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Modified on-column interface for coupled high-performance liquid chromatography–gas chromatography and its application to the determination of levamisole in milk

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ABSTRACT

A modified on-column interface is reported for the coupling of high-performance liquid chromatography with gas chromatography, incorporating an adapted, commercially available multidimensional gas chromatography switching system. Novel features include cryogenic analyte focusing, total solvent exclusion from the analytical column and independent carrier gas supplies to the analytical GC column and uncoated pre-column. The instrumentation was used for the determination of the veterinary anthelmintic drug levamisole in milk with analyte detection by both flame ionisation and nitrogen–phosphorus detectors. Detection limits for the assay were 2.2 $\mu g\ l^{-1}$ and 0.4 $\mu g\ l^{-1}$ by flame ionisation and nitrogen–phosphorus detectors, respectively. The assay was applied to a survey of fourteen milk samples from different dairy outlets.

INTRODUCTION

Multidimensional high-performance liquid chromatography–gas chromatography (HPLC–GC) is an emerging technique for the automated analyses of analytes in complex matrices. HPLC–GC has been applied to a wide variety of analyses including polychlorinated biphenyls (PCBs) in coal tar and water [1,2], herbicides and pesticides in sorghum and butter [3], wax esters in olive oil [4], chlorinated pesticides in water [2,5], diethylstilbestrol in bovine urine [6], and oil from jute sacks in various foodstuffs [7]. These and other applications have been reviewed by Davies *et al.* [8].

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Coupled HPLC–GC systems utilise HPLC to provide a sample clean-up step prior to on-line GC analysis with analyte detection by any of the wide variety of GC detectors available. Traditional off-line purification methods such as solid-phase extraction and liquid–liquid partition are relatively inefficient compared to HPLC, and yet form the basis for sample preparation in most assays involving complex matrices. The coupling of HPLC with GC not only provides a means of automation, but an inherently more efficient assay since the clean-up is more selective towards the analyte of interest.

In this paper we report a modified on-column interface for the coupling of HPLC with GC, incorporating cryogenic cold trapping for analyte focusing at the head of the analytical GC column and a dual carrier gas supply enabling the column to be

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isolated from the uncoated pre-column. The system has been applied ot the analysis of the drug levamisole in milk, with detection by flame ionisation and nitrogen–phosphorus detectors.

Levamisole, [*l*(−)-2,3,5,6-tetrahydro-6-phenylimidazo [2,1-*b*]thiazole] is the *l*(−) isomer of the racemic drug preparation tetramisole, the anthelmintic (de-worming) activity of which was first reported by Thienpont *et al.* in 1966 [9]. Levamisole is widely used with a variety of livestock animals including pigs, sheep and cattle, and can be administered (as the hydrochloride salt) as a bolus injection via the feed, by subcutaneous injection or in the form of a drench. The compound is also used in clinical chemotherapy for the treatment of cancer [10]. Animal products intended for human consumption are covered by recommendations restricting the permissible level of drug residue to 10 $\mu g\,l^{-1}$ [10,11].

Levamisole has been analysed by HPLC [12–14], and by GC with detection by nitrogen–phosphorus detection (NPD) [15,16] and mass spectrometry (MS) [17,18]. Analysis of the compound commonly requires extensive and time consuming sample preparation before measurement by any of the systems above. Since the free base is amenable to GC [19], the overall procedure can be shortened, simplified and automated by the use of on-line HPLC–GC.

EXPERIMENTAL

Materials

Levamisole hydrochloride was purchased from Sigma (Poole, UK). Methanol (Aristar), dichloromethane (pesticide residue analysis grade), ethyl acetate (AnalaR), hydrochloric acid (GPR), and 40% sodium hydroxide solution (AnalaR), were all purchased from BDH (Poole, UK). Sodium aluminosilicate 10 Å molecular sieve, over which the dichloromethane was dried, was purchased from Sigma. Water was obtained from an Elga UHP purification unit.

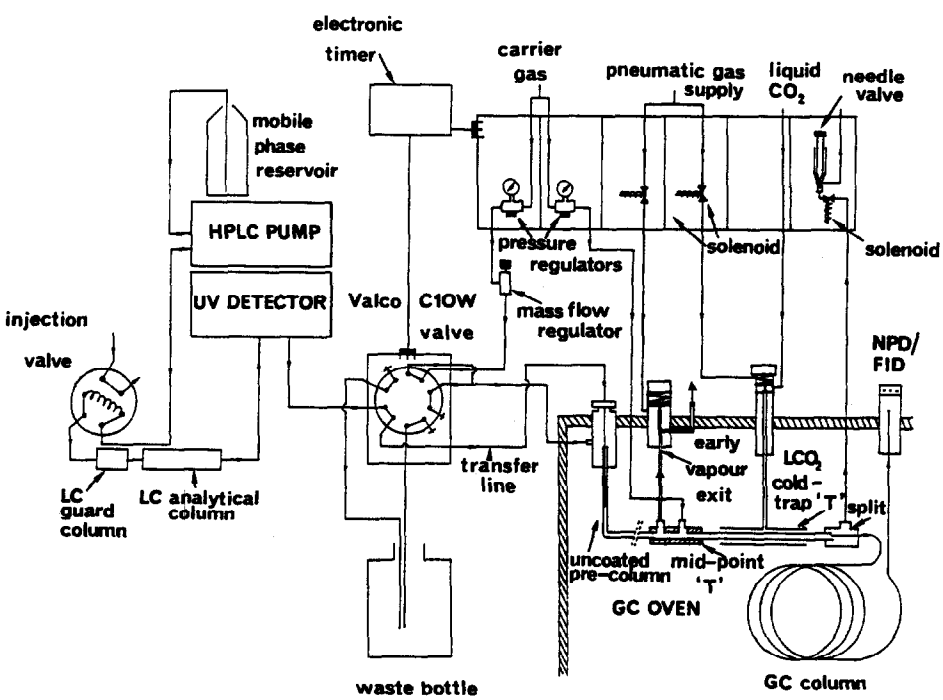

Fig. 1. The schematic layout of the normal-phase HPLC–GC system showing the switching position for the loading of a fraction from the liquid chromatograph to the gas chromatograph.

HPLC–GC instrumentation

The HPLC–GC system, shown schematically in Fig. 1, consisted of an LKB 2150 dual-piston pump and a Waters 455 UV detector interfaced to a Carlo Erba Fractovap 4160 GC via an on-column interface. The HPLC column (25 cm × 2.1 mm I.D., Spherisorb S5W normal-phase silica), was eluted with methanol–dichloromethane (5:95) at a flow-rate of 200 μl min^{-1}. The column was protected by a normal-phase universal guard cartridge kit (Whatman, Maidstone, UK) positioned immediately downstream of the injection valve (Negretti-Zambra 6-port with 20-μl loop). Following injection of a sample extract or standard, the appropriate fraction (10–14 min) was diverted to the gas chromatograph by means of the HPLC–GC interface.

A Valco C10W electronically actuated 10-port valve was positioned downstream of the UV detector used to monitor the LC effluent at 254 nm. This allowed the HPLC effluent to be directed either to waste or to the gas chromatograph via a 45 cm × 0.22 mm I.D. deactivated fused-silica transfer line threaded through the septum of the injector and into the uncoated pre-column positioned within the GC oven. The system was arranged to allow forward flushing of the transfer line as well as back-flushing with helium carrier gas.

The GC oven contained a 10 m × 0.53 mm I.D. uncoated non-polar deactivated fused-silica pre-column (Scientific Glass Engineering, Milton Keynes, UK), into which the transfer line from the C10W switching valve was threaded to depth of 10 cm. The inlet end of the pre-column was secured within the body of the injector, enabling carrier gas to enter around the outside of the transfer line. The carrier gas supply was regulated by a mass flow valve downstream of a pressure regulator to prevent solvent backflush.

The outlet of the pre-column was connected to the mid-point "T" assembly as shown in Fig. 1. The side arm of the "T" functioned as an "early vapour exit" [5,20] to allow HPLC mobile phase vapour to be vented away from the analytical column (30 m × 0.25 mm I.D., 0.25-μm film thickness DB17, J&W, Folsom, CA, USA) after vaporisaton in the pre-column. A liquid CO_2 cooled cryogenic cold trap (Scientific Glass Engineering) was positioned immediately downstream of the early vapour exit. This permitted focusing of the analyte on a short piece of coated column (15 cm × 0.53 mm I.D. DB5, 1.5-μm film thickness, J&W) at the head of the analytical column, following solvent evaporation, and closure of the early vapour exit. A relatively thick film coating was chosen to ensure efficient trapping of the analyte, although some band broadening was expected on analyte transfer from the thick film wide bore column (phase ratio *ca.* 88) to the analytical column (phase ratio *ca.* 249).

Control of fully concurrent mobile phase evaporation and analyte focusing was achieved using an automated pneumatic column switching (PCS) sys-

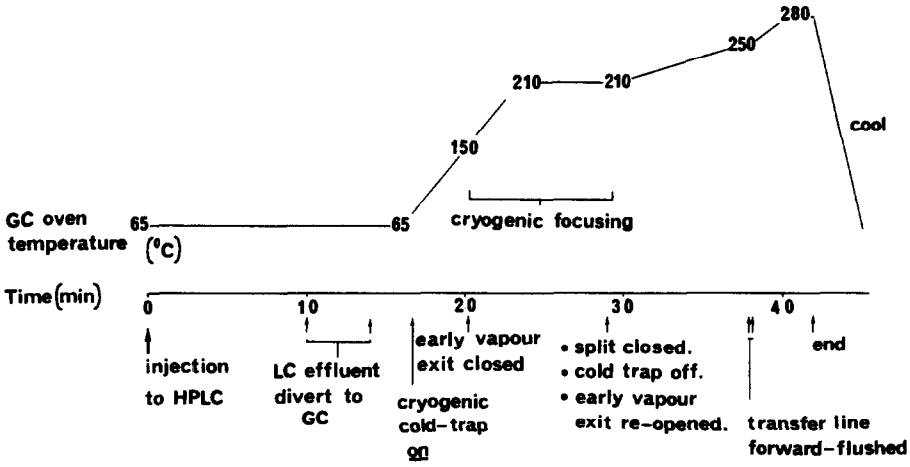

Fig. 2. Sequence of events within the HPLC–GC system relative to the GC temperature programme.

tem (Scientific Glass Engineering) designed for GC–GC, but adapted for multidimensional HPLC–GC. The automated operation of the cryogenic cold trap (pneumatic), the early vapour exit (pneumatic), the mid-point split (electronic) and the Valco C10W switching valve (electronic) was controlled by an electronic timer (Chrontrol CD-45, Lindberg Enterprises, San Diego, USA). Introduction of sample to the HPLC was by manual injection.

Fig. 2 shows diagramatically the course of events from the injection of sample into the liquid chromatograph to the end of the GC programme. The initial temperature of the gas chromatograph and hence the temperature at which the HPLC "cut" was introduced to the gas chromatograph was 65°C.

Standard solutions

Standard solutions of levamisole hydrochloride for fortification of milk samples were prepared at 1 mg ml^{-1} and 100 μg ml^{-1} concentrations in water. Standards for HPLC–GC analysis were prepared in methanol and HPLC mobile phase at 1 mg ml^{-1} concentration, and diluted as necessary.

Milk extraction procedure for levamisole determination

Milk (50 ml) was acidified to pH 4.6 with 6 *M* hydrochloric acid, then centrifuged at 4200 g for 15 min at at 10°C, and the supernatant decanted from the protein pellet and the fat layer (whole and semi-skimmed only). The supernatant volume was measured, and the pH adjusted to 11.0–11.2 by the addition of a few drops of 40% sodium hydroxide solution. A 25-ml aliquot of supernatant was loaded onto a C_{18} reversed-phase solid-phase extraction cartridge (Sep-Pak; Waters, Milford, MA, USA), pre-conditioned with 10 ml each of ethyl acetate, methanol and water. The cartridge was eluted with 10 ml of water-saturated ethyl acetate, and the eluate taken to dryness by rotary evaporation at 35°C. The residue was dissolved in 200 μl methanol–dichloromethane (5:95) for HPLC–GC analysis.

RESULTS AND DISCUSSION

Instrumentation

The HPLC–GC interface used in the application reported here was of the on-column type using fully concurrent evaporation. The principles of operation of this type of interface have been fully described elsewhere [2,21].

The commercially available PCS system consists of a series of solenoid activated valves designed for the regulation of pneumatic column switching in multidimensional GC–GC. With simple adaptation, however, it provided a convenient way of controlling and automating the valve switching requirements associated with the modified HPLC–GC interface. The unit directly controlled the opening/closure of the early vapour exit, the on/off control of the cryogenic cold trap, the opening/closure of the split at the head of the analytical GC column, and provided the independent pressure regulation of the carrier gas supplies to the column and the pre-column (via the mass flow valve). The pneumatic control arrangement is shown schematically in Fig. 1.

The early vapour exit was opened during sample introduction from HPLC to GC, permitting the venting of solvent vapour away from the analytical column, with the analyte condensing on the pre-column in a similar manner to the system described by Noy *et al.* [5]. Closure of the early vapour exit, after complete solvent evaporation, allowed analyte (s) remaining behind on the pre-column to be transferred to and focused on the 15 cm length of coated column held within the cryogenically cooled "T" piece cold trap. This novel feature, was capable of maintaining sub-ambient temperatures within the hot GC oven (210°C) for peak focusing. This differs from the cold trap described by Noy *et al.* [5], which was held at 70°C (*i.e.* initial GC oven temperature), and was "cold" with respect to an in-line vapouriser at 250°C upstream.

The splitter positioned between the cryogenic cold trap and the head of the analytical column, enabled the flow-rate through the pre-column (not compatible with the analytical column at *ca.* 7.5 ml min^{-1}) to be maintained whilst the early vapour exit was closed. The analytes were quantitatively retained on the cold trap even with the split open. Closure of the liquid CO_2 supply to the cold trap and the splitter, allowed the analysis to proceed on the analytical column.

The additional supply of helium carrier gas to the mid-point "T", immediately downstream of the early vapour exit, but upstream of the cold trap, is

an important novel feature of the system. The pressure at this point was set at 93 kPa, *i.e.*, a pressure slightly exceeding the equilibrium pressure at the outlet end of the pre-column when the inlet pressure at the GC injector port was set to 104 kPa, with the split open. This supply had two principal functions; firstly to ensure complete exclusion of the HPLC mobile phase from the analytical column (and subsequently the detector), by sweeping all vapour out of the early vapour exit, and secondly to provide the analytical column with its own carrier gas supply independent of that serving the pre-column. This meant that after transfer of analyte(s) from the pre-column to the cryogenic cold trap, the early vapour exit could be re-opened and the transfer line forward flushed with fresh HPLC mobile phase whilst analysis proceeded on the GC column. This facility also allowed less volatile materials still remaining behind on the pre-column following analyte transfer, to be vented out of the early vapour exit rather than through the analytical column.

During conventional GC–GC operation the side arm of the mid-point "T" would normally allow effluent from a coated pre-column to be directed to a monitor detector, when in the open position, or allow a "heartcut" to be diverted to the analytical column when closed. For the HPLC–GC operation reported here, the side arm functioned as the early vapour exit, with the uncoated pre-column replacing the coated pre-column. In HPLC–GC, a length of coated pre-column is frequently connected to the end of the uncoated column, prior to the early vapour exit [22], to prevent loss of volatile analytes during solvent evaporation and assist in analyte focusing, but was unnecessary in this instance.

The supply of carrier gas to the injector and hence the uncoated pre-column was via a pressure regulator (part of PCS system) positioned upstream of a mass flow regulator (Porter VCD-1000 series). This arrangement ensured that a positive flow of carrier gas was maintained through the pre-column during sample introduction despite the back pressure exerted by the evaporating mobile phase. During normal operation, when no HPLC fraction was being diverted to the gas chromatograph, approximately 30 ml min^{-1} carrier gas was used to backflush the transfer line, whilst a flow of 7.5 ml min^{-1} was maintained through the pre-column. On diversion of the HPLC fraction to the gas chromatograph, the entire flow from the mass flow valve passed through the pre-column to assist fully concurrent evaporation (*ca.* 37.5 ml min^{-1}).

HPLC–GC determination of levamisole in milk extracts

Initial attempts to analyse levamisole without cryogenic focusing revealed problems with band broadening. Since the compound is prone to tailing during GC [15,17,19], further deterioration in the form of excessive band broadening could not be tolerated. It was found, by monitoring the early vapour exit line, that levamisole did not start to elute from a new pre-column until a temperature of 160°C was reached. However, the apparent adsorptivity of the pre-column increased with use, probably due to re-activation by moisture and deposition of non-volatile material, such that in time a temperature of 210°C was needed to obtain complete recovery. This resulted in the transfer of the levamisole to the head of the analytical column in a broad band leading to poor chromatography. Having succesfully demonstrated the HPLC–GC system with no broadening problems using more volatile compounds under partially concurrent evaporation conditions, cryogenic focusing was considered as a possible way to prevent the broadening observed for levamisole.

The chromatograms obtained for 50-ng standard injections of levamisole hydrochloride (42.5 ng free base) made to the HPLC–GC system with cryogenic focusing, and using flame ionization detection (FID) and NPD, are shown in Fig. 3. In each case, 800-μl volumes of HPLC effluent (between 10–14 min after injection) were diverted to the gas chro-

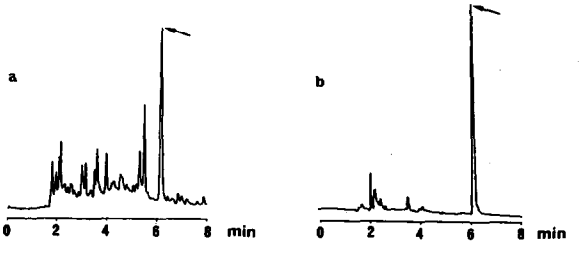

Fig. 3. GC chromatograms obtained for 50-ng injections of levamisole hydrochloride standards (42.5 ng free base) made to the HPLC–GC system, with (a) FID (attenuation 32) and (b) NPD (attenuation 32). Conditions as for Figs. 2 and 4.

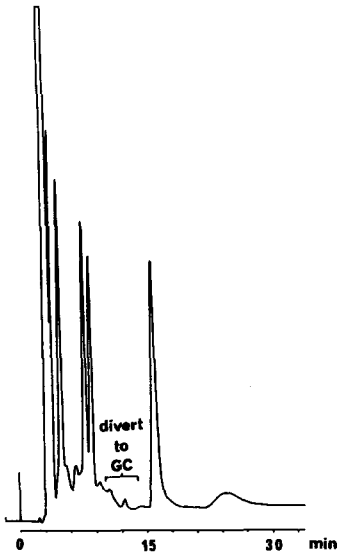

Fig. 4. HPLC chromatogram with UV detection at 254 nm (AUFS = 0.08) obtained from a 20-μl injection of milk extract onto a 25 cm × 2.1 mm I.D. normal-phase silica (Spherisorb S5W) column, eluted with methanol–dichloromethane (5:95) mobile phase at 200 μl min^{-1} flow-rate. The fraction diverted to the gas chromatograph for analysis is indicated.

matograph, and the use of cryogenic focusing enabled excellent peak shapes to be obtained. Additional peaks are present in both chromatograms arising from impurities in the HPLC solvents. The responses for both FID and NPD were linear for standard injections of levamisole hydrochloride in methanol over the range 50–400 ng, with correlation coefficients of $r = 0.999$ and $r = 0.998$, respectively (8 data points).

Cryogenic focusing provided a convenient method to ensure a good chromatographic peak shape for levamisole, and such an approach, in general, ensures that GC resolution is not sacrificed by the process of transferring the analyte(s) to the analytical column. Since all solvent is diverted away from the analytical column, cryogenic focusing of any solvent along with the analytes is not a problem.

Fig. 4 shows the HPLC trace obtained for a milk extract, where the HPLC fraction indicated was diverted to the gas chromatograph. The sample clean-up achieved by the HPLC step is demonstrated in Fig. 5, which compares HPLC–GC with GC analysis of the same extract. The GC chromatograms of extracts obtained with and without the HPLC clean-up are illustrated for both FID and NPD. For the analysis without HPLC clean-up, the HPLC column was replaced by a 40 cm length of 0.010 in. I.D. (1 in. = 2.54 cm) tubing and the entire extract was introduced into the gas chromatograph. Without the HPLC clean-up, even the extra selectivity of NPD was insufficient for reliable quantitative analysis. However, the clean-up afforded by the extraction and HPLC–GC procedure was sufficiently selective to allow the use of FID for analyte detection in milk extracts, demonstrating the benefits of the multidimensional approach. NPD showed improved selectivity and sensitivity and gave a detection limit of 0.4 μg l^{-1} for levamisole in milk compared with 2.2 μg l^{-1} with FID (S/N = 3:1). The

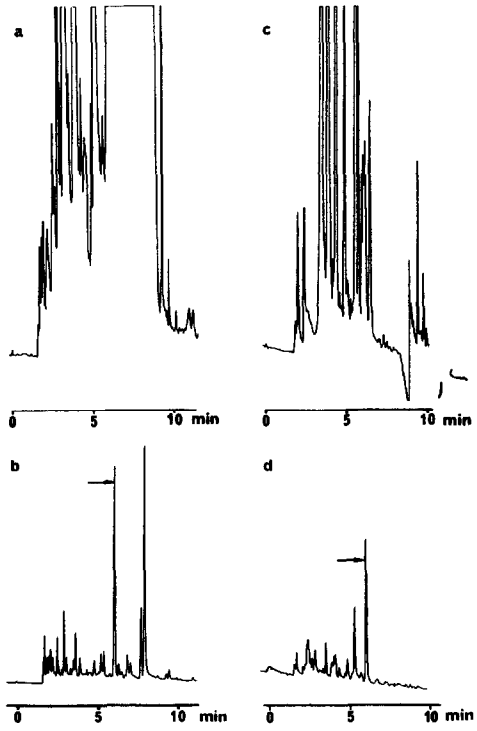

Fig. 5. GC chromatograms showing (a) GC–FID response (attenuation 32) for an extract from milk spiked with levamisole hydrochloride at 50 μg l^{-1} (42.5 μg l^{-1} free base) with no on-line HPLC clean-up and (b) HPLC–GC–FID response for the same extract. (c) and (d) show the equivalent traces with NPD (attenuation 8) for an extract from milk spiked at 10 μg l^{-1} (8.5 μg l^{-1} free base) without and with HPLC clean-up respectively. Conditions as for Figs. 2 and 4.

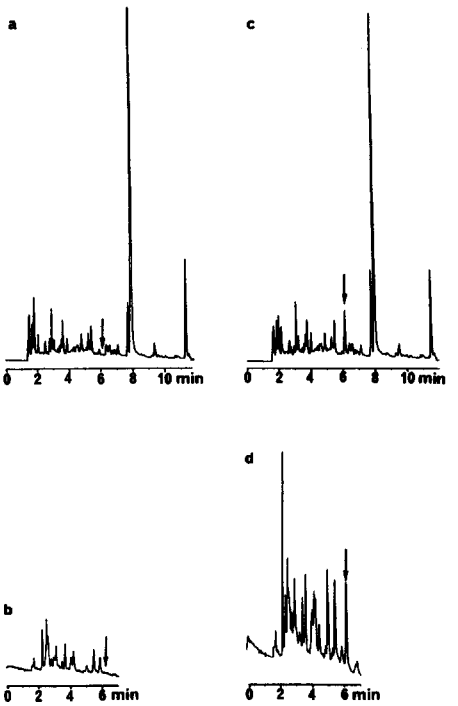

Fig. 6. GC chromatograms following normal-phase HPLC–GC analysis of extracts from unspiked milk with (a) FID (attenuation 32) and (b) NPD (attenuation 8). (c) FID (attenuation 32) and (d) NPD (attenuation 4) show chromatograms for extracts from milk spiked with levamisole hydrochloride at 10 $\mu g\ l^{-1}$ and 2 $\mu g\ l^{-1}$ (8.5 $\mu g\ l^{-1}$/1.7 $\mu g\ l^{-1}$ free base) for FID and NPD respectively. Conditions as Figs. 2 and 4.

chromatograms obtained for the analysis of milk extracts with both FID and NPD are shown in Fig. 6a and b. In each case, the concentration of levamisole was below the respective detection limits. The chromatogram for an extract of milk spiked at 10 $\mu g\ l^{-1}$ with levamisole hydrochloride using FID is also shown (Fig. 6c), together with an extract spiked at 2 $\mu g\ l^{-1}$ with NPD (Fig. 6d). The mean recovery for the assay (*i.e.* through the initial extraction and the HPLC–GC system) was 88.1 ± 3.9% (standard deviation) for 5 milk samples spiked with levamisole hydrochloride at 10, 25, 50, 75 and 100 $\mu g\ l^{-1}$ (Table I). Assay precision (coefficient of variation) for the extraction and HPLC–GC analysis was 4.9% for 7 whole milk samples spiked at 50 $\mu g\ l^{-1}$ with levamisole hydrochloride.

Fourteen different whole milk samples, obtained from a variety of dairy outlets, were extracted and

TABLE I

RECOVERY OF LEVAMISOLE FROM MILK THROUGH EXTRACTION AND NORMAL-PHASE HPLC–GC SYSTEM AT VARIOUS SPIKED LEVELS

Spiked level of levamisole hydrochloride ($\mu g\ l^{-1}$)	Corresponding level levamisole ($\mu g\ l^{-1}$)	Recovery (%)
10.00	8.50	87.1
25.00	21.25	94.9
50.00	42.50	85.1
75.00	63.75	86.4
100.00	85.00	87.0
		Mean 88.1 ± 3.9

analysed for levamisole using the HPLC–GC system. All fourteen samples contained less than the 10 $\mu g\ l^{-1}$ advisory limit set for levamisole by the joint FAO/WHO expert committee on food additives [10] and Statutory Instrument 1991 No. 2843 [11], and all except one were below the 0.4 $\mu g\ l^{-1}$ level set as the detection limit for this assay using NPD. One sample did show a peak at a retention time consistent with levamisole, corresponding to a concentration close to the detection limit. Further HPLC–GC–MS studies established that this signal was not due to the presence of levamisole [23].

CONCLUSIONS

A novel refinement in HPLC–GC interfacing is described which allows improved chromatographic clean-up and analysis. The use of the pneumatic column switching system with the liquid CO_2 cryogenic facility proved a convenient approach to peak focusing, enabling quantitative transfer of the analyte to the head of the analytical column with good GC resolution. The presence of methanol as modifier in the HPLC mobile phase also helped to eliminate problems of adsorption in the HPLC parts of the system such as the injection valve.

The HPLC–GC interface described has enabled the problems associated with a difficult analyte (levamisole) to be overcome, and the cryogenic focusing approach should greatly facilitate the analysis of difficult analytes in general. The on-line chromatographic purification is highly effective, allow-

ing a specific detection method such as NPD or a broad spectrum detection method such as a FID to be used, with detection limits in the range 0.4–2.2 $\mu g\ l^{-1}$ for levamisole.

The method reported, if fully automated with an autosampler, could be expected to handle a throughput of 20 samples per day. Current off-line methods typically handle around 6–7 samples per day.

The system could be modified to determine analytes which are not quantitatively retained on the uncoated pre-column, by connection of a short coated section, or by complete replacement with a coated column.

REFERENCES

1 H. J. Cortes, C. D. Pfeiffer and B. E. Richter, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 469–474.
2 E. Noroozian, F. A. Maris, M. W. F. Nielen, R. F. Frei, G. J. de Jong and U. A. Th. Brinkman, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 17–24.
3 R. E. Majors, E. L. Johnson, S. P. Cram, A. C. Brown III and E. Freitas, *Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, OH, 1979*, Abstract 116.
4 K. Grob Jr. and T. Laubli, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 593–594.
5 Th. Noy, E. Weiss, T. Herps, H. van Cruchten and J. Rijks, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 181–186.
6 K. Grob, Jr., H. P. Neukom and R. Etter, *J. Chromatogr.*, 357 (1986) 416–422.
7 K. Grob, M. Lanfranchi, J. Egli and A. Artho, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 506–512.
8 I. L. Davies, K. E. Markides, M. L. Lee, M. W. Raynor and K. D. Bartle, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 12 (1989) 193–207.
9 D. Thienpont, O. F. J. Vanparijs, A. H. M. Raeymaekers, J. Vandenberk, P. J. A. Demoen, F. T. N. Allewijn, C. J. E. Niemegeers, K. H. L. Schellekens and P. A. J. Janssen, *Nature (London)*, 209 (1966) 1084–1086.
10 *36th Report of The Joint FAO/WHO Expert Committee On Food Additives*, Cambridge University Press, Cambridge, 1990, pp. 31–37.
11 Statutory Instrument 1991 No. 2843, *The Animals, Meat and Meat Products (Examination for Residues and Maximum Residue Limits)*, Regulations, HMSO, London, 1991.
12 S. Marriner, E. A. Galbraith and J. A. Bogan, *Analyst (London)*, 105 (1980) 993–996.
13 M. Alvinerie, P. Galtier and G. Escoula, *J. Chromatogr.*, 223 (1981) 445–448.
14 B. G. Osterdahl, H. Johnsson and I. Nordlander, *J. Chromatogr.*, 337 (1985) 151–155.
15 J. E. Smith, N. R. Pasarela and J. C. Wyckoff, *J. Assoc. Off. Anal. Chem.*, 59 (1976) 954–958.
16 R. Westenborghs, L. Michielsen and J. Heykatis, *J. Chromatogr.*, 224 (1981) 25–32.
17 S. J. Stout, A. R. DaCunha, R. E. Tondreau and J. E. Boyd, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 1150–1153.
18 J. R. Perkins, D. E. Games, J. R. Startin and J. Gilbert, *J. Chromatogr.*, 540 (1991) 257–270.
19 M. J. Shepherd, in C. S. Creaser and R. Purchase (Editors), *Food Contaminants: Source and Surveillance*, Royal Society of Chemistry, Cambridge, 1991 pp. 109–175.
20 K. Grob, H. G. Schmarr and A. Mosandl, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 12 (1989) 375–382.
21 K. Grob, Jr., D. Frohlich, B. Schilling, H. P. Neukom and P. Nageli, *J. Chromatogr.*, 295 (1984) 55–61.
22 K. Grob, *Trends Anal. Chem.*, 8 (1989) 162–166.
23 C. G. Chappell, C. S. Creaser, M. J. Shepherd and J. W. Stygall, *Biol. Mass Spectrom.*, in press.