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ON-LINE LIQUID CHROMATOGRAPHY–GAS CHROMATOGRAPHY IN RESIDUE ANALYSIS

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SUMMARY

A fully automated high-performance liquid chromatograph with column-switching capabilities interfaced on-line to a gas chromatograph is described. An autosampler with a flow-through syringe injection system was modified for control by a laboratory data system or a programmable event timer, which allowed sampling into the liquid chromatograph and on-line transfer of a fraction of the effluent to the gas chromatograph. This system, involving standard gas chromatographic detectors, performed highly efficient on-column sample enrichment and clean up of complex samples, together with specific and sensitive detection by gas chromatography (GC). It was demonstrated to be superior to either liquid chromatography (LC) or GC alone, and has high potential for the determination of pesticide residues in complex biological matrices. The technique is not limited to adsorption chromatography. The GC columns could handle aqueous effluents from of the LC system. The efficiency of the LC–GC system was demonstrated by application to the trace analysis of folpet in hop samples.

INTRODUCTION

High- and low-pressure liquid chromatographic systems are highly efficient clean-up systems for samples of biological origin or are efficient class fractionation systems[1–3]. In class fractionation, components having similar characteristics are eluted as a single peak from the liquid chromatographic system.

Pesticide residue analyses are usually performed by off-line low-pressure column chromatographic clean up, collection of a fraction containing the component(s) of interest, concentration of the eluate to a few millilitres by evaporation of the solvent and injection of a few microlitres into a gas chromatograph equipped with a specific detector.

In the present paper an on-line high-pressure liquid chromatography–gas chromatography (LC–GC) system is described, in which a liquid chromatograph is used for sample concentration and clean up prior to the introduction of relevant fractions into a gas chromatograph. The liquid chromatographic concentration, followed by peak cutting to a second LC column, provides an efficient sample clean up, reducing the number of off-line clean-up steps and the sample size[4–7]. The on-line transfer from

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the first to the second LC column and to the gas chromatograph avoids sample losses during evaporation steps.

Many authors advocate injection of the largest possible aliquot of the available LC peak for GC. However, the volumes of conventional LC peaks greatly exceed those suitable for direct GC introduction. Therefore special techniques are required for handling large amounts of solvents in GC. Grob and co-workers[8–10] proposed the use of retention gaps in capillary GC. This enabled several hundred microlitres of LC eluates to be handled. The necessity to evaporate such large volumes and to reconcentrate the analyte at the top of the capillary column at low oven temperatures increases the GC analysis time enormously. These drawbacks restricted this technique to low boiling solvents and to analytes with a boiling point at least 50°C above that of the mobile phase. The retention-gap technique is therefore not compatible with volatile compounds and aqueous mobile phases. In the present technique the small fraction of the eluate transferred to the gas chromatograph behaves like a conventional GC injection and the technique is not restricted to adsorption chromatography.

The efficiency of this LC–GC system is demonstrated for clean up and electron-capture detection (ECD) of traces of the plant protection agent folpet in hop samples.

EXPERIMENTAL

Instrumentation

The principle of the LC–GC system is illustrated in Fig. 1. The flow switching of the high-pressure liquid chromatographic system was achieved by pneumatically actuated valves. A Packard GC autosampler effected the filling of the sampling loop and the GC injection. The LC system was interfaced between the sampling needle and the injection syringe, originally directly connected by a PTFE tube. The principle of the modified GC autosampler is illustrated in Fig. 2.

The pneumatic controller of the autosampler was replaced by the interface shown in Fig. 3 which was controlled by a laboratory timer or a data system. It started the loop filling cycle, effected the transfer of the LC fraction from the first to the second column and initiated the injection of the top of the peak eluted from the second LC column in the gas chromatograph. The principle of operation of the flow-through syringe injection for LC–GC was first described by Majors[11] and by Apffel and McNair[3].

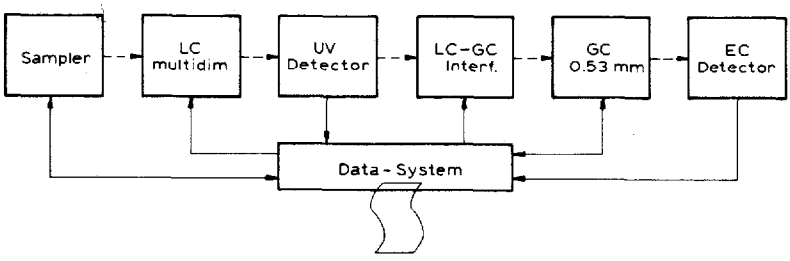

Fig. 1. Schematic diagram of the LC–GC system. -------→, Sample flow; ——→, data and control lines.

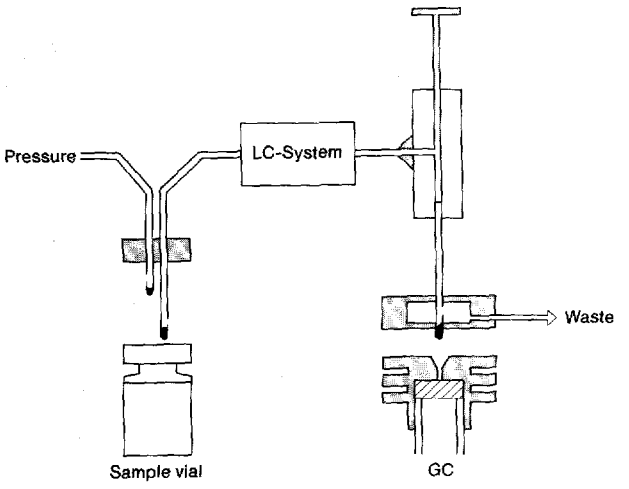

Fig. 2. Flow-through syringe autosampler with on-line HPLC system.

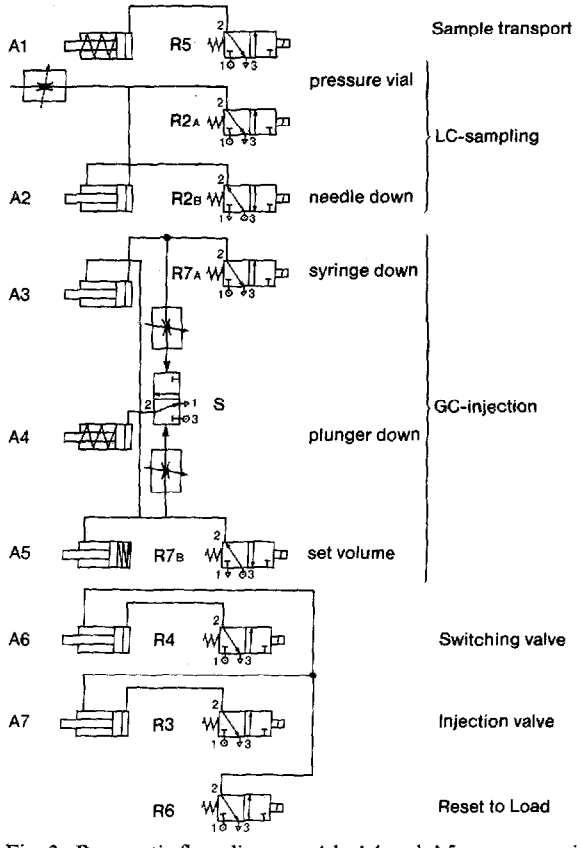

Fig. 3. Pneumatic flow diagram. A1, A4 and A5 = pneumatically actuated cylinders with spring return; A2 and A3 = double acting cylinders; A6 and A7 = double acting pneumatic high-pressure valves; R2–R7 = solenoid valves; S = spooling valve pneumatically actuated through restrictors (time delay); ⊙, pressure inlet line; 2 = "consumer" (user of pressurized air); ▽, vent exit. All valves and relays are in the stand-by position. R2b and R7b are pressurized in the stand-by position.

Mode of action of the interface (Fig. 3). Sample loading into the loop of the LC injection valve A7 was initiated by solenoids R2A and R2B. The actuator A2 forces a needle into the sample vial, and at the same time the sample vial is pressurized by solenoid R2A. A variable restrictor in the pressure line was used to set the sample flow-rate. At the end of the loop-filling cycle, solenoid R3 was actuated, moving the injection valve (A7) into the inject position. Solenoid R4 actuates the switching valve A6 to begin the transfer of the LC fraction to the second LC column. At the end of this transfer period, solenoid R6 resets the injection valve (A7) and the switching valve (A6) to their respective stand-by positions.

In the stand-by position, the effluent from the second LC column passes through the UV detector, enters the body of the flow-through syringe and exits through the needle to the waste (Fig. 2). When the injection is initiated by the data system just as the top of a peak passes through the syringe, the solenoid R7B depressurizes the actuator A5, the syringe plunger is lowered to set the injection volume and the inlet port at the syringe body is closed. At the same time, the ten-port injection valve (A7) is actuated by solenoid R3 to divert the effluent from the second LC column to the waste. This prevents an overpressure at the connector of the glass syringe inlet or in the UV-detector cell. For injection, solenoid R7A is pressurized also and rapidly lowers the injection syringe by means of actuator A3 until the needle pierces the GC septum. The syringe-plunger actuator A4, slightly delayed by the restrictors acting on the spooling valve, becomes pressurized and the GC injection is executed. Finally the injection syringe is returned to the stand-by position when solenoids R7A and R7B are shut off. Solenoid R6 is actuated to reset the LC loop-injection valve to the load position.

Solenoid R5 was used to transport the sample vial into the load position by means of actuator A1. The vial position was controlled by the data system, which therefore allowed random access to samples. In the absence of the data system, the laboratory timer moved the next vial into the load position. Relay number 1 was used to initiate the start/stop function of the system and to mark the point of sample injection on a strip chart recorder.

An UV detector was generally used to monitor the sample elution. The delay time between the top of the LC peak passing the UV detector and its entering the flow-through syringe could be calculated from the diameter of the interconnecting tube and was fixed exactly by experimental evaluation. The interconnecting tube was made from PTFE tubing of 0.3 mm I.D.

LC–GC network. Fig. 4 shows the column network, the switching valves and the GC injection syringe. Large volumes of sample, up to 1.5 ml, limited only by the capacity of the vials of the autosampler, were concentrated on the first LC column. The samples were dissolved in a solvent of lower elution strength than the mobile phase of the first LC column. The analyte bands were resharpened on the second LC column again by suitable choice of the elution strength of the second mobile phase and the stationary phase.

Owing to the fact that, because of the low flow-rate the linear velocity of the eluate in the syringe was low, extremely rapid and precisely times injections were not mandatory, *i.e.*, a deviation of a few seconds was tolerable.

The LC system consisted of two Model LC 250/1 reciprocating piston pumps (Kratos Analytical Instruments, Westwood, NJ, U.S.A.), a ten-port injection valve

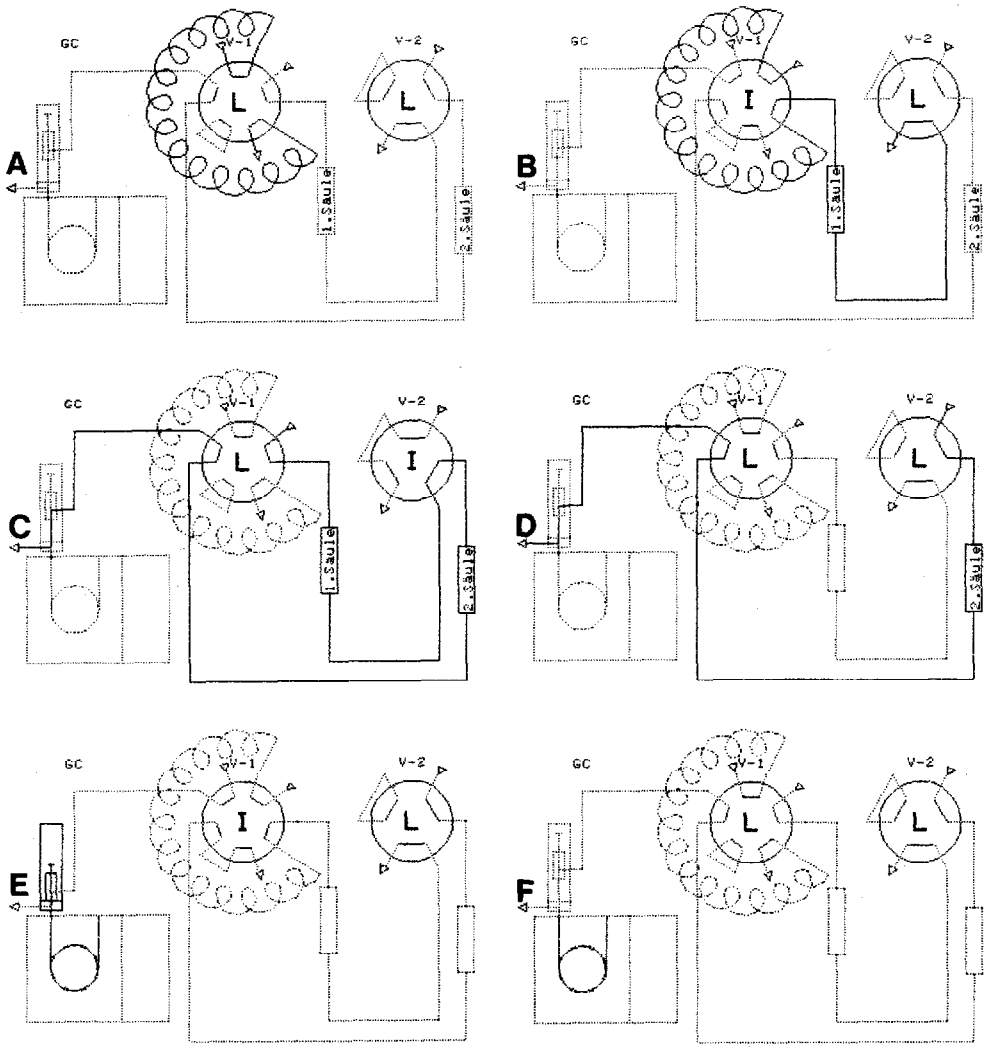

Fig. 4. Liquid and gas flow diagram of the LC–GC system. Dotted lines are stand-by positions; solid lines show the actual sample flow. (A) Stand-by position, LC loop being filled. (B) Injection and elution of the first LC column. (C) Transfer to the second LC column. (D) Elution of the second LC column. (E) Injection of a fraction of the eluent stream into the gas chromatograph. (F) GC elution and detection. V-1 and V-2 are valves, L = load position, I = inject position, Säule = column.

with a 250-μl loop (Valco, Houston, TX, U.S.A.); a six-port switching valve and a Model UVICON LCD-725 variable-wavelength UV detector (Kontron, Zürich, Switzerland). The GC system consisted of a Model 439 gas chromatograph, (Packard Instrument, Delft, The Netherlands) equipped with an electron-capture detector and a fused-silica mega bore (0.53 mm I.D.) capillary column.

The laboratory data system was a Model 3357 (Hewlett-Packard, Avondale, PA, U.S.A.)[12]. It was equipped with electronic control modules (ECM Model

18653B) and analog to digital converters (A/D Model 18652A) for data acquisition. The ECM provided a binary input of the bottle number and seven 110-V a.c. power outputs. These outputs were switched on/off independently by the system software (chromatographic integration methods) or by post-analytical user programs initiated by the chromatographic integration system software.

The laboratory timer with eight timed relay exits of 110 V a.c. was from Alpha Electronic (Stutensee, F.R.G.). The modified autosampler Model LS 607 was from Packard.

The hardware for the electropneumatic interface consisted of six three-way solenoids (stand-by position: pressure inlet closed) and two three-way solenoids (stand-by position: pressure inlet open), 110 V a.c., from local suppliers and a spooling valve Model 44-220 from Kuhnke (Malente, F.R.G.).

Chromatographic columns. The first LC column (30 mm × 4 mm) was packed with LiChrosorb DIOL (E. Merck, Darmstadt, F.R.G.). *n*-Hexane was used to dissolve the sample and as an eluent at a flow-rate of 1 ml/min. The second LC column (120 mm × 4.6 mm) was packed with LiChrosorb CN (E. Merck). Ethanol–*n*-hexane (20:80) was used as the eluent at a flow-rate of 200 μl/min.

A fused-silica capillary column (15 m × 0.53 mm I.D.) (SGE, Ringwood, Australia) coated with methyl silicon was used for the GC separation. The column temperature was 220°C, the detector temperature 280°C and the injector temperature 250°C. Helium at a flow-rate of 8 ml/min was used as the carrier gas.

Determination of folpet in hop samples by LC–GC

Folpet (Fig. 5) is used to protect against fungal diseases in a wide variety of crops. The analysis of folpet residues in biological samples by GC and HPLC has been described (ref. 13 and references cited therein).

Folpet has a high electron affinity and is routinely detected in crop samples by GC–ECD after partitioning into hexane and cleanup on a silica gel column or by gel permeation. Hop extracts are always difficult to clean up for GC of trace compounds. Such extracts were therefore chosen to demonstrate the effectiveness of LC–GC for residue analysis.

Sample preparation. The entire hop sample was macerated and a 20-g aliquot was extracted with 250 ml of acetone. A 2-ml volume of this extract was filtered and diluted in 2 ml of water and 1 ml of brine. The mixture was transferred onto a Clin Elut® 1005 column (Analytichem International, Harbor City, CA, U.S.A.). After 15 min of penetration into the column support, folpet was partitioned into *n*-hexane by rinsing the column with 15 ml of this solvent. The eluent was concentrated to 2 ml and the sample was filtered into the sample vials for LC–GC detection.

Chromatographic separation. Fig. 6a shows the ECD chromatograms obtained after on-line LC clean up. Hop extracts (250 μl) equivalent to 10 mg of sample were

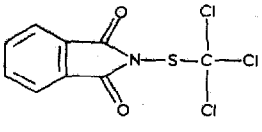

Fig. 5. Structure of folpet, N-(trichloromethylthio)phthalimide.

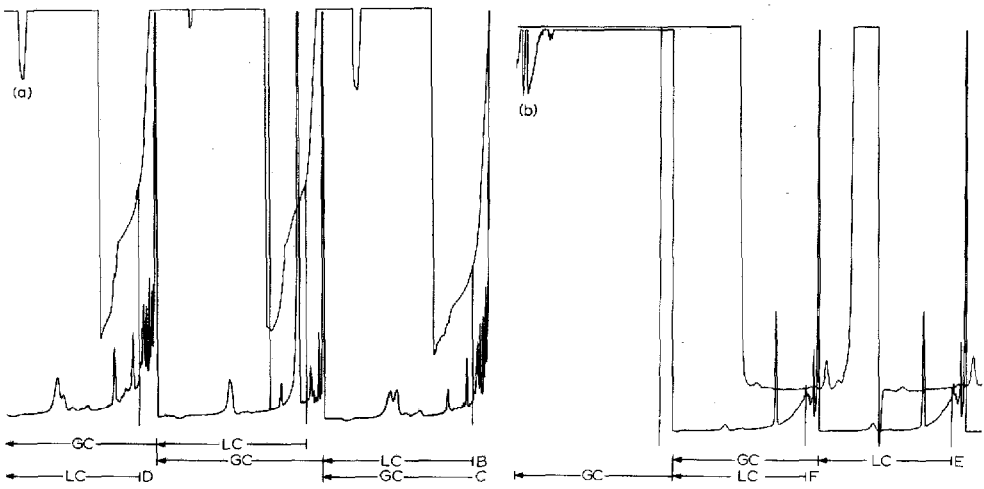

Fig. 6. (a) Gas chromatogram of an hop extract after on-line HPLC clean up. Sample injected into the LC column: 250 μl. Sample transferred to the GC column: 2 μl. Top: UV trace (LC). Bottom: ECD trace (GC). A = Hop sample spiked at 0.1 mg/kg. B = standard LC injection, 2 ng folpet; C, D = previous sample injection for GC and next sample injection for LC. The injection of the next sample for LC was made *ca.* 2 min after the GC injection. The UV trace after the second LC column is off-scale. (b) Gas chromatogram of an hop extract without LC column switching (sample directly injected into the second LC column). E = Standard injection, 10 ng folpet; F = hop sample, spiked at 0.5 mg/kg; no detection possible.

injected into the liquid chromatograph; 3.3 min after injection, a fraction of 2.7 ml was transferred to the second LC column. A 2-μl volume of the effluent from this column was injected for GC about 16.8 min after the begin of the chromatography. The LC peak of folpet, eluted from the second LC column, showed a base width of 1.2 min (240 μl at a flow-rate of 200 μl/min). The peak compression factor after the second LC column was therefore more than 10, based on the volume transferred (2.7 ml) to the column.

The GC injection was performed within ± 3 s of the preselected time. The resulting standard deviation of the peak height was ± 6.8% (25 ng injected into the liquid chromatograph, N = 15), which is quite acceptable for trace and residue determination. A calibration plot constructed from 2–40 ng of folpet injected for LC, which resulted in GC peak heights of from 1 to 20 cm (six triplicate injections), had a correlation coefficient of 0.999 (Fig. 7).

The detection limit of the method was estimated to be 0.05 mg/kg of folpet in hops. It was not fully exploited, as the LC sampling volume may be increased up to 1.5 ml.

The percentage of folpet transferred from the LC peak to the gas chromatograph in this experiment was about 2%, which was about 8–10 times the percentage injected after off-line clean up.

Fig. 6b shows a gas chromatogram of the same hops sample without LC column switching. The GC signal was off scale at the retention time of folpet. The sample clean up with only the second LC column was insufficient.

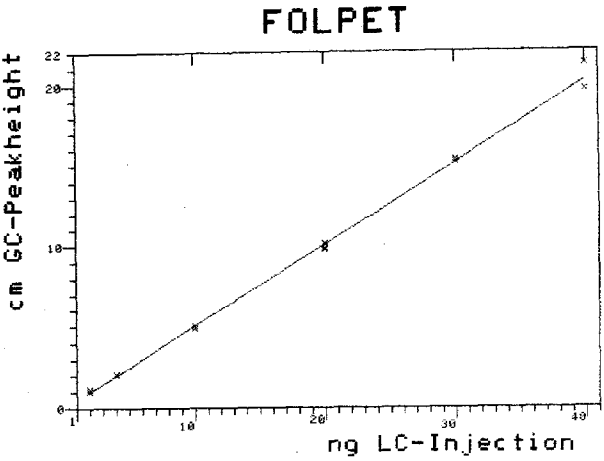

Fig. 7. Calibration plot for folpet obtained by LC–GC.

LIMITATIONS AND PROBLEMS

To monitor the effluent of the LC columns an UV detector was employed. All solvents used had to be UV grade and ECD compatible. The range of solvents available for LC–GC with ECD was therefore limited.

The GC detection mechanism depended upon the ability of the eluents to capture thermal electrons. Thus solvents such as chloroform, carbon tetrachloride, etc., which have high affinities for electrons, could not be used, despite their relatively good UV properties and moderate elution strength in liquid adsorption chromatography.

During the experiments it became apparent that most of the brands and grades of saturated hydrocarbons (*n*-hexane, isooctane, etc.) tested, when used as received from suppliers, were insufficiently pure for ECD after on-line liquid chromatography. They contained strongly electron capturing impurities, which resulted in broad solvent peaks and peaks interfering with the detection of the analyte. Similar behaviour was found by Brinkman and co-workers[14,15] when they interfaced the LC effluent directly with an electron-capture detector. They proposed a procedure for eliminating the ECD-active contaminants. Polar solvents or polar modifiers (methyl isopropyl ether, acetonitrile, ethanol, etc.) did not contain such contaminants.

The addition of non-volatile acids or bases or ion-pairing agents to the mobile phase had also to be avoided, to prevent clogging of the insert or capillary GC column with non-volatile materials.

CONCLUSIONS

The LC–GC system described is easily constructed with minor modifications of commercially available instruments (autosampler, laboratory timer or data system). The electropneumatic interface between the data system/laboratory timer and the autosampler/HPLC switching valves could be constructed from parts available in modern chromatographic laboratories.

The combination of LC with GC involving standard detectors has potential for residue and trace analysis. The highly efficient sample clean up by LC reduces the intensity of matrix effects, enabling detection at very low limits and a reduction in the overall sample size. The LC column switching allows the injection of large portions of sample extracts for which no clean up has been carried out. Especially when volatile trace components are to be analyzed, the concentration of the sample on the LC column is a convenient way of reducing losses during evaporation of solvents. Crude sample extracts were fractionated on the first column precisely and transferred to the second column for a further separation.

The ratio of analyte/interferences at the top of the LC peak was most favourable. Increasing the fraction transferred to the gas chromatograph reduces this ratio by diluting the analyte and increasing the interferences. As a consequence of the sample concentration and peak compression, only a minute fraction (2%) of the LC peak needed to be transferred to the gas chromatograph for trace analysis.

The technique described is superior to low-pressure LC–off-line GC. It will be useful for automated determination of pesticide residues in complex biological matrices.

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