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Analysis of pesticides in red wines by on-line coupled reversed-phase liquid chromatography–gas chromatography with vaporiser/precolumn solvent split/gas discharge interface

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Abstract

Pesticides in red wines were analysed by on-line coupled reversed-phase liquid chromatography–gas chromatography where a vaporiser/precolumn solvent split/gas discharge interface enabled direct transfer of aqueous eluent to the GC system. The LC part of the system provided sample clean-up and re-concentration, and the GC the final analytical step. The method developed allowed automated and quantitative analysis of the wine samples, where the only manual step was filtration. The limits of quantification were clearly below the maximum residue limits established for grapes, being lower than 10 $\mu g\,l^{-1}$ for all pesticides studied. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Wine; Vaporiser interface; Interfaces, LC–GC; Liquid chromatography–gas chromatography; Pesticides

1. Introduction

A wide range of insecticides, fungicides and herbicides are used in grape production. Although the vinification process, especially alcoholic fermentation, greatly reduces the levels of pesticides, residues persist in wine and could be a source of toxicity to the consumer [1]. No uniform maximum residue limits (MRL) have been established for wines, but the limits for grapes are in the range 1.0–0.01 $mg\,l^{-1}$. Lower MRL limits have been suggested for wines.

At present, pesticide residues in wine are most commonly determined by chromatographic methods, mainly liquid and gas chromatography [2–7]. Efficient clean-up of the wine samples is always necessary before the actual analysis. A variety of sample preparation methods are reported in the literature, the traditional but tedious liquid–liquid extractions [2–4] gradually being replaced by solid-phase extraction methods relying on C18 [4], porous carbon [8] or diatomaceous earth [7] as stationary phase. The sample pre-treatment is not only time consuming, but it also increases the risk of sample loss and contamination. Recently, therefore, much attention has been paid to the development of LC–LC [9], SPE–GC [10], and LC–GC [11,12] and other automated sample pre-treatment methods.

Hyphenated chromatographic methods, such as on-line coupled LC–GC, incorporate the best features of both techniques: the large sample capacity and good preseparation capabilities of LC, and the

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efficient separation and sensitive detection techniques of GC. In the analysis of aqueous samples, coupling of RPLC to GC minimises the sample preparation. However, direct injection of aqueous solvents into the GC creates a problem, and special techniques are required. There are two possible approaches: the first is to avoid direct injection of water by using phase-switching techniques, e.g. RPLC–LLE–GC [11,12], and the second is to inject aqueous eluents directly while using special retention gaps or stationary phases, micro-LC, or a vaporiser interface. Special columns have proved to be problematic in use, and this approach has been adopted less widely. A major disadvantage of micro-LC is the low sample capacity.

In this study, we have developed an RPLC–GC method requiring minimal amount of manual sample preparation for the determination of pesticide residues common in red wines. The method utilises RPLC for sample clean-up and enrichment and GC for the final separation. A vaporiser/pre-column solvent split/gas discharge interface was used coupling of the RPLC and the GC.

The vaporiser interface allows direct introduction of the aqueous fraction from the LC to the GC. The fraction of interest is vaporised in the heated vaporising chamber under high gas flow-rate. The solvent vapours are removed through the retaining pre-column via a solvent vapour exit (SVE), while the analytes of interest are retained on the stationary phase of the pre-column. The critical parameter for the retention of the volatile analytes is the oven temperature during the transfer. The minimum transfer temperature is the dew point of the eluent, which is determined by the eluent composition, LC flow-rate, inlet pressure and the gas flow-rate during transfer [13,14].

2. Experimental

2.1. Apparatus

An automated LC–GC system Dualchrom 3000 Series from CE Instruments (Milan, Italy) equipped with UV and flame ionisation detectors was used. The programmable temperature vaporiser (PTV) was controlled with an MFA 815 (Multi-Function Actuator). The vaporising chamber consisted of a glass liner partially filled with Carbofrit and internally coated with polyimide [13,15]. PTV and detector temperatures were 300 and 310°C, respectively and the wavelength of the UV detector was 210 nm.

A 1 m×0.53 mm (I.D.) retaining pre-column (BGB-5, film thickness 0.25 μm, BGB Analytic, Zurich, Switzerland) was connected with a press-fit T-piece to the solvent vapour exit line and to a 30 m×0.25 mm (I.D.) analytical column (BGB-5, film thickness 0.25 μm, BGB Analytik, Zurich, Switzerland). The 10 mm×2 mm (I.D.) LC column was dry-packed in our laboratory with C18 (AsahiPak, Japan), of particle size 5 μm. The analytical procedure is described in Fig. 1.

2.2. Analytical procedure

The analytical conditions and procedure are set out in Fig. 1. Before injection the LC column was conditioned with an ethanol–water mixture (10:90, v:v) for 2 min at a flow-rate of 700 μl min^{-1} (solvent volume 1.7 ml), and after the LC analysis the column was back-flushed with methanol with the same flow-rate (5 min corresponding 3.5 ml of methanol). The conditioning and methanol wash can

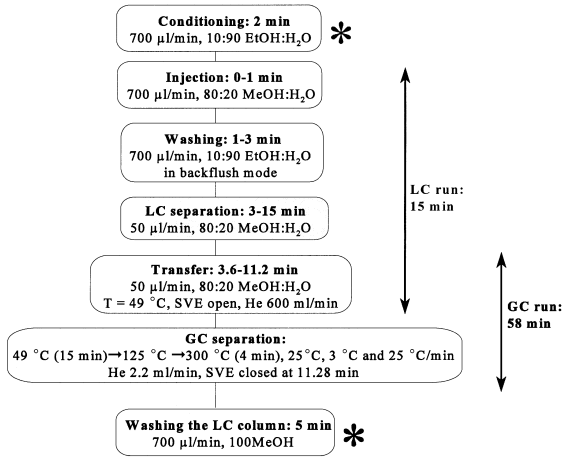

Fig. 1. Analytical procedure for analysis of pesticides in red wine. * the step can be during precious GC program.

be done during the GC analysis. The whole procedure took 62 min.

2.3. Chemicals

HPLC-grade methanol was purchased from JT Baker (Deventer, The Netherlands) and 96% ethanol from Primalco Oy (Rajamäki, Finland). Water was distilled, ion-exchanged and filtered. The pesticide standards (carbophenthion, endosulfan isomers α and β, procymidone, quinalphos, tetradifon and vinclosolin) were from Accustandard Inc. (New Haven, CT, USA). Polyimide was from HNU- Nordion (Helsinki, Finland).

2.4. Samples

The samples were red wines of different origin (Table 1). Spiked wine samples were prepared by adding a standard solution of the pesticides in methanol to wine containing no pesticides. The internal standard, carbophenthion, was added to all samples, after which they were diluted with distilled and de-ionised water (3:1, v/v) to reduce the ethanol concentration, and filtered. The size of the injection loop was 1000 μl and, from this, 700 μl of the sample was injected into the LC column. The total content of the loop was not injected in order to avoid mixing of the sample with the eluent during injection.

3. Results and discussion

3.1. LC clean-up

It was necessary to wash the LC column to remove matrix compounds that would seriously disturb the GC analysis. Ethanol–water (10:90, v/v) and methanol–water mixtures (10:90, v/v) were tested as washing eluents, and the former, which resulted in more effective clean-up was chosen. Two min washing, corresponding 1.4 ml of solvent, was sufficient.

Possible loss of the analytes in the LC clean-up procedure was investigated by comparing the results obtained in the total RPLC–GC procedure with the results of direct large-volume injections of standard solutions to the GC. The injections were made from a loop, from which the sample was pumped with the LC pump to the vaporising chamber. The sample volume and concentration were calculated to correspond to the LC fraction of interest. The results showed good recovery of all other pesticides (100–130%) except procymidone, for which the recovery was only 35%.

In the choice of flow-rate in LC, the limiting factor is the transfer step. Because of the large vapour volume and the high heat capacity of water, the transfer flow-rate to the vaporising chamber should not exceed about 200 $\mu l\,min^{-1}$. At higher flow-rates, the evaporating water will cool the vap-

Table 1
Red wines studied

Wine: country and grape	Pesticides found
USA Carigan, Barbera, Ruby Cabarnet, Carnelian	NF
Spain: Garnacha	NF
Italy: Corina Veronese, Rondinella, Molinara, Rossignola, Negtata	NF
Chile Cabarnet Sauvignon	NF
Kypros -	NF
Hungary: Kékfrankos	vinclozolin 8 $\mu g\,l^{-1}$
Bulgary: Merlot and Pinot Noir	NF
Spain: Tempranillo, Garnacha	vinclosolin 10 $\mu g\,l^{-1}$
Italy: mixture	vinclosolin 10 $\mu g\,l^{-1}$, tetradifon 25 $\mu g\,l^{-1}$, procymidone 36 $\mu g\,l^{-1}$
France: mixture	tetradifon 30 $\mu g\,l^{-1}$
Italy: Schiava, Merlot, Lambrusco	tetradifon 27 $\mu g\,l^{-1}$

NF=no pesticides found.

orising chamber to the boiling point of water, causing flooding inside the chamber. Furthermore, the transfer flow-rate is one of the parameters determining the lowest possible transfer temperature, as will be discussed below. A low flow-rate, however, would prolong the LC analysis, so a higher flow-rate of 700 $\mu l\,min^{-1}$ was chosen for the injection and washing period, and the flow was reduced to 50 $\mu l\,min^{-1}$ for the LC separation and transfer to the GC.

The composition of the eluent is another important parameter that has to be considered in the optimisation of the LC part. Besides affecting the transfer temperature, it affects the fraction volume of the analytes and the separation of matrix compounds from the analytes. The analytes should be in as small a fraction volume as possible, to reduce the band broadening associated with large amount of mobile phase, i.e. carrier gas eluent vapour mixture, which would push the analytes further into the GC pre-column. At the same time, total separation of the matrix compounds from the analytes of interest would be ideal. As a compromise, 80:20 methanol–water mixture was chosen as eluent (Fig. 2A). A decrease in methanol concentration to 70% resulted in more efficient clean-up, and practically no disturbing compounds retained in the fraction of interest (Fig. 2B). However, the fraction volume was then increased 70%, and furthermore, the dew point of the mixture was increased by several degrees, causing vinclosolin, the most volatile of the analytes of interest, was almost totally lost during the transfer. The elution order for the analytes was procymidone, quinalphos, endosulfan, vinclosolin, tetradifon, with fraction volume of 220 μl. Addition of internal standard, carbophenthion, increased the fraction volume from 220 to 380 μl At 80:20 methanol–water, the matrix compounds eluted with the fraction of interest did not interfere with the GC analysis of the pesticides.

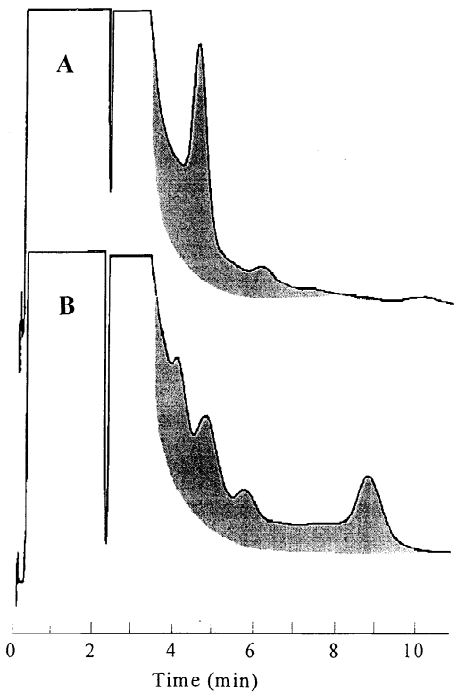

Fig. 2. The LC separation of pesticides in red wine with methanol–water (A) 70:30 and (B) 80:20 as eluent.

3.2. GC analysis

The vaporiser/pre-column solvent split/gas discharge interface is well suited for direct injection of aqueous eluents so long as no buffer salts are used. Solvent effects of water, i.e. solvent trapping and phase soaking effects, are poor, and water rapidly destroys the deactivation layer of the retention gaps. Re-condensation of water into the pre-column has to be avoided, therefore, and the oven temperature during the transfer should exceed the dew point of the eluent. Too high temperatures, on the other hand, will lead to losses of volatile compounds and the transfer temperature should rather be as close to the dew point of the eluent as possible.

Traditionally, the best conditions for the transfer have been determined according to the peak shapes or losses of analytes, and recently, programs calculating the conditions have become available. As shown in our previous study [13] it is not always possible to determine optimum conditions according the peak shapes. Dependence of the conditions on several, interdependent parameters nevertheless makes it difficult to find the optimal conditions. In our previous paper [13], we describe two ways to determine the conditions directly. The dew point of an eluent can be measured from the temperature changes on the outer column wall or by adding suitable compounds to the sample as indicators of

possible re-condensation [13]. Carbowax compounds, which dissolve well in water make good indicators for aqueous eluents.

The dew point, and thus the lower temperature limit for transfer, was determined by adding Carbowax compounds as indicators of re-condensation. Different LC flow-rates, and inlet pressures versus the carrier gas flow-rates, were studied in the optimisation of the transfer conditions. At 100 $\mu l\,min^{-1}$ LC flow-rate, the dew point could not be lowered sufficiently to allow analysis of vinclosolin, even with high gas flow-rates. When the LC flow-rate was only 50 $\mu l\,min^{-1}$, the dew point was lowered by several degrees and vinclosolin could be quantitatively analysed although the peak was slightly broadened. The broadening was due to the high flow-rate of the carrier gas. A thicker film in the retaining pre-column and also a longer (2 m) retaining pre-column were tested to improve the retention of vinclosolin. However, as in our work [13], the decreased reconcentration effect between the thicker film retaining column and analytical column broadened the peak of the most volatile analyte, here vinclosolin, unacceptably. The lengthening of the precolumn also required increase in the inlet pressure to obtain sufficient gas flow-rate through the pre-column, and since this raised the dew point there was no improvement in retention power.

Different inlet pressures were tested to obtain sufficiently high gas flow-rates. The short retaining pre-column had large I.D. to keep resistance inside the pre-column as low as possible. This enabled high carrier gas flow-rates with relatively low inlet pressures. 120 kPa pressure gave a carrier gas flow-rate through the SVE of 600 $ml\,min^{-1}$, corresponding to a transfer temperature slightly above the dew point, i.e. 49°C. No further improvement was obtained by increasing the gas flow-rate, and thus the inlet pressure beyond this. SVE was closed 10 s after the end of transfer, giving a flow-rate of 2.2 $ml\,min^{-1}$ through the analytical column.

3.3. Quantitative analysis

To evaluate the analytical method, we determined recovery in the LC clean-up procedure, linearity, repeatability, and limits of detection and quantification. Carbophenthion was used as an internal standard.

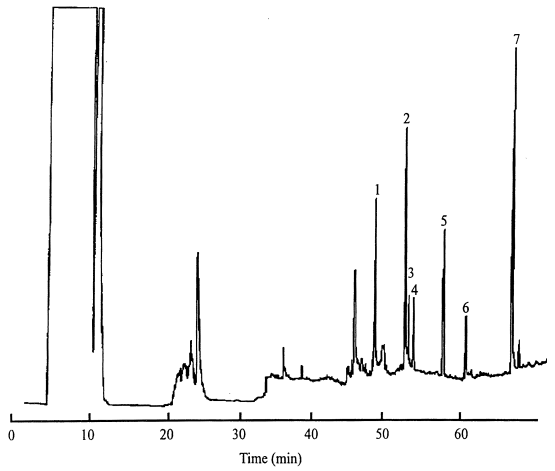

Fig. 3. LC–GC analysis of a wine sample spiked with the pesticides 100 $\mu g\,l^{-1}$, endosulfan isomers 50 $\mu g\,l^{-1}$, both. Peaks: 1=Vinclosolin, 2=Quinalphos, 3=Procymidone, 4=Endosulfan α, 5=Endosulfan β, 6=Carbophenthion (i.s.) and 7=Tetradifon.

The LC clean-up step gave a clean GC chromatogram free of disturbing matrix compounds (Fig. 3). For all the pesticides studied, the linearity of the method was good in the range 10–1000 $\mu g\,l^{-1}$ (Table 2). The repeatablities of retention times and peak areas were also good, although the R.S.D. value for the peak area of procymidone was relatively high. Compared to the large volume GC injections (R.S.D. 7% between the injections), the relatively poor repeatability of procymidone in the RPLC–GC analysis was due to the low recovery in the LC step. The R.S.D. values of vinclosolin and quinalphos were also better for the large volume injections than

Table 2
Linearities (10–1000 $\mu g\,l^{-1}$), repeatabilities (relative standard deviations, R.S.D. %, $n=4$) of relative retention times and peak areas, and limits of quantification ($\mu g\,l^{-1}$)

Analyte	Linearity	Repeatability		LOQ
		t_r	Area	
Vinclosolin	0.9994	0.11	16	5
Quinalphos	0.9991	0.12	17	8
Procymidone	0.9998	0.10	27	10
Endosulfan α	0.9990	0.10	9	5
Endosulfan β	0.9991	0.09	3	5
Tetradifon	0.9967	0.06	3	10

for the whole analysis procedure, showing that the volatility of the analytes did not have a major effect on the deviation of peak areas. The low recovery of procymidone was probably due to analyte eluting too close to the bulk of matrix being partly discarded with the matrix compounds as the washing step did not affect on the recovery of the analyte. A slightly longer LC column could probably increase the recovery, but it would also increase the analysis time. If analysis of more polar pesticides than included to this study is required, change in column material should be considered, for example C_8 might in that case be better than C_{18}. Limits of quantification were in the range 4–10 $\mu g\,l^{-1}$ ($S/N=5$). Relative to the MRL values given for grapes (>100 $\mu g\,l^{-1}$), the limits obtained are sufficient for residue analysis.

3.4. Stability of the system

Since the wine samples were directly injected into the short LC column, it was necessary to flush the column with pure methanol in back flush mode after each injection to avoid plugging of the column and the frits. However, cleaning of the frits in methanol ultrasonic bath was required after some 200 injections of wine samples, as indicated by increasing pump pressure. Part of the packing material was changed at the same time.

The inner surface of the vaporising chamber had to be well coated with the polyimide layer. When the layer was very thin, there was a noticeable adsorption of the analytes on the chamber after about 20 analyses. With a relatively thick film, the stability of the coating was much better and the chamber had to be replaced only after some 100 injections. The thickness of the polyimide layer could be estimated according to the colour of the vaporising chamber: the ticker the layer, the darker brown the glass chamber was. Relative to earlier results with water samples, the stability of the coating was not as good. Apparently the nonvolatile matrix components of the wine samples caused some deterioration of the polyimide layer.

The GC column system worked well during the analysis, as long as there was no significant recondensation of the eluent. During the optimisation of transfer temperatures, conditions allowing recondensation of water were tested. According to these experiments, slight recondensation of water (less than some 20 μl) at low temperatures did not have a serious negative effect, but a large amount of recondensed water caused deterioration of the stationary phase of the retaining precolumn, seen in the decreased retention power of the precolumn.

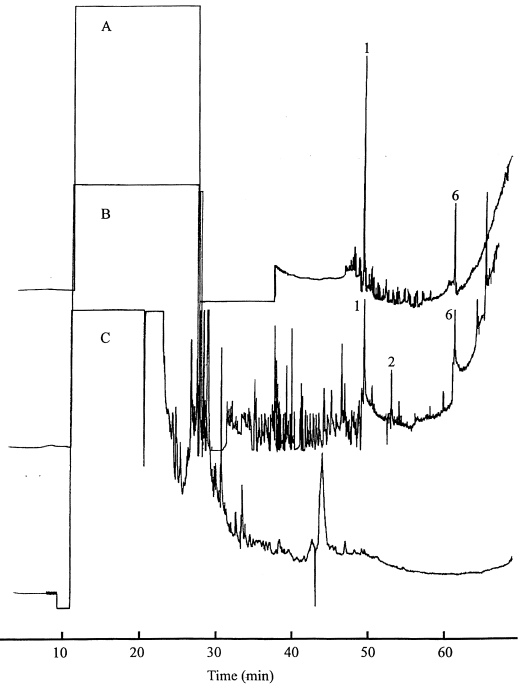

Fig. 4. LC–GC chromatograms of wine samples containing (A) vinclosolin (Hungarian wine, 6) and (B) vinclosolin, procymidone and tetradifon (Italian wine, 9), and (C) a blank wine sample (Spanish wine, 2). Conditions and peaks as in Fig. 3.

3.5. Analysis of red wines

Several red wines from different countries were tested for pesticides. Contaminants were found at trace levels of 8–36 $\mu g\,l^{-1}$ (Table 1). LC–GC chromatograms of two red wine samples containing pesticides, and one blank sample are shown in Fig. 4A–C.

4. Conclusions

The method developed allowed automated and

quantitative analysis of pesticides in red wines. The limits of quantification were much better than the maximum limits set for pesticide residues in grapes. No tedious sample preparation was required; the samples were merely diluted and filtrated before injection.

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