective thermionic or nitrogen-phosphorus detector (TID) is the detector of choice. There are several types of TIDs, e.g., the Kolb-Bisschof [11] and Patterson [12, 13] types, which possess different response modes for the detection of nitrogen and phosphorus-containing compounds. This is achieved by changing the polarity of the jet and/or the alkali source with respect to the collector electrode. In the case of P detection the alkali source is charged oppositely to the collector electrode while in the case of N or NP detection both the jet and the alkali source are charged oppositely to the collector electrode. All detectors contain an alkali source heated by a flame (alkali flame ionisation detector), and/or an electrically heated source (flameless alkali-sensitised detector) [14].

In 1983 Fjeldsted et al. [15] were the first to describe the use of a TID for SFC. Next, West and Lee [16] evaluated a Patterson-type TID and David and Novotny [17] characterised a Kolb-Bisschof-type TID, while Ashraf et al. [18]

described the use of a flame-based TID. Both in the flame-based [17, 18] and in the flameless [16] P mode sloping baselines were observed when pressure programming was applied. This limits the use of pressure programming when low levels of analytes have to be determined. Only few applications have been reported so far. These include the determination of α -keto acids (as quinoxalinol derivatives) [8], steroids (as their thiophosphinic ester derivatives) [19], amino compounds (e.g., toluene diamines and their perfluorobutyl derivatives and trialkylamines) [20], and agrochemicals (e.g., triazines, pyrethroids, benzophenyl urea, and vinyl-phosphone) [18]. All these studies were performed with OTC-SFC.

On account of the advantages of packed-capillary column SFC (above), the combination with selective TID is studied. Using this detector modified carbon dioxide (e.g., methanol, 2-propanol) can be used as mobile phase. Phosphorus-containing pesticides (for structures, see Figure 1) of varying polarity are used as model compounds because so far only limited research has been done on the detection of such compounds with SFC-TID. The effect of several parameters on the detector performance is evaluated. The selectivity and sensitivity of the system is demonstrated by the determination of organophosphorus pesticides in onions and tomatoes.

Experimental

Instrumentation

A Phoenix-20 syringe pump (Carlo Erba Strumentazione, Milan, Italy) was used for delivery of the mobile phase and pressure control. Samples were injected manually using a 60 nl Valco injection valve (Type CI4W, Schenk, Switzerland). The 130 $\times$ 0.32 mm I.D. fused silica capillary column (Chrompack, Middelburg, the Netherlands) was packed with 5 μ m LiChrosorb RP-18 (Merck, Darmstadt, Germany) and connected with a zero dead volume union (Valco) to a 13.5 cm $\times$ 10 μ m I.D. fused silica, linear restrictor (SGE, Ringwood, Australia) for pressure restriction. The main part (11 cm) of the restrictor was situated in the detector base which was heated by an Ether type 17-90B heat controller. For detection a Carlo Erba NPD-40 thermionic detector described by Verga [21] with a Carlo Erba Model 180 electrometer and a BD 40 recorder (Kipp & Zonen, Delft, the Netherlands) were used. The NPD-40 contains a rubidium salt bead located between the polarised jet and a cylindrical collector electrode. The position of this thermionic source with respect to the flame can be adjusted for selectivity and sensitivity regulation. The NPD-40 was used in the P mode only. The column temperature was controlled by a thermostatted waterbath.

The syringe of the Phoenix pump was filled with carbon dioxide from a cylinder with dip tube. Modified mobile phases were prepared by adding a known volume of modifier at the top of the syringe followed by introduction of the carbon dioxide. During filling, the syringe was water-cooled (4 $^{\circ}$ C) to obtain higher filling percentages. All modifier percentages are expressed as % mol mol⁻¹. These percentages were calculated with interpolated carbon dioxide

Figure 1

Structures of model pesticides: (1) malathion, (2) phoxim, (3) ethion, (4) dimethoate, (5) azinphos-methyl.

densities at 140 bar and various temperatures, from the tabulated data of Angus et al. [22], and the known densities for methanol and 2-propanol. Critical temperature, T_c , and critical pressures, P_c , for binary mixtures were calculated by the Chueh-Prausnitz method and the Kreglewski-Kay method, respectively [23]. T_c and P_c values for 1.5 % methanol, 5.7 % methanol and 3.5 % 2-propanol were 309 K/78 bar, 323 K/91 bar, and 320 K/84 bar, respectively. Freshly prepared mixtures in the syringe were allowed to stabilise overnight and the column was equilibrated for 45 min (180 bar) before use. Linear velocities were calculated from t_0 values of pentane or acetone. Mobile phase flow rates were calculated assuming the porosity of the stationary phase, ϵ , to be 0.6.

Preparation of Vegetable Extracts

A portion of 50 g tomatoes or onions was chopped and blended with 90 ml ethyl acetate in a universal food cutter for 15 min. After vacuum filtration the aqueous layer was discarded. The organic layer was dried over anhydrous sodium sulphate and filtered through a 0.2 μ m filter (Schleicher & Schuell, Dassel, Germany). Aliquots of 5 ml were evaporated at 50 $^{\circ}$ C under a stream of dry nitrogen and the residues redissolved in 4.5 ml acetone. The extracts – which were clear orange-red and yellow for tomatoes and onions, respectively – were spiked by adding 0.5 ml of a standard mixture of the pesticides in acetone. Injections of 60 nl were performed.

Chemicals

Carbon dioxide (99.5 %, technical-grade) was obtained from Hock Loos (Schiedam, the Netherlands). HPLC-grade acetone, methanol, ethyl acetate, hexane, pentane and sodium sulphate were from Baker Chemicals (Deventer, the Netherlands). 2-Propanol was from Rathburn (Walkerburn, Scotland, UK). Phoxim [α [(diethoxyphosphinothionyl)oxy]imino]benzeneacetonitrile and dimethoate [O,O-dimethyl-S-[2-(methylamino)-2-oxoethyl]phosphorodithioate] were by Riedel de Haen (Seele, Germany) and malathion [diethyl(dimethoxyphosphino-

thionyl)-thiobutanedioate} by Chrompack. Ethione [O,O,O',O'-tetraethyl-S,S'-methylene di(phosphorodithioate)] and azinphos-methyl [O,O-dimethyl-S-[4-oxo-1,2,3-benzotriazin-3(4H)yl)methyl]phosphorodithioate] were gifts from the Governmental Food Inspection Service (Alkmaar, the Netherlands). Ethione and azinphos-methyl were of technical-grade and the other pesticides of analytical-grade quality. N,N-dimethylaniline (99 %), hexadecane and triphenylphosphate were from Aldrich (Brussels, Belgium), Poly Science (Niles, IL, USA) and Merck, respectively.

Results and Discussion

Detector Characterisation

The rubidium source in the Carlo Erba NPD-40 is heated by a flame. The bead temperature and therefore the response, depends on the hydrogen and air flow rates and the bead position with respect to the jet [14]. The hydrogen and air flow rates determine the flame energy and the flame geometry. First the influence of the mobile phase composition on detector performance was studied. Then, in order to optimize detector performance, the hydrogen flow rate was varied at a constant air flow rate; next the air flow was varied at a constant hydrogen flow and, finally, the hydrogen/air flow rates were varied while keeping their ratio constant. The detector performance was studied with respect to background current, noise level and signal intensity.

Comparison of detector noise and background current (I_{bg}), under different sets of TID conditions (cf. above) showed that the detector noise is a fixed percentage of I_{bg} , viz. $(0.15 \pm 0.05) \%$, as long as I_{bg} is over 10 pA. At lower background currents the noise increased relative to I_{bg} .

Influence of Modifier on Background Current

Using pure carbon dioxide as the mobile phase (Figure 2A, curve 1), I_{bg} increased when the bead was moved downward towards the jet – i.e., when $d_{(jet-bead)}$ was decreased – independent of the hydrogen and air flow rates, which were varied between 25–50 ml min⁻¹ and 200–425 ml min⁻¹, respectively. The responses observed upon injecting 2-propanol, ethanol and hexane depended on the bead position and hydrogen and air flow. For a given hydrogen flow, the response vs. $d_{(jet-bead)}$ curves for the three organic solvents were essentially the same. Equal curves were found when the air flow was changed from 350 to 425 ml min⁻¹. In all cases a positive response with a maximum at relatively high bead positions and a slight but significant negative response at low bead positions (Figure 2B) were found. Negative responses for carbon-containing compounds can be advantageous in terms of detection selectivity. The jet-bead distance providing maximum response and the position where the response changed from positive to negative, varied with the detector conditions. For example, the larger the excess of air, the smaller $d_{(jet-bead)}$ has to be in order to obtain a negative response. Negative responses for carbon-containing compounds have been observed with the Carlo Erba NPD-40 also in GC [21], and have been reported for alkali flame ionisation detectors [14].

Figure 2

Background current (I_{bg}) and response of carbon compounds ($p = 140$ bar, $T = 46^\circ\text{C}$) versus distance between flame jet and bead ($d_{(jet-bead)}$). (A) 1, mobile phase 100 % CO_2 , $\text{H}_2 = 40$ ml min⁻¹, air = 400 ml min⁻¹; 2 and 3, mobile phase $\text{CO}_2/3.5\%$ 2-propanol, $\text{H}_2 = 35$ ml min⁻¹; 2, air = 350 ml min⁻¹; 3, air = 425 ml min⁻¹. (B) Mobile phase $\text{CO}_2/3.5\%$ 2-propanol, $\text{H}_2 = 35$ ml min⁻¹; 1, hexane, air = 350 ml min⁻¹; 2, 2-propanol, air 350 ml min⁻¹; 3, 2-propanol, air 425 ml min⁻¹; Injection volume, 60 nl.

When using carbon dioxide modified with 2-propanol (3.5 %), decreasing $d_{(jet-bead)}$ initially caused I_{bg} to increase, and for still lower bead positions, to decrease (Figure 2A, curves 2 and 3). The decrease started to occur at bead positions for which diminishing responses for carbon compounds were observed (cf. Figure 2B). That is, when using modified carbon dioxide, the system I_{bg} is the combined result of the I_{bg} obtained with pure carbon dioxide as mobile phase and the positive or negative modifier response. As the modifier response changes in a complicated way when varying the TID parameters, accurate prediction of the I_{bg} behaviour is rather difficult.

Influence of Detector Parameters on Detection

In Figures 3A and B the effects of changing $d_{(jet-bead)}$ on the phosphorus response (analyte, malathion) and the signal-to-noise (S/N) ratio are shown for three different hydrogen flow rates at a constant air flow of 300 ml min⁻¹. The malathion response sharply increases with increasing hydrogen flow due to increasing flame energy (Figure 3A).

Figure 3
Effect of detector parameters on response (A, C) and signal-to-noise ratio (B, D) versus distance between flame jet and bead ($d_{(\text{jet-bead})}$). Mobile phase CO₂/3.5 % 2-propanol, $p = 140$ bar, $T = 46$ °C; A and B, effect of varying H_2 flow at constant air flow (300 ml min⁻¹); C and D, effect of air flow at constant H_2 flow (35 ml min⁻¹).

However, under these conditions I_{bg} increased even more and, consequently, the best S/N ratios were found at intermediate values, i.e. 30–35 ml min⁻¹ hydrogen (Figure 3B).

With increasing hydrogen flow the response maximum shifted to higher bead positions (Figure 3A). This indicates that a certain flame energy is needed to heat the bead to obtain optimum phosphorus response. The energy transfer can be regulated by changing $d_{(\text{jet-bead})}$. Increasing hydrogen flow will lead to higher flames and therefore higher optimum bead positions.

Next, the effect of the air flow was studied at constant hydrogen flow of 35 ml min⁻¹. Figure 3C shows that the malathion response increased strongly with increasing air flow. The influence of $d_{(\text{jet-bead})}$ on the phosphorus response is rather high in case of high air flow; besides the response maximum shifts to lower bead positions with increasing air flow. This can again be explained by the energy transfer from flame to bead which is influenced by the air flow. The variation of the air flow causes changes in flame geometry, leading to a higher response at higher air flow, and the cooling effect of the air leads to a shift in response maximum. High as well as low air flow caused relatively low S/N ratios (Figure 3D) due to high I_{bg} and a low phosphorus response, respectively. Therefore it is necessary to optimize both air flow and bead position in order to obtain a high S/N ratio.

Finally, the detector performance was studied for constant-ratio flames. Changing the hydrogen and air flow (hydrogen/air, 25/195, 35/275 and 45/355 ml min⁻¹) while keeping their ratio constant (1/7.9) resulted in similar response curves as obtained in Figure 3A. However, the differences in height of the response maxima were significantly larger. When going from 25 to 35 and from 35 to 45 ml min⁻¹ hydrogen, the malathion response increased 11-fold and 6-fold, respectively. In similar series of experiments with other hydrogen/air ratios the response also increased upon going to larger flames. When also taking into account the dependence of the noise on the hydrogen/air ratio (data not shown), one finally arrives at the results in Table I. As seen from this table, the same optimum S/N ratio (given as the calculated limit of detection) can be obtained for almost every hydrogen rate between 30 and 50 ml min⁻¹ by properly adjusting the air flow and the bead position. By adjusting these parameters the energy transfer from flame to bead is regulated (cf. above). In order to obtain high S/N ratios when going to higher hydrogen flow rates it is necessary to use less air and to increase $d_{(\text{jet-bead})}$. However, at hydrogen flows higher than about 40 ml min⁻¹ significant wander of the background signal occurred; therefore, hydrogen rates of 30–35 ml min⁻¹ were used in all further experiments.

Restrictor

Owing to the dimensions of packed-capillary columns, the same restrictors can be used as in OTC-SFC. Although in OTC-SFC tapered restrictors are generally preferred [3], a linear restrictor is satisfactory provided efficient heating is applied, e.g. by an extra heating block [24]. The major part of the linear restrictor used in this study, is situated in the

Table 1 Calculated limits of detection (via $S/N = 3$) in SFC-TID using different hydrogen flow rates*.

H ₂ (ml min ⁻¹)	Air (ml min ⁻¹)	d _(jet-bead) (mm)	Calculated limit of detection (pg)
25	305	1.8	3.0 ± 0.5
32	255	2.4	2.0 ± 0.5
35	300	2.4	1.5 ± 0.5
40	275	2.6	2.0 ± 0.5
45	230	3.2	2.0 ± 0.5
55	230	3.9	2.5 ± 0.5

* Conditions: detector temperature, 350 °C; column: 120 × 0.32 mm I.D. capillary packed with 10 µm LiChrosorb RP-18; mobile phase: CO₂/3.5 % 2-propanol, 140 bar, 55 °C; 60 nl injections containing 25 ng malathion in hexane

Figure 4
Effect of restrictor temperature on peak area of 150 ng injections of malathion and triphenyl phosphate. Restrictor outlet 5 mm below jet. Mobile phase: CO₂/5.7 % methanol at 140 bar and 55 °C.

detector block and can therefore be almost entirely heated. The influence of the restrictor temperature – i.e., the detector temperature – on peak area and peak shape has been studied for malathion and triphenyl phosphate (Figure 4). The response decreased for detector temperatures below 300 °C, probably due to precipitation of the compounds in the restrictor. Especially with triphenyl phosphate, spikes were observed in the chromatogram at temperatures below 200 °C. By diluting the sample or increasing the pressure, the response could be partly restored and spiking could be reduced. Spikes are thought to be caused by cluster formation in the restrictor during decompression [25].

The influence of the distance of the restrictor outlet to the jet tip was investigated by studying the response to 60 ng injections of malathion at restrictor temperature of 350 °C; other conditions were as in Figure 4. When the restrictor outlet was situated at or above the jet tip there was no signal at all: the analyte molecules are apparently blown through the flame without being decomposed or detected. When the outlet was situated slightly below the jet tip (between 1 and 10 mm) the response was constant and a maximum. For positions more than 10 mm below the jet the response decreased again. The position of the restrictor outlet was kept at between 1 and 5 mm below the jet tip in all further experiments.

Figure 5
Linear flow rate (cm s⁻¹) and mass flow rate (ml min⁻¹) versus pressure of 130 mm × 10 µm I.D. linear restrictor.

The effect of the detector temperature and the applied pressure on the linear flow and the mass flow of the system are shown in Figure 5. As can be seen, as a result of the compressibility of the supercritical fluid, when going to higher pressures the increase in mass flow is larger than the increase in linear flow. The decrease of both parameters upon temperature increase is caused by the increasing viscosity of the mobile phase which leads to higher back pressures. The linear flows are below the typical value of 0.6 cm s⁻¹ reported by Schoenmakers [26]; for this packed-capillary column that is, faster analyses should be possible using wider linear restrictors or other types of restrictors. This aspect was not further studied in the present project.

Chromatography

A 130 × 0.32 mm I.D. fused silica capillary packed with 5 µm LiChrosorb RP-18 was used for SFC. Using pure carbon dioxide as the mobile phase the five pesticides selected as test compounds could not be eluted within a reasonable period or did not even elute at all. Moreover, severe peak tailing was observed caused by interaction of the polar functional groups of the analytes – i.e., their secondary amine function – with residual silanol groups of the silica packing [10, 27]. Therefore, a modifier had to be used. In contrast with SFC-FID [6], the use of modifiers in

SFC-TID is not expected to limit significantly detector performance because of the detector selectivity.

With a low proportion of methanol (1.5 %), all compounds could be eluted relatively fast at moderately high pressures ($t_r < 6$ min at $p = 150$ bar and $T = 55^\circ\text{C}$). Deactivation of the active sites of the packing material by the polar modifier is mainly responsible for this effect; the increase in solvent strength upon addition of small amounts of modifier is only slight and will play only a minor role in reducing retention [9]. In agreement with this, capacity factors, k' , generally decreased only slightly when the methanol content was increased from 1.5 to 5.7 % (Table II). Two exceptions were noted, viz. azinphos-methyl and dimethoate. These compounds each possess a highly polar site which probably can compete effectively with modifier molecules for the active sites on the packing material. It is also obvious from Table II that the density of the mobile phase is an important parameter when optimizing the SFC separation. Dimethoate and azinphos-methyl, with their polar sites, also showed the largest effects when changing the pressure: using carbon dioxide at 150–180 bar modified with 3.5 % 2-propanol, reversed the elution order of these compounds compared to separations with carbon dioxide modified with 5.7 % methanol at 150–180 bar. Such effects are difficult to predict, but they clearly demonstrate that modifiers can be used to improve selectivity. Malathion, phoxim and ethion gave symmetrical peaks (asymmetry factors ≤ 1.4), but dimethoate and azinphos-methyl showed some tailing. With methanol as modifier tailing was less than with 2-propanol; for example, for dimethoate the asymmetry factor was 2.3 with carbon dioxide/1.5 % methanol and 3.7 with carbon dioxide/3.5 % 2-propanol. The asymmetry factor varied slightly ($\pm 10\%$) with the applied pressure.

All five test compounds could be separated with the three modified mobile phases mentioned above. Methanol was preferred as modifier because of the better peak shape for dimethoate and azinphos-methyl. The separations are discussed in the section on analytical aspects below.

As pressure programming is commonly applied in SFC, the influence of pressure on the background current, I_{bg} , was examined. Using the conditions found after optimization (see above)—i.e., a hydrogen flow of 35 ml min^{-1} , an air flow of 300 ml min^{-1} , and a $d_{(\text{jet-bead})}$ of 2.4 mm—carbon com-

pounds showed a negative response. The presence of a modifier in pure carbon dioxide will therefore reduce the background current compared with pure carbon dioxide as mobile phase. Consequently, an even larger background reduction was expected for higher percentages of modifier or higher flow rates (higher pressures). However, the opposite effect was observed. This is probably due to a change in the flame size and/or flame geometry caused by combustion of the organic modifier. A pressure increase from 120 via 150 to 180 bar resulted in an increase of I_{bg} from 1.4 via 3.4 to 8.6 pA, from 4.3 via 9.9 to 23 pA, and from 0.6 via 0.9 to 1.9 pA for carbon dioxide modified with 1.5 % methanol, 5.7 % methanol and 3.5 % 2-propanol, respectively. That is, pressure programming will lead to positively sloping baselines. With methanol as modifier this will severely complicate the use of pressure programming when trace-level amounts of analytes have to be determined. With 2-propanol the slope is much less pronounced (cf. refs. [16, 17]) and pressure programming seems possible without really adverse effects on detection sensitivity.

Analytical Aspects

The linearity of the SFC-TID system was studied using the same experimental conditions as above. It should be emphasized here that, in order to obtain a large linear dynamic range, the central hole in the disc-shaped bead should be carefully positioned over the jet orifice. Linearity was studied for malathion and phoxim dissolved in acetone and in a tomato extract, using carbon dioxide modified with 5.7 % methanol as mobile phase. The calibration plots (0–100 ng injected, $n = 11$) are given by:

Malathion

acetone $y = 41 (\pm 23) + 137.8 (\pm 0.7) x$ ($r = 0.9998$)

tomato extract $y = 1.3 (\pm 6.5) + 144.1 (\pm 0.2) x$ ($r = 0.9997$)

Phoxim

acetone $y = 29 (\pm 29) + 119.9 (\pm 0.9) x$ ($r = 0.9997$)

tomato extract $y = -28 (\pm 15) + 121.8 (\pm 0.4) x$ ($r = 0.9997$)

where x is the amount of analyte injected and y is the peak height. No significant differences in the slopes of the calibration curves were observed, indicating the absence of matrix effects. Under the conditions used, the relative standard deviation in peak height for 310 pg injections of

Table II. Capacity factors under different chromatographic conditions*,**.

Analyte	k' value at								
	120 bar			150 bar			180 bar		
	methanol 1.5 %	5.7 %	IPA 3.5 %	methanol 1.5 %	5.7 %	IPA 3.5 %	methanol 1.5 %	5.7 %	IPA 3.5 %
Mal	5.4	4.9	4.3	1.2	1.0	1.1	0.6	0.5	0.6
Phox	7.0	6.2	5.8	1.5	1.4	1.4	0.8	0.8	0.8
Eth	12.0	10.1	9.8	2.3	2.2	2.2	1.3	1.2	1.2
Dim	18.7	12.7	18.8	5.7	3.5	7.6	3.4	2.2	4.2
APM	33.4	25.1	26.7	6.1	5.0	5.9	3.2	2.8	3.2

* Column and TID conditions as Figure 7.

** Abbreviations: Mal = malathion, Phox = Phoxim, Eth = ethion, DIM = dimethoate, APM = azinphos-methyl and IPA = 2-propanol.

Table III Limits of detection in SFC-TID using different chromatographic conditions*, **.

Analyte	Limit of detection (S/N = 3) for					
	1.5 methanol/140 bar		5.7 methanol/130 bar		3.5 % IPA/150 bar	
	(pg)	(fg P/s)	(pg)	(fg P/s)	(pg)	(fg P/s)
Mal	18	68	44	130	15	69
Phox	26	110	69	210	19	82
Eth	15	68	43	150	11	55
Dim	40	99	82	190	60	115
APM	62	120	210	280	63	130

* Column and TID conditions as Figure 7.

** Abbreviations: Mal = malathion, Phox = Phoxim, Eth = ethion, DIM = dimethoate, APM = azinphos-methyl and IPA = 2-propanol.

malathion was 3.7 % ($n = 10$). Both with 2-propanol and methanol-modified carbon dioxide (130–140 bar, 55 °C), the detector response was linear over four orders of magnitude.

The lowest detection limits were obtained with carbon dioxide modified with 1.5 % methanol or 3.5 % 2-propanol, viz. 55–128 fg P/s ($S/N = 3$) depending on the compound tested (Table III). The higher I_{bg} for 5.7 % methanol-modified carbon dioxide caused a two-fold decrease in sensitivity compared to the other mobile phases used. As combustion is carried out in a flame-based TID, the original molecular structure of the analyte and the matrix have little effect on response; therefore, the detection limits do not differ significantly for the five pesticides. This is in marked contrast with systems using flameless TIDs for which large variations in detection limits have been reported [16].

In order to study the selectivity of the TID for phosphorus-containing compounds over carbon-containing and nitrogen-containing compounds, peak areas of malathion, hexadecane and N, N-dimethylaniline were compared. To achieve optimum selectivity, carbon compounds should be identified as negative peaks (Figure 6). N, N-dimethylaniline did not show up as a negative peak; plotting the response against $d_{(jet-bead)}$ resulted in essentially the same curve as obtained for malathion (cf. Figure 3A). Good selectivity of phosphorus over carbon and nitrogen was obtained, viz. $9 \cdot 10^4$ g C/g P and 75 g N/g P, respectively, which is in marked contrast with the rather poor selectivity reported by other workers [17]. An additional advantage of the low or negative response of carbon compounds is the absence of a large solvent peak which allows the determination of early eluting analytes.

The five pesticides used as test compounds in this study could be analysed under the chromatographic conditions given in Table III. For all mobile phase compositions mentioned in the table it was possible to select a pressure which led to good separation of the five compounds within 8 min. As can be seen from Figure 7 the elution order is changed by using 2-propanol. (Figure 7B) instead of methanol (Figure 7A) as modifier in carbon dioxide.

In order to demonstrate the selectivity and sensitivity of the system for real samples, pesticides dissolved in a vegetable extract were analysed. These were obtained by a single

Figure 6

Selectivity of SFC-TID system. (1) 5.9 ng N,N-dimethylaniline, (2) 0.5 ng malathion, (3) 6100 ng hexadecane. Column: 130 × 0.32 mm I.D. capillary packed with 5 μ m LiChrosorb RP-18. Mobile phase: CO₂/5.7 % methanol, 130 bar, 55 °C. TID conditions: (A) 35 ml H₂ min⁻¹, 425 ml min⁻¹, $d_{(jet-bead)}$, 3.4 mm; detector range, 160 pA full scale; (B) 35 ml H₂ min⁻¹, 300 ml air min⁻¹, $d_{(jet-bead)}$, 2.4 mm; detector range, 16 pA full scale.

extraction with ethyl acetate, a simple sample treatment which is far less time-consuming than the clean-up procedures normally used for GC [28]. Since acetone was a better solvent for some of the pesticides, the ethyl acetate was evaporated and the residue dissolved in acetone. Even in 10-fold concentrated blank extracts no positive or negative peaks other than the acetone signal were observed. The detection limits for all pesticides tested were the same as with the standard solution (see Table III). The chromatograms shown in Figure 6 for spiked onion and tomato extracts nicely illustrate that packed-capillary SFC with flame-based TID detection is a promising approach for the trace-level determination of organophosphorus pesticides in biological samples.

Figure 7

SFC-TID of organophosphorus pesticides. Column: 130×0.32 mm I.D. capillary packed with 5 μ m LiChrosorb RP-18. TID conditions: detector temperature, 300 °C; 35 ml H_2 min^{-1} ; 300 ml air min^{-1} ; $d_{(\text{jet-bead})}$, 2.4 mm. (0) contamination in ethion, (1) malathion, (2) phoxim, (3) ethion, (4) dimethoate, (5) azinphos-methyl. (A) 1 ng/compound in onion extract. Mobile phase: CO_2 /1.5 % methanol, 140 bar, 55 °C. Detector range, 4 pA full scale. (B) 0.2 ng/compound in tomato extract. Mobile phase: CO_2 /3.5 % 2-propanol, 150 bar, 55 °C. Detector range, 2 pA full scale.

Conclusions

The performance of a flame-based TID for use in SFC has been systematically studied and optimized for the detection of phosphorus-containing compounds. From these studies it is clear that the performance of the TID depends on the energy transfer from flame to alkali bead. This energy transfer can be regulated by the flow rates of air and hydrogen and by the distance between jet and bead, $d_{(\text{jet-bead})}$. For each setting of the hydrogen flow between 25–50 ml min^{-1} it is possible to obtain the same optimum S/N ratio by adjusting the air flow and $d_{(\text{jet-bead})}$. In this study a hydrogen flow of 35 ml min^{-1} was used while the air flow and $d_{(\text{jet-bead})}$ were 300 ml min^{-1} and 2.4 mm, respectively. Under these conditions the selectivity of phosphorus over carbon is $9 \cdot 10^4$ g C/g P and of phosphorus over nitrogen 75 g N/g P. The sensitivity is excellent with typical values of 50–150 fg P/s and the response is linear over four orders of magnitude.

The packed-capillary SFC system used allows the separation of a series organophosphorus pesticides of varying polarity within 8 min. The limits of detection for the pesticides range from 15 to 65 pg. The good selectivity of the system was demonstrated by determining the pesticides in

onion and tomato extracts, which were obtained by a one-step extraction procedure. In view of the promising results obtained in the present study, future research will be devoted to extend the application range of the present SFC-TID system, both with regard to the analytes and the sample types studies.

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