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IMPROVED DESIGN AND APPLICATIONS OF AN ON-LINE THERMIONIC DETECTOR FOR NARROW-BORE LIQUID CHROMATOGRAPHY

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SUMMARY

The improvement and application of a previously reported on-line thermionic detection system for reversed-phase liquid chromatography (LC–TID) are described. Modifications have been made to simplify the introduction of the LC effluent to the evaporation interface. The vaporized effluent is directed into the base of a gas chromatographic–thermionic detection (GC–TID) system via a heated fused-silica capillary and is swept into the detector using a minimal flow of nitrogen gas. Minimum detectable amounts of 0.2–0.5 pg/s of phosphorus were achieved for a variety of phosphorus-containing compounds, including several polar pesticides. Repeatability, at a level of 5 ng of injected compound, gives relative standard deviations ranging from 2 to 5%. Applications are reported for the determination of polar phosphorus pesticides in complex plant matrices. Results obtained with the unmodified detector are compared with those obtained using the present modified system.

INTRODUCTION

The use of gas chromatographic (GC) detectors in high-performance liquid chromatography (LC) has recently received increasing attention owing to the growing need for high detection sensitivity and selectivity in LC. Unfortunately, the continuous introduction of aqueous and organic eluents at the millilitre per minute flow-rates typical of conventional LC has caused numerous technological problems. Over the years, both belt[1] or wire transport[2] and direct effluent introduction[3] devices have been employed in attempts to couple the popular flame-based GC detectors or the mass spectrometer to LC systems.

The advent of miniaturized LC has alleviated many of the difficulties associated with direct mobile phase introduction, as its very low flow-rates cause only minor detection disturbances. The utilization of flame ionization[4,5], flame emission[6], thermionic[7–10], electron-capture[11,12] and mass spectrometric[13] detectors for microcolumn LC has been described previously. However, the application of these systems to compounds that are not readily amenable to GC analysis has proved both difficult and

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expensive. In addition, coupling these typical gas-phase detectors to liquid-stream analyses frequently results in a loss of sensitivity in comparison with GC.

A thermionic detector based on the design of Kolb and Bischoff[14] was recently described[10] for the element-specific detection of phosphorus-containing compounds. While this detector was linear over 3.5 orders of magnitude, gave a minimum detectable amount in narrow-bore reversed-phase LC of 3 pg/s of phosphorus for triethyl phosphate and could easily handle mobile phase flow-rates of up to 40 μl/min[10], its sensitivity remained considerably less than the 0.02 pg/s of phosphorus specified as the GC detection limit of the thermionic detector. Also, the background noise level tended to vary with time, and only applications involving analytes of high volatility were reported.

In this investigation, modifications were made to the geometry of the liquid chromatograph–thermionic detector evaporation and transport interface such that significant improvements in both sensitivity and stability have been achieved. In addition, several polar pesticides were determined in spiked fruit and vegetable samples. Comparisons are made between UV and thermionic detection (TID) for these mixtures and the results obtained using the unmodified, compared with the improved, LC–TID system are reported.

EXPERIMENTAL

Materials

The solvents were of HPLC-grade quality (Baker, Deventer, The Netherlands) and were passed through a filter of 0.45 μm pore diameter before use. Analytical grade triethyl phosphate and triethyl thiophosphate were obtained from Aldrich Europe (Beerse, Belgium). Analytical grade malathion and ethyl-parathion, tributyl phosphate and fenitrothion were purchased from Chrompack (Middelburg, The Netherlands), Merck (Darmstadt, F.R.G.) and Riedel-de Haen (Seelze, F.R.G.), respectively. Technical grade coumaphos, demeton-O, dimethoate, ethyl-azinphos, ethyl-paraoxon, methyl-paraoxon, o-methoate and trichlorfon were obtained as gifts from the Governmental Food Inspection Service (Alkmaar, The Netherlands).

Sample preparation

Sample pre-treatment was carried out using a modified procedure derived from that reported by the Association of Official Analytical Chemists[15] and routinely used by the Governmental Food Inspection Service of The Netherlands (Alkmaar)[16]. The modified procedure, which avoids column clean-up steps, is as follows. A 15-g amount of either red cabbage or tomato (including skin and seeds) was ground with 10 ml of acetone in a small blender, 5 ml each of light petroleum and dichloromethane were added and the mixture was spiked with the pesticide standards (*ca.* 3–5 ppm). After thorough mixing and centrifugation, the organic layer was removed and evaporated nearly to dryness. Methanol was added to yield a final solution volume of 0.5 ml for analysis using the original system and UV detection and 1.0 ml when the modified detector was employed.

Chromatographic system

Glass-lined stainless-steel columns (GLT; 200 × 0.7 mm I.D.; SGE, Melbourne, Australia), laboratory-packed with 5 μm LiChrosorb RP-18 (Merck), were used. Band-broadening studies were conducted using a flow injection system directly coupled to the evaporation interface. A high-pressure pump (Model 302; Gilson, Villiers-le-Bel, France) coupled with a laboratory-made membrane pulse damper provided stable eluent delivery. Samples were directly introduced via a laboratory-made micro-injection valve having both 0.05 and 0.5 μl internal loops. The gas chromatograph (Packard Model 427; United Technologies, Delft, The Netherlands) coupled to the unmodified detector contained a glass GC column (100 cm × 2 mm I.D.; Chrompack; packed in-house with 3% OV-101 on 80–100 mesh Chromosorb W HP). The GC carrier gas was nitrogen at 30 ml/min.

Thermionic detector

As described previously[10], the thermionic detector was a Packard Model 905 TID, the rubidium silicate bead of which was electrically heated by a Packard Model 612 detector controller. The evaporation interface was located in the detector block, which was maintained at 300°C. In the original system, this interface was coupled to the LC column by an unheated fused-silica capillary (SGE) with an inner diameter of 120 μm[10]. The modified system differed in its interface and coupling to the LC column as described below and illustrated in Fig. 1. The air flow-rate was maintained at 200 ml/min, the hydrogen flow-rate at 5 ml/min and the background current (i_{bc}) at 2 pA in both detectors. The GC oven temperature was maintained at 275°C throughout.

RESULTS AND DISCUSSION

LC–TID interfacing

Several interface modifications were examined in the optimization of the LC–TID system. Initially, it was desired to maintain a flexible system having both GC and LC capabilities. The first alteration made to the old system (see Fig. 1) was to carefully separate and independently regulate the nitrogen flows through the GC column and the LC interface. It was subsequently noted that the signal-to-noise ratios were improved by approximately a factor of 2 owing to increased response sensitivity as the GC column flow-rate approached that of a capillary column (*i.e.*, 1–4 ml/min). As an extension of this finding, the packed GC column was replaced with a capillary column terminating within the quartz flame jet. Unfortunately, the detector response in the LC–TID mode was not stable in this configuration, and excessively high noise levels were frequently obtained. Consequently, the GC column was removed. Instead, the vaporized effluent from the LC column was introduced into the flame jet via a fused-silica capillary (150 × 0.12 mm I.D.) in place of the capillary GC column, as shown at the bottom of Fig. 1B. This capillary (Fig. 1) was connected to the termination of the stainless-steel interface within the GC oven and was inserted from the bottom of the thermionic detector approximately half way up the flame jet.

Finally, in order to maximize the heat transfer efficiency and to simplify coupling, the termination of the LC column was directly connected to the stainless-steel interface. This change is illustrated by comparing the top parts of Fig. 1A and B.

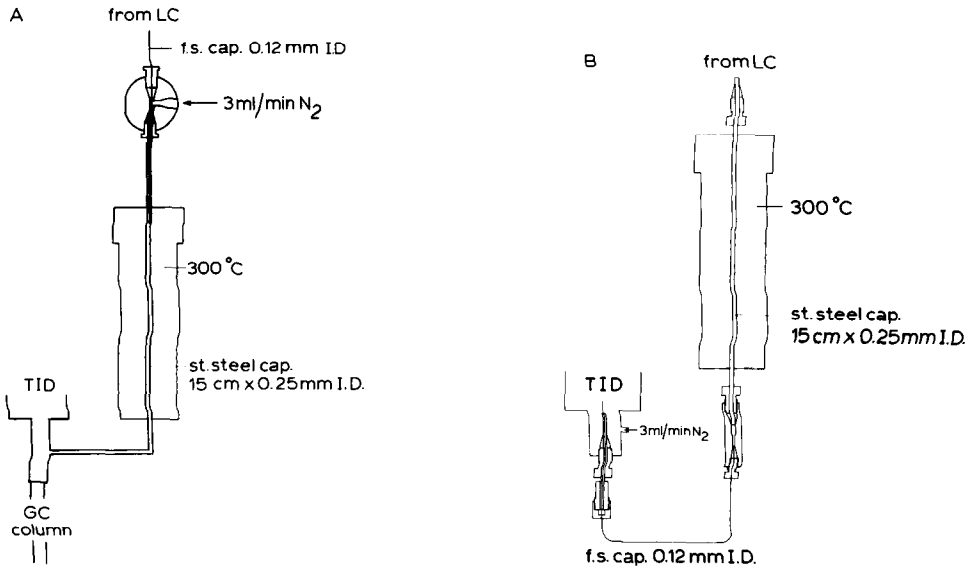

Fig. 1. Schematic diagrams of (A) the original and (B) the modified evaporation interface.

The inlet of the 3 ml/min nitrogen flow, which previously entered with the LC column effluent at the top of the interface to help transport the solutes toward the detector, could therefore be relocated. Using a T-piece just below the detector body in the GC oven (Fig. 1), this flow was introduced after the evaporation interface and pre-heated in the oven in order to minimize its cooling effect, thus ensuring the continued vaporization of low-volatility solutes.

Unfortunately, this configuration no longer combined LC and GC systems into a single unit[10]. However, the potential for higher sensitivity and response stability was felt to outweigh this disadvantage. In addition, the outlet of a packed GC column can replace the nitrogen flow at the base of the thermionic detector, although some loss of LC detection sensitivity will probably result from the higher flow-rates. Alternatively, using a two-holed vespel–graphite ferrule, a capillary GC column may be installed in the detector and enter the flame jet alongside the fused-silica capillary coming from the evaporation interface.

Detector characterization

The sensitivity and selectivity of the thermionic detector are known to depend on a variety of operating parameters[17], many of which vary with the specific geometry of the individual detector. Among these, the gas flow-rates and the bead heating current are probably the most significant. In improving the vaporization interface of the LC–TID system, the detector itself remained unchanged and preliminary checks using methanol–water (80:20) at 20 μl/min showed that the optimal operating conditions were, indeed, identical with those reported previously[10], *i.e.*, approximately 5 ml/min of hydrogen, 180–300 ml/min of air and a bead heating current adjusted to give an i_{bc} of 2 pA.

Changing the interface design, however, created a greater dependence of i_b on

the interface nitrogen flow-rate, as this flow comprised a larger percentage of the total gas mixture reaching the bead in the modified system. The sharp decline in i_b seen in this system with increasing nitrogen flow arose, no doubt, from a greater cooling effect. While a cooler environment induced by higher nitrogen flow-rates decreased the background noise level, the phosphorus response fell to an even larger extent, leading to lower overall signal-to-noise ratios. The smallest nitrogen flow effective in moving solutes sufficiently rapidly to reduce band broadening within the detector was determined to be approximately 3 ml/min.

The contribution of the modified interface and detector to band broadening was measured using flow injection and was calculated by means of the Foley–Dorsey[18] experimental approximation to the second moment (M_2). The polar pesticides ethyl-paraoxon and coumaphos served as model solutes. At a mobile phase flow-rate of 20 μl/min, an M_2 value of 5.9 sec^2 was measured for ethyl-paraoxon. This corresponded to a volumetric variance, σ_v^2, of 0.66 μl^2, a value almost identical with that obtained for the more volatile triethyl thiophosphate using the unmodified detector. The less volatile compound coumaphos, however, gave an M_2 value of 16.1 sec^2 (σ_v^2 = 1.78 μl^2) with the new system. At a mobile phase flow-rate of 35 μl/min, ethyl-paraoxon had an M_2 of 3.5 sec^2 (σ_v^2 = 1.1 μl^2), while coumaphos exhibited an M_2 of 4.08 sec^2 (σ_v^2 = 1.38 μl^2). All band-broadening data were calculated to have a relative standard deviation of 3.5–4.5% (n = 4).

A probable explanation of these findings is that the major source of peak broadening within the system is the vaporization efficiency of the interface, particularly with regard to compounds of low volatility. In the modified system, the LC column is connected with the heated interface using a stainless-steel capillary without insulation. Therefore, heat from the 300°C interface can easily radiate towards the column, creating a temperature gradient along the connecting capillary. Compounds leaving the column experience a gradual warming, instead of a sharp temperature increase, and are vaporized in a broad zone. This problem can be especially severe for high-boiling substances, which may be incompletely vaporized in the low-temperature regions of the tube. Increasing the effluent velocity reduces this effect by moving the solutes rapidly through the thermal gradient into the hot interface. Indeed, it is the less volatile analytes, such as coumaphos, whose bands are sharpened the most at increased flow-rates.

Analytical data

A variety of phosphorus-containing compounds were chromatographed with a methanol–water (80:20) mobile phase at a flow-rate of 40 μl/min. A typical chromatogram of five polar pesticides is shown in Fig. 2. In addition, the pesticides demeton-O, dimethoate, *o*-methoate and methyl-paraoxon were measured with similar results.

As shown in Table I, the minimum detectable amount (MDA, based on average mass flux, S/N = 3) of the system at an LC flow-rate of 40 μl/min ranged from an injected amount of 30 pg (0.2 pg/s phosphorus) of ethyl-paraoxon to 100 pg (0.3 pg/s phosphorus) of the more polar and higher molecular weight coumaphos. The MDA of 40 pg of injected compound (0.5 pg/s phosphorus) for triethyl phosphate represents a eight-fold increase in sensitivity over the MDA of 3 pg/s of phosphorus obtained for triethyl phosphate at a flow-rate of 20 μl/min or a sixteen-fold increase

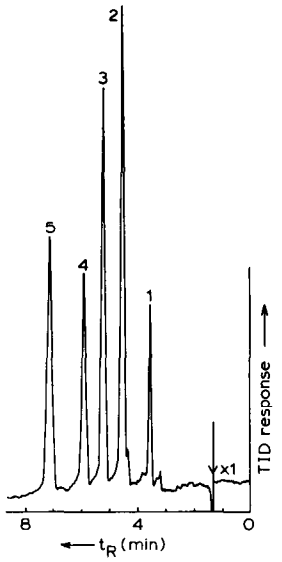

Fig. 2. LC trace of phosphorus-containing pesticide standards obtained with the modified LC–TID system. Column, glass-lined stainless steel (200 × 0.7 mm I.D.) packed with 5 μm LiChrosorb RP-18; mobile phase, methanol–water (80:20) at a flow-rate of 40 μl/min. Solutes: 1 = trichlorfon; 2 = ethyl-paraoxon; 3 = malathion; 4 = fenitrothion; 5 = ethyl-parathion (3–6 ng of compound injected).

over the MDA of 6 pg/s of phosphorus at 40 μl/min in the unmodified system.

It is interesting that while the sensitivity of the original LC–TID system decreased by a factor of 2 per given time unit for all compounds in going from an LC flow-rate of 20 μl/min to one of 40 μl/min, the modified system showed differential

TABLE I

DETECTION LIMITS FOR PHOSPHORUS-CONTAINING STANDARD COMPOUNDS OBTAINED WITH THE MODIFIED LC–TID SYSTEM

Mobile phase, methanol–water (80:20); column, 200 × 0.7 mm I.D., 5 μm RP-18; flow-rate, 40 μl/min; injection volume, 0.5 μl.

Compound	*MDA**	
	pg injected compound	*pg/s of phosphorus*
Triethyl phosphate	40	0.5
Triethyl thiophosphate	40	0.4
Ethyl-parathion	80	0.4
Trichlorfon	40	0.3
Ethyl-paraoxon	30	0.2
Malathion	40	0.4
Methyl-azinphos	90	0.3
Ethyl-azinphos	90	0.3
Coumaphos	100	0.3

* MDA = minimum detectable amount (based on average mass flux; signal-to-noise ratio = 3).

increases in sensitivity with increasing LC flow-rate for compounds of different volatility. For example, the present system had an MDA of 0.5 pg/s of phosphorus for both the pesticide ethyl-paraoxon and the readily volatile compound triethyl phosphate at an LC flow-rate of 20 μl/min. It is only the sensitivity to the pesticide, however, which increases by approximately a factor of 2 at the higher flow-rate of 40 μl/min.

The modified LC–TID system did not respond linearly to compounds of low volatility. Rather, there seemed to be saturation effects in the interface when large amounts were injected such that only a portion of the sample was vaporized, creating a concave response curve. Readily volatile substances, on the other hand, had a response linearity of 10^3, which is similar to that measured previously[10] for the LC-TID system.

The repeatability of the system was determined for a 5-ng injected amount of three pesticides. Ethyl-paraoxon and ethyl-parathion both showed a relative standard deviation of 1.8% (n = 9), compared with 5.2% (n = 9) for fenitrothion. Also, the modified detector had a measured baseline noise level half that of the original system and could handle mobile phase flow-rates of up to 70 μl/min without a significant increase in the background level.

Organophosphorus pesticide residue analysis

Capillary GC with TID is frequently the method of choice for the trace level determination of organophosphorus pesticides in environmental samples[19,20]. The low detection limits, selectivity and easy availability of instrumentation are attractive. However, frequent difficulties arise when analysing thermally labile or polar substances. For example, some phosphorus-containing pesticides are thermally labile (*e.g.*, trichlorfon) and many are fairly polar, typically exhibiting broad or tailing peaks and/or long retention times and yielding poor separations by GC[20]. It has also been noted that in order to determine low concentrations of pesticides by GC, the column temperature should be optimized in order to minimize bleeding of the liquid phase[21]. Because of these problems, the LC analysis of such compounds is an area of growing interest, and selective LC detectors, such as the thermionic detector, are becoming particularly important.

Coumaphos, ethyl-azinphos and trichlorfon are potent pesticides commonly used to control ectoparasites and insects on both livestock and fruit, vegetable and grain crops[22]. Owing to their wide use and potentially high toxicity, it is of interest to develop a sensitive, rapid and reliable method for their determination in complex sample matrices. Figs. 3 and 4 show the LC chromatograms of cabbage and tomato extracts, spiked with the three pesticides, separated by reversed-phase LC and detected with (A) the LC–TID system and (B) UV absorbance at 254 nm. In Fig. 3 the unmodified thermionic detector was employed in conjunction with a GC column flow-rate of 30 ml/min of nitrogen. In Fig. 4 the improved thermionic detector was utilized.

Although the sensitivity of the unmodified LC–TID system was approximately an order of magnitude less than that of the modified system, the ability to make simultaneous GC and LC measurements with the original system may outweigh this disadvantage when sample amounts are not limited. The selectivity of the LC–TID system was previously[10] calculated to be $1 \cdot 10^5$ g of carbon per gram of phosphorus.

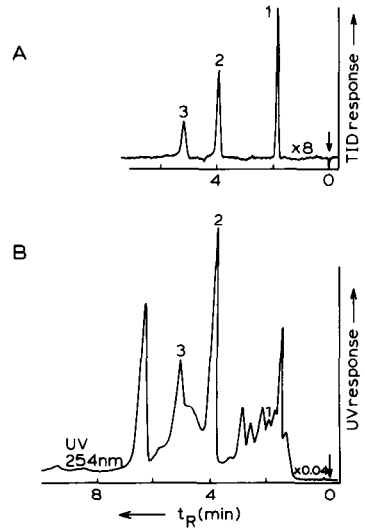

Fig. 3. LC trace of cabbage extract spiked with 3–5 ppm pesticide standards as monitored by (A) the original LC–TID system and (B) UV absorbance at 254 nm. Chromatographic conditions as in Fig. 2, except mobile phase flow-rate 30 μl/min. Solutes: 1 = trichlorfon (74 ng injected); 2 = ethyl-azinphos (59 ng injected); 3 = coumaphos (53 ng injected).

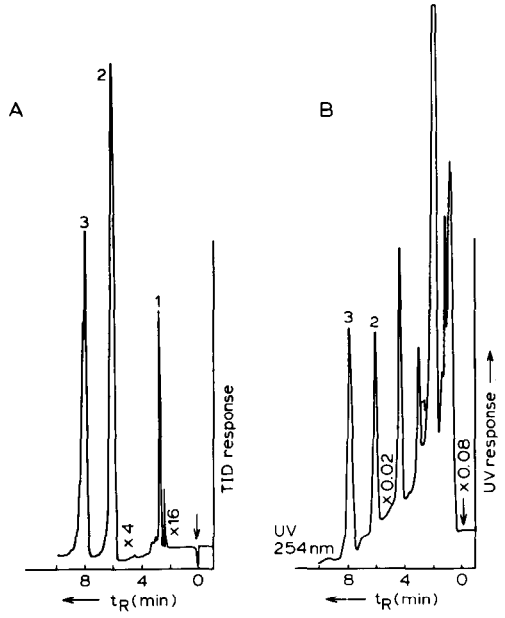

Fig. 4. LC trace of tomato extract spiked with pesticide standards as monitored by (A) the modified LC–TID system and (B) UV absorbance at 254 nm. Chromatographic conditions as in Fig. 3. Solutes: 1 = trichlorfon (36 ng injected); 2 = ethyl-azinphos (30 ng injected); 3 = coumaphos (26 ng injected) for TID. Injected amounts as in Fig. 3 for UV detection.

Both chromatograms show this high selectivity for the phosphorus-containing pesticides and are in striking contrast to the complex traces obtained by UV absorbance, where the absorptivity is often low. Indeed, this selectivity allows simple clean-up procedures to be used, and even relatively poor chromatographic efficiency can be tolerated. The spike evident on the coumaphos peak in Fig. 4 is caused by the thermodynamic instability of relatively involatile compounds as they move from the small inner diameter interface capillary into the body of the detector[23–25]. This instability leads to immediate molecular association and wall condensation, especially at low mobile phase flow-rates, and is evident from the short ion bursts seen as molecular clusters are detected[24]. Perhaps one promising way to eliminate these spikes will be through the creation of small, volatile molecular fragments by the pyrolysis of large compounds immediately prior to detection[24].

CONCLUSIONS

Recent modifications and applications of an on-line LC–TID system are reported. Improvements in the liquid introduction interface lowered the detection limit for an LC flow-rate of 40 μl/min by a factor of 16 compared with that of the previously reported LC–TID system[10]. This reduction in the MDA can probably be attributed to three primary factors: (i) a sensitivity increase naturally arises from the closer proximity of the interface fused-silica termination to the bead; (ii) sample molecules are also more precisely directed toward the bead owing to this positioning and because the reduced interface nitrogen flow experiences less expansion at the flame jet orifice than did the higher flow previously reported; and (iii) the use of a small I.D. fused-silica transfer line following sample vaporization creates a back-pressure within the detector which stabilizes signal fluctuations caused by density changes in the mobile phase. The net effect of this decrease in baseline noise is a proportional increase in the signal-to-noise ratio. Also, by optimizing the heat transfer efficiency inside the detector, compounds of poor volatility can be detected.

The improved LC–TID system has been successfully employed to monitor polar pesticides in environmental matrices at the Environmental Protection Agency (EPA, U.S.A.) tolerance level (high ppb or low ppm). Higher sensitivity and less matrix interference were achieved than previously experienced and reported[26] using an LC–UV system for pesticide residue determinations in vegetables.

Low-volatility or high-molecular-weight compounds, such as nucleic acids and phospholipids, remain difficult to vaporize and seem to experience increased broadening in the thermal gradient existing in the connection capillary between the LC column and the evaporation interface. In the future, effective cooling and insulation of the connection region and installation of a higher temperature interface should lessen these problems.

Large and polar biological molecules will be the focus of continuing work, as their analysis by GC frequently requires prior derivatization to increase both their volatility and their thermal stability. In addition, acidic mobile phases will be tested in an effort to further expand the range of compounds to which the LC–TID system may be applied.

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