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COUPLED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–GAS CHROMATOGRAPHY FOR THE DETERMINATION OF PESTICIDE RESIDUES IN BIOLOGICAL MATRICES

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SUMMARY

A fully automated high-performance liquid chromatography–gas chromatography (HPLC–GC) network is described. A ten-port valve set up as a loop type LC–GC interface allowed the transfer of large LC effluent fractions into the gas chromatograph by concurrent solvent evaporation. The system performed highly efficient sample enrichment and clean up by LC and on-line GC separation with sensitive electron-capture detection. The efficiency of the system was demonstrated by application to the trace analysis of N-(3-chloro-2,6-dimethylphenyl)-N-(2-oxotetrahydrofuranyl)-2-methoxyacetamide (CGA 80000) in various crops and soil samples. The residue level determined was 0.02 mg/kg for crop samples and 0.01 mg/kg for soil samples. The relative standard deviations of the calibration graphs were in the range 2–5%; the mean recovery was >85%.

INTRODUCTION

Recent trends towards automated analyses combine on-line sample preparation with the final determination[1]. High-performance liquid chromatography (HPLC) is a very efficient method to separate trace components from coextractives of the matrices. Coupled HPLC–gas chromatography (GC) systems allow the direct transfer of a selected LC fraction into a GC capillary column; they produce high analyte/matrix selectivities, lower the detection limits and improve quantitation[2]. The coupling of LC and GC has recently been made more feasible by the development of evaporation techniques for large solvent volumes in capillary GC retention gaps[3]. Using

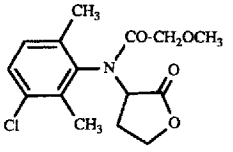

Fig. 1. Structure of CGA 80000, N-(3-chloro-2,6-dimethylphenyl)-N-(2-oxotetrahydrofuranyl)-2-methoxyacetamide.

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a loop-type LC–GC interface[4] in combination with automated column switching techniques, routine analyses are less time consuming and more reproducible.

CGA 80000, *i.e.*, N-(3-chloro-2,6-dimethylphenyl)-N-(2-oxotetrahydrofuranyl)-2-methoxyacetamide is a systemic experimental fungicide (Fig. 1). Residue methods developed so far use GC with electron-capture detection. The rather poor sensitivity of the component requires several labour intensive clean up and concentration steps.

EXPERIMENTAL

HPLC conditions and equipment

The LC system consisted of a 200 mm × 4.6 mm I.D. column packed with Nucleosil CN 5 μm (Macherey-Nagel, Düren, F.R.G.). Injections were made with a Valco injection valve (Model C6W; Valco, Houston, TX, U.S.A.); the injection volume was 500 μl. The solvent delivery system consisted of a piston pump (Model 420; Kontron Instruments, Zurich, Switzerland); the flow-rate was 1 ml/min. The mobile phase used was *n*-hexane–ethanol (8:2, v/v); the monitoring UV detector was a Pye Unicam LC-UV-detector (Pye Unicam, Cambridge, U.K.), operated at 220 nm.

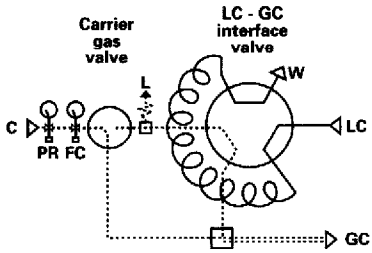
GC conditions and equipment

The GC system consisted of an Hewlett-Packard Model 5700 gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with an electron-capture detector. The analytical column was a 15 m × 0.53 mm I.D. fused-silica column (DB-5, film thickness 1.5 μm; J&W Scientific, Cordova, CA, U.S.A.). A retention gap of 3 m (530 μm I.D., fused-silica column phenyl-deactivated; Macherey-Nagel) coupled to the GC column with a press-fit connection (J&W Scientific) was used to reconcentrate the components of interest. The carrier gas was helium at a flow-rate of 8–10 ml/min during GC analysis and 1–2 ml/min during introduction of the LC fraction. Nitrogen at a flow-rate of 40 ml/min was used as a make-up gas for the electron-capture detector operated at 300°C. The GC oven temperature was held at 120°C during solvent evaporation, then programmed to 240°C at 16°C/min and held at 240°C for 8 min.

LC–GC interface

The HPLC–GC interface was assembled with commercially available components following suggestions by Grob[4] but modified for automated routine analysis. It consisted of a ten-port switching valve (Model C10W, Valco), a low dead volume T-piece (Model ZTl, Valco), a variable restrictor (Model 47220; Kuhnke, Malente, F.R.G.), a pressure regulator (Model 8286 ANVS-30; Porter Instruments, Hatfield, PA, U.S.A.), a flow controller (Model VDC-1000 AVF-10, Porter) and two pressure gauges (Model 111.10.40; WIKA, Klingenberg, F.R.G.).

Fig. 2 shows the loop-type LC–GC interface suggested by Grob[4] consisting of a sample valve and a carrier gas valve. The carrier gas is regulated by a pressure regulator followed by a flow controller. During GC analysis the carrier gas is flow controlled. However, while the LC fraction evaporates, the column inlet pressure increases. To accelerate the discharge of solvent vapours through the column and to maintain a constant solvent flow into the retention gap against the increased inlet pressure, a relatively high overall pressure setting is required[5]. As a consequence the



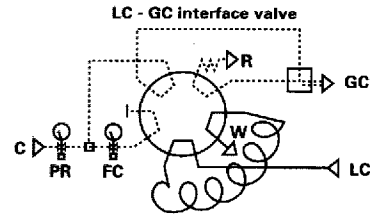


Fig. 2. Loop-type HPLC–GC interface according to suggestions by Grob[4]. The carrier gas switching valve and the interface valve are set to the mode for GC elution. C = Carrier gas supply; PR = pressure regulator; FC = flow controller; L = leak; GC = flow direction of carrier gas (to GC column); LC = flow direction of mobile phase (exit from LC column); W = waste.

Fig. 3. Loop-type HPLC–GC interface used in the present study. The ten-port interface valve is set to the mode for GC elution; the carrier gas flow is pressure regulated. R = Variable restrictor.

boiling point of the solvent increases. This must be compensated by higher initial transfer temperatures to maintain concurrent solvent evaporation conditions[6]. Hence, the minimum temperatures for eluting sharp peaks from the GC column are increased due to the reduced efficiency of the phase soaking effect[3]. The sample valve and its connection tubes are backflushed after solvent introduction to prevent contamination of the carrier gas with solvent residues.

Fig. 3 shows the modified LC–GC interface. A ten-port interface valve replaces the separate sample valve and carrier gas valve. Between the pressure regulator and the following flow controller a T-piece is installed which allows pressure regulation of the carrier gas during GC analysis. The connections between the interface valve and the T-piece above the GC oven are made of deactivated fused-silica capillaries or of inert polymeric capillaries. In contrast to the system set up by Grob using a 310-μm retention gap and analytical column, the interface valve described here is connected to 530-μm capillaries. The larger column diameter allows a low pressure setting of the carrier gas at 0.4 bar (with a resulting flow-rate of 8–10 ml/min at the initial transfer temperature of 120°C). The transfer flow-rate was set to 1–2 ml/min. During solvent evaporation the column inlet pressure is $\approx$0.3 bar permitting low initial transfer temperatures. The full power of the phase soaking process can be reached at lower GC oven temperatures and extends therefore the use of LC–GC loop transfer with concurrent solvent evaporation to lower boiling components.

LC–GC network

A sampler (Model 7671 or 7672, Hewlett-Packard) was adapted for HPLC loop sampling and for sample transfer into the gas chromatograph. A detailed description is given in ref. 7.

Fig. 4A–D show the LC–GC network used in this study with a single LC column. In Fig. 4A and B the sample loop (500 μl) is filled and, after rotating the sampling valve into the ON position, LC elution starts. The effluent of the LC column passes through the GC injection loop (size 1000 μl) of the ten-port interface valve. After rotating the interface valve to the injection position (Fig. 4C, position ON) the flow controlled carrier gas forces the solvent fraction containing the analyte of interest from the loop

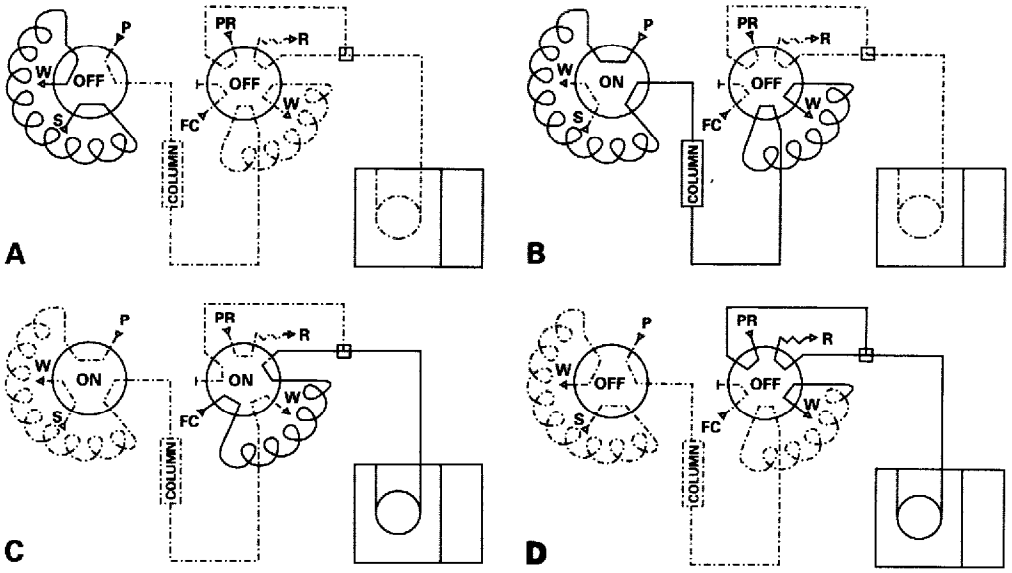


Fig. 4. HPLC–GC network. LC sampling valve and loop-type HPLC–GC interface valve. W (six-port sampling valve) = Waste exit of the LC sampling loop connected to the sucking pump; S (six-port sampling valve) = sample inlet port from the automated liquid sampler; P (six-port sampling valve) = eluent pump; PR = pressure regulated carrier gas inlet; FC = flow controlled transfer gas inlet; R = variable restrictor; W = waste exit of the LC–GC transfer loop; ON = injection position; OFF = standby position. Solid lines show the actual liquid sample and gas flow, dashed lines are standby positions. (A) Standby position, LC loop being filled; (B) injection and elution of the LC column; (C) transfer of the loop fraction into the gas chromatograph with the flow controlled carrier gas flow; (D) GC elution and detection with the pressure regulated carrier gas flow, purging of the liquid transfer line.

into the retention gap. As the column inlet pressure increases during solvent evaporation, the flow controller shuts the gas line and prevents a back flow of solvent vapours. Part of the solvent is pressed through the T-piece connector into the carrier gas line, but the compressed gas prevents the solvent reaching the interface valve. The increase of the pressure during the transfer of the LC fraction is monitored by the pressure gauge of the flow controller. At the end of the transfer period the pressure decreases, the flow controller opens the gas line and residual solvent is flushed into the retention gap. The interface valve is now rotated back (Fig. 4D, position OFF), the carrier gas supply returns to pressure regulation and the elution of the GC column is started. The solvent transfer line is backflushed through the variable restrictor (flow-rate ≈ 0.3 ml/min) to prevent residual solvent vapours from entering the GC column during GC analysis.

This LC–GC network with a single LC column can be extended to multiple column networks[8] for trace enrichment[9] and clean up of complex sample matrices[10]. The sampler with the ten-port interface valve can also be used as a large volume GC sampler suitable for direct sample enrichment on the GC column. This GC injection mode can easily be achieved by connecting the interface valve directly to the liquid sampler.

Sample preparation

The whole crop samples were homogenized with a cutter (horizontal cutter Model H4/4-2R; Schwabenland, Zurich, Switzerland). Subsamples of 10 g were extracted with 150 ml methanol–0.1% phosphoric acid (9:1, v/v) by shaking for 120 min. A 9-ml volume of the extract was transferred to a round-bottom flask and concentrated to 4 ml using a rotating evaporator. After dilution in 10 ml methanol–0.1% phosphoric acid (2:8, v/v) the solution was transferred to a Chem Elut® 1020 column (No. AI CE 1020; Analytichem, Harbor City, CA, U.S.A.). The round-bottom flask was rinsed twice with 2 ml methanol–0.1% phosphoric acid (2:8, v/v) and the rinsing solutions were transferred to the column. CGA 80000 was eluted with 80 ml toluene–*tert.*-butyl methyl ether (8:2, v/v); the eluate was evaporated to dryness and redissolved in 6 ml hexane–ethanol (8:2, v/v).

RESULTS AND DISCUSSION

Injection of large volumes in GC

To test the performance of the LC–GC interface, standard samples were injected directly into the retention gap by the loop interface and compared to manual injections made with a micro syringe into the standard GC injection port. The interface valve was connected to the liquid sampler and 500 μl of standard solutions containing 5 ng CGA 80000 were injected. The repeatability of this injection technique was determined from a set of ten injections. Mean and standard deviation were calculated to be 4.91 $\pm$ 0.16 ng ($N = 10$). Volumes of 2 μl of standard solutions containing 5 ng CGA 80000 were injected manually and mean and standard deviations were calculated to be 5.34 $\pm$ 0.38 ng ($N = 10$). The results demonstrate the possibility to enrich trace samples on the GC column from large solvent volumes without disturbing the performance of the electron-capture detector flushed with large volumes of solvent vapours.

The GC system was calibrated by injecting 500 μl of standard solutions (loop injection) in the range 1–25 ng CGA 80000. The resulting peak heights were used to calculate a linear regression. The standard deviations of the relative deviations of the measured peak heights from the calculated regression lines were in the range of 2–5%.

Direct GC injection with the loop type interface was also used to optimize the transfer time and the solvent evaporation temperature. The initial transfer temperature was set to 120°C for routine analysis. A reduction of the transfer temperature to 100°C did not increase the sensitivity significantly. Using a transfer flow-rate of 1–2 ml/min, good reproducibility was achieved with transfer times of about 5 min for 500-μl samples in hexane–ethanol (8:2, v/v) and of about 11 min for 1000-μl samples.

The solvent evaporation temperature and transfer times are not very critical for the routine analysis of CGA 80000 as the elution temperature of the compound is relatively high. In our experience the crucial factor is the purity of the solvents when using electron-capture detection (ECD) and large injection volumes. Similar problems were also reported in the application of ECD in LC[11]. The authors recommend a purification procedure to remove electron-absorbing contaminants from solvents. So far these clean up procedures have not been tested because changing to another batch or to another quality of the solvents solved the problem.

TABLE I

CALIBRATION OF THE HPLC–GC SYSTEM AND RECOVERIES OF SAMPLES FORTIFIED WITH CGA 80000

Mean and standard deviation of recoveries: 87 ± 8% (N=42).

Crop analyzed	*Range of standard deviations (%)*	*Range of recoveries (%)*
Pepper	2.6–5.2	70–96
Strawberry	1.4, 4.5	84–97
Citrus	1.2, 3.3	86–114
Soy bean	3.9	79, 85
Soil	2.7–4.8	80–91

Coupled HPLC–GC

For the determination of low pesticide concentrations in soil and crop samples, off-line LC techniques are commonly used for pretreatment and clean up purposes. Since CGA 80000 is separated into the isomeric components on silica columns, prepacked cartridges packed with C_{18} chemically modified silica gel were used. These columns however showed irreproducible performance due to batch to batch variablity caused by residual silanol groups. Reusable LC colunns for sample clean up are therefore an attractive alternative to prefilled cartridges.

The HPLC and GC conditions were optimized separately. The sensitivity of CGA 80000 was of the order of 10 ng by UV detection, while the sensitivity by GC with ECD was 0.5 ng.

The repeatability of the overall LC–GC system was determined from ten injections of standard solutions with 5 ng CGA 80000. Mean and standard deviation were calculated to be 5.17 ± 0.04 ng (N= 10). The system was calibrated by injecting standard solutions of CGA 80000 ranging from 1 to 25 ng. The resulting GC peak heights were used to calculate the linear regression with the relative standard deviation. The data for the calibrations are summarized in Table I.

Analysis of residue samples

The described LC–GC network is being used for routine trace analysis of CGA 80000 and a wide variety of crops and soil samples has been analyzed. To check the performance of the system, samples were fortified with 0.04 and 0.2 mg/kg of CGA 80000. Soil samples were also fortified at levels of 0.02 and 0.1 mg/kg. Standard and sample injections were made alternately. The linear regression, the relative standard deviation of each calibration graph and the recoveries of the fortified samples were calculated. The results are given in Table I.

Fig. 5A and B show the chromatograms of orange peel samples. Compared with the standard injection in Fig. 5C, only few peaks from crop coextractives influence the GC separation with the sensitive electron-capture detector.

The residue level for quantitation used for routine analyses is 0.02 mg/kg of CGA 80000 in various crops and 0.01 mg/kg in soil samples. The limit of determination was not fully exploited as the LC sampling volume may be increased to several millilitres and the efficiency of the LC clean up can be increased by column switching depending on the crop to be analyzed.

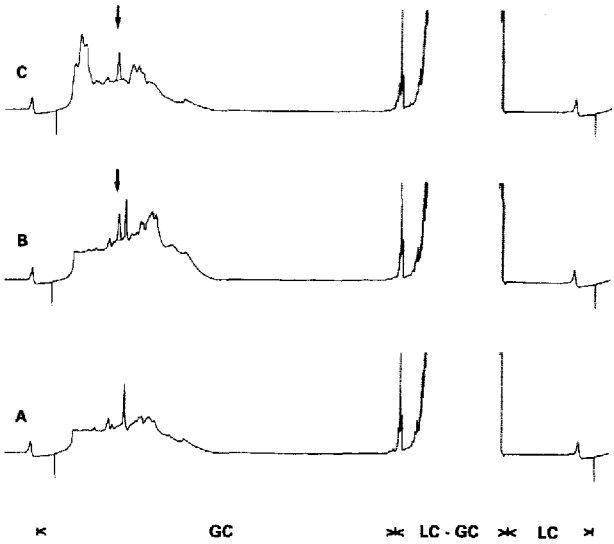


Fig. 5. (A) Gas chromatogram of an extract of orange peels after on-line HPLC clean up. The aliquot injected corresponds to 50 mg of the extract. (B) Gas chromatogram after on-line HPLC clean up of an extract of orange peels spiked before extraction with 0.04 mg/kg of CGA 80000. The aliquot injected corresponds to 50 mg of the extract. (C) Standard injection of 2 ng of CGA 80000 into the coupled HPLC–GC system.

CONCLUSIONS

Coupled HPLC–GC has been shown to be highly suitable for automation of the trace analysis of CGA 80000. On-line sample concentration and LC clean up prior to GC detection reduced the analysis times. Using a loop-type LC–GC interface with the possibility of injecting large sample volumes into capillary columns, the required limits of determination were obtained with good reproducibility.

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