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Matrix effects in the determination of acaricides and fungicides in must by gas chromatography with electron-capture and nitrogen–phosphorus detection

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Abstract

The gas chromatographic determination of 12 pesticides, often used in viticulture, usually presents higher values than the real ones, arising from matrix effects, when must samples are analyzed. In order to reduce those errors, a method involving solid-phase extraction on octadecylsilane cartridges followed by a Florisil clean-up is developed. Moreover, a calibration with must extracts containing increasing amounts of pesticides and subjected to the same treatment as the samples also contributes to diminishing such errors. © 1997 Elsevier Science B.V.

Keywords: Matrix effects; Sample handling; Pesticides

1. Introduction

The need for phytosanitary treatments in vineyards has the disadvantage of the potential appearance of pesticide residues in musts and wines. This affects not only the wine elaboration but also the later commercialization of the product. In fact, there is a lot of literature on this topic, particularly with regard to the presence of pesticides in wine, however, this has been scarcely documented in the case of must.

The analysis of these residues usually entails a prior step of isolation that, until now, was carried out almost exclusively by liquid–liquid extraction with solvents such as benzene, ethyl ether or isooctane [1–4]. More recently, the use of solid-phase extraction (SPE), mainly on octadecylsilane (ODS) [5–7] has been used for the same purpose, allowing a clean-up step on the same extraction cartridge to be included [6]. The analysis of the extracts is predominantly performed by gas chromatography (GC) or high-performance liquid chromatography (HPLC), depending on the physico–chemical features of the analytes [1–3,5–7]. Our experience in this field suggests to us that wine and all must GC analyses for pesticides present considerable errors by excess. This phenomenon is attributed to the so called matrix effect which has so far been described in the GC analysis of diverse pesticides on samples such as wine [6], milk and butter [8] and, more recently, fruits and vegetables [9–11]. This effect is explained by a higher transference of analytes from the injection port to the chromatographic column either as a result of the presence in the extract of adjuvant carrier substances from the matrix, or of a protective effect in the injection port performed by those substances.

Taking into account the above considerations, this paper reports the results of the experiments intended

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to identify the source of these errors and their possible correction. For this purpose, an extraction procedure based on ODS cartridges followed by a clean–up on a Florisil packed-column and determination by GC with electron-capture (ECD) or nitrogen–phosphorus detection (NPD) has been developed for the analysis of 12 fungicides and acaricides often used in viticulture.

2. Experimental

2.1. Reagents

Trichlorphon, triadimefon, dichlofluanid, dicofol, triadimenol, procymidone, captan, folpet, nuarimol, oxadixyl, tetradifon and cypermethrin standards were obtained from Riedel-de Haën (Hannover, Germany) and Promochem (Wessel, Germany). Residue analysis-grade acetonitrile, ethyl acetate, ethyl ether, methanol, dichloromethane, acetone and *n*-hexane were supplied by Lab-Scan (Dublin, Ireland). Ultrapure water was obtained by using a Milli-Q apparatus from Millipore (Milford, MA, USA). Florisil of 60–100 mesh was purchased from Baker (Deventer, The Netherlands). Octadecylsilane 500-mg cartridges from Merck (Darmstadt, Germany) were used for solid-phase extraction.

2.2. Study of the solid-phase extraction

A study about the extraction of the target-compounds by ODS cartridges has been made. The study was carried out on a synthetic sample, water–ethanol (95:5, v/v) containing 24 g/l of glucose and 16 g/l of fructose and spiked with 4 μg/l of each pesticide.

The use of ethyl acetate, acetone, acetonitrile and methanol as eluents, the variation of the recovery with the solvent volume (2, 3 or 4 ml), and the influence of an equilibrium (or soaking) time between the eluent and stationary phase on the recovery were studied after eluting 500 ml of the synthetic sample through the cartridge.

The influence of the equilibrium time above mentioned was evaluated by pouring 2 ml of solvent into a cartridge, allowing the stationary phase to soak for 2 min before elution by gravity. If needed, 1 or 2 ml of the same solvent were subsequently added to the cartridge and eluted.

After the cartridge elution procedure was optimized, different volumes of solution (100, 200, 300, 400 and 500 ml) were eluted to study the breakthrough volume. Also, different amounts of each pesticide, 0.25, 0.5, 1, 1.5, 2 and 2.5 μg, were added to 100 ml of solution to check the possible saturation of the cartridges.

2.3. Study of the elution through a Florisil packed-column

A study to verify the pesticide elution through a Florisil packed-column, as clean-up, was also carried out. Florisil was conditioned by heating at 120°C for 4 h. The column, 10-cm long×1-cm I.D., was prepared from a Florisil (about 5 g) slurry in *n*-hexane and compacted with a rod. Then, the column was loaded with 2 ml of a pesticide solution in acetone (0.5 mg/l each one) and eluted by gravity with 15, 20, 25, 30, and 35 ml of *n*-hexane-dichloromethane (1:1, v/v), avoiding the column from drying at any time. Subsequently, the eluate was evaporated in a rotary evaporator from Büchi (Plawil, Switzerland) at 35°C and the residue dissolved in 2 ml of acetone.

2.4. Preparation of must samples

Spiked must samples were prepared just before extraction. For this purpose, a must volume of 50 ml, previously filtered on a glass plate, was mixed with 1 ml of acetone containing the fungicides and acaricides in known concentration, and with 200 ml of water to facilitate the elution through the cartridge.

2.5. Solid-phase extraction of must

A solid–liquid extraction system supplied by Varian (Harbor City, CA, USA) was used. ODS cartridges were conditioned by successive elution of 15 ml of methanol and 10 ml of water, by means of a gentle vacuum, and were not allowed to run dry during the procedure. Then, the sample was percolated at a flow-rate of 5 ml/min and the cartridge dried with nitrogen for about 30 min. Then, 2 ml of acetone were added to the cartridge, keeping them

for 2 min, after that the 2 ml volume was percolated and another 2 ml of acetone were later eluted, all by gravity. Finally, both portions were combined and concentrated at 2 ml in a rotary evaporator at 35°C.

2.6. Clean-up of must extracts

Must extracts were cleaned up by passage through a Florisil-packed glass column, made as above described. The column was loaded with 2 ml of extract which was left to elute by gravity, and then, 30 ml of *n*-hexane–dichloromethane (1:1, v/v) mixture was percolated. Finally, the eluate was evaporated in a rotary evaporator and the residue dissolved in 2 ml of acetone.

2.7. GC system

An Hewlett–Packard (Avondale, PA, USA) 5890 gas chromatograph equipped with an HP7673 autosampler, two detectors, electron-capture and nitrogen–phosphorus, and a 60-m×0.25-mm capillary column coated with a 0.25-μm thick film of 50% phenylmethylpolysiloxane (007–17) from Quadrex Scientific (Surrey, UK) was used. The oven temperature programme was as follows: initial temperature 50°C, held for 1 min, 15 C°/min ramp to 200°C, and finally 1 C°/min ramp to 275°C, held for 34 min. The carrier gas (He) flow-rate was 0.7 ml/min, measured at 50°C. Splitless injection of a 2 μl volume was carried out at 200°C with the purge valve on at 1 min. Hydrogen, air and helium were used as auxiliary gases for the NPD, and argon–methane (90:10) for the ECD. Both detector temperatures were 300°C.

3. Results and discussion

3.1. Extraction with ODS

Fig. 1 shows the recovery of the pesticides obtained by eluting the ODS cartridges with 2 ml of ethyl acetate, acetone, acetonitrile or methanol. Ethyl acetate seemed to be the poorest option. On the other hand, acetone and methanol provided higher recoveries than acetonitrile, except for tetradifon. In general and on the basis of the highest recoveries,

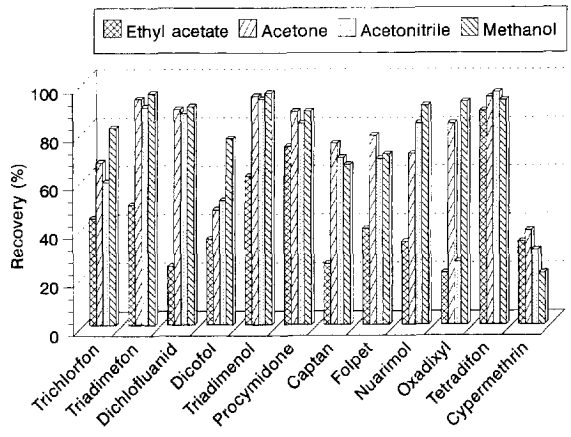

Fig. 1. Recovery of pesticides from 500 ml of synthetic sample spiked with 4 μg/l by ODS cartridges eluted with 2 ml of ethyl acetate, acetone, acetonitrile or methanol (n=5).

methanol was a more suitable eluent than acetone for most of the pesticides, and was selected to carry out the following experiences.

The inclusion of an equilibrium time of 2 min enhanced the analyte extraction, according to data published [12]. So, including the equilibrium time, the recoveries increased from 1.1% for triadimefon up to a 4.8% for procymidone and nuarimol. On the other hand, the recoveries were gradually higher if the eluent volume increased from 2 to 4 ml. The increase was about 3–5% for oxadixyl, tetradifon, triadimenol and triadimefon and about 11% for dicofol and folpet. The recoveries obtained in the elution with 4-ml after an equilibrium time of 2 min were higher than 90%, except for captan, folpet and cypermethrin, (75%, 83% and 27%, respectively).

A study about the breakthrough volume revealed that trichlorphon was the most affected compound, varying its recovery from 98.3% for 100 ml to 93.6% for 500 ml, while for the other pesticides the variation of the recoveries was only about 2% or less. A different behaviour was observed for dicofol, tetradifon, and in less extent, for cypermethrin whose recoveries increased when the sample eluted volume was higher. So, the recoveries of dicofol, tetradifon and cypermethrin for a 500-ml sample volume were 92, 96 and 27%, while they were 48, 64 and 20% for a sample volume of 100 ml.

When the pesticide amount was increased from

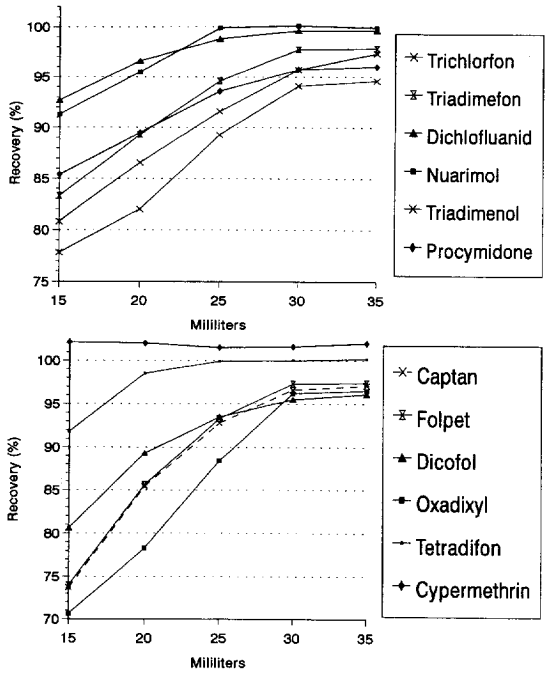

Fig. 2. Recovery of pesticides from a Florisil column for different volumes of *n*-hexane–dichloromethane (1:1) ($n=5$).

0.25 to 2.5 μg, variations in the recovery of less than 2% were found, excepting oxadixyl (6.6%) and cypermethrin (11%).

3.2. Elution through Florisil

Fig. 2 shows the results of the elution study on a Florisil packed-column. The recovery was gradually higher with increasing volumes of *n*-hexane–dichloromethane (1:1), except for cypermethrin, whose recovery did not vary from 15 ml. Experiments carried out on must extracts revealed the presence of a larger number of interfering compounds in the chromatograms for an excessive solvent volume, particularly in ECD. An eluent volume of 30 ml was considered satisfactory for the multiresidue analysis because it ensured recoveries of above 95%, and it avoided the number of co-extracted substances.

3.3. Must analysis

3.3.1. Influence of clean-up on matrix-effect

Table 1 lists the recovery and precision (expressed as relative standard deviation, R.S.D.) obtained in the determination of acaricides and fungicides on spiked must samples (20 μg/l of each compound) by the ODS–SPE procedure, eluting the cartridge with methanol or acetone, and including or excluding a Florisil clean-up step. Quantitation of the pesticides in the extracts was performed by a conventional external standard calibration. Enormously

Table 1
Recovery and precision obtained from must and must+water samples by conventional standard-solvent calibration ($n=7$)

ODS eluent:	Methanol	Methanol	Acetone	Acetone
Clean-up:	None	Florisil	Florisil	Florisil
Sample:	Must	Must	Must	Spiked water +must
	Recovery (%) ± R.S.D. (%)			
Trichlorphon	150±11.0	108±9.7	90±7.3	91±7.0
Triadimefon	431±5.6	212±5.7	102±8.0	100±8.1
Dichlofluanid	454±10.2	162±11.8	129±7.2	141±7.4
Dicofol	114±11.3	99±8.7	99±9.2	93±6.9
Triadimenol	420±13.3	230±11.0	78±8.9	77±7.3
Procymidone	243±9.8	152±6.4	99±6.3	99±6.0
Captan	1011±19.6	297±7.9	293±9.1	340±11.0
Folpet	931±15.0	100±7.5	250±8.0	271±9.3
Nuarimol	347±6.5	211±10.3	88±4.6	88±5.6
Oxadixyl	373±8.3	223±10.6	92±6.7	93±7.2
Tetradifon	199±4.2	129±6.3	94±3.3	95±2.6
Cypermethrin	319±7.2	151±8.6	74±3.3	31±3.4

R.S.D.: relative standard deviation

high recoveries were obtained in some instances and attributed to matrix effects. So, recoveries of about 900–1000% were obtained when the extracts were not submitted to clean-up, 150–230% for the most compounds in the extracts obtained with methanol and treated with Florisil, and 130–290% for some compounds in the extract eluted with acetone and also on Florisil. Dichlofluanid, captan and folpet were the most influenced by the matrix.

The clean-up efficiency is directly related to the matrix- effect, as can be deduced from the observation of the values given in Table 1 and their corresponding chromatograms in Fig. 3. It can be stated that recoveries calculated from the extracts with more complex chromatograms, which present larger amounts of substances from the matrix, did exceed the predictions. By contrast, values very much closer to the expected ones were obtained in the extracts that supplied simpler chromatograms.

According to the results obtained, elution with acetone and subsequent Florisil clean-up, is the best alternative for the analysis of fungicides and acaricides on must. The assays described below were carried out in this way. On the other hand, Figs. 4 and 5 show the chromatograms of extracts in GC–NPD and GC–ECD, respectively.

In order to ascertain that the high recoveries were due to an alteration in the transference of analytes from the injection port to the chromatographic column, a dual extraction was devised. A must sample without pesticides (50 ml+200 ml of water) was extracted; simultaneously, a water sample (250 ml) spiked with the analytes was subjected to a similar sample preparation. Subsequently, both extracts were combined and injected in the GC system. The recoveries obtained from the combined extract were analogous to those obtained for a spiked must sample, except for cypermethrin (see Table 1). Unexpectedly, this compound was less recovered in the dual extraction, its recovery (31%) being similar to that one obtained by SPE from the synthetic sample. In this case, the matrix does not affect the

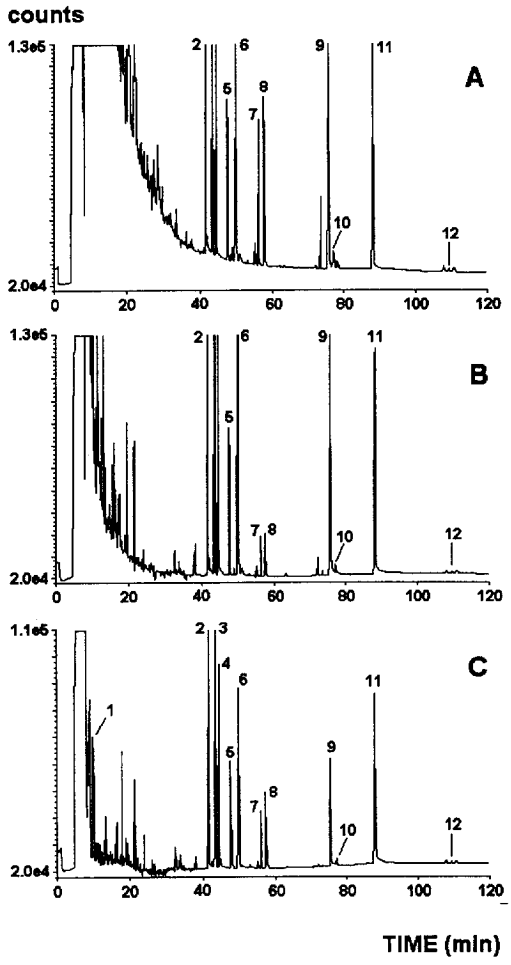

Fig. 3. Chromatograms of a spiked must extract in ECD. (A) Cartridge eluted with methanol. No Florisil clean-up. (B) Cartridge eluted with methanol. Florisil clean-up. (C) Cartridge eluted with acetone. Florisil clean-up. Peaks: 1=Trichlorphon, 2=triadimefon, 3=dichlofluanid, 4=dicofol, 5=triadimenol, 6=procymidone, 7=captan, 8=folpet, 9=Nuarimol, 10=oxadixyl, 11=tetradifon, 12=cypermethrin.

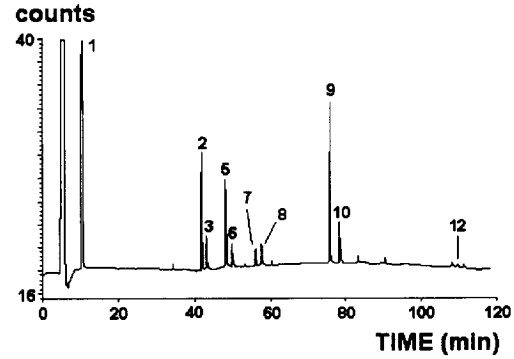

Fig. 4. Chromatogram of a spiked must extract obtained by NPD. Cartridge eluted with acetone. Florisil clean-up. See Fig. 3 for peak identification.

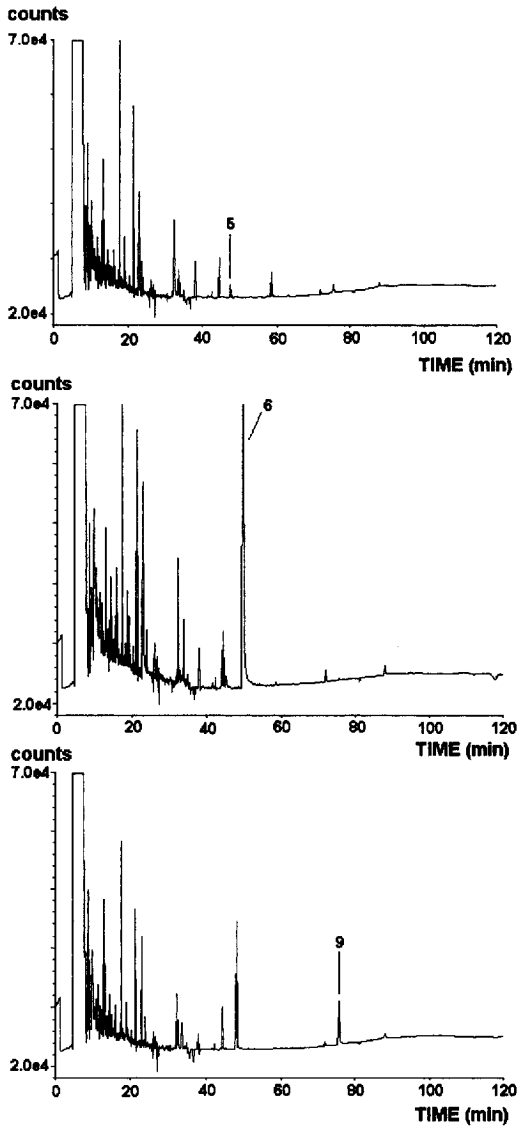

Fig. 5. Chromatograms of some must extracts obtained in ECD. Cartridge eluted with acetone. Florisil clean-up. See Fig. 3 for peak identification.

GC injection, but it seems to help the retention of cypermethrin on ODS.

3.3.2. Correction of the matrix effect

To reduce the quantitative errors from the matrix effect, a standard-addition method or an external standard calibration with the standards dissolved in a non-spiked sample extract could be used [6]. However, a third possibility has been considered in this work. Must spiked with the pesticides was treated identically as the samples, making a calibration of 5 levels with the extracts obtained. In Table 2, the validity of the quantitation procedure can be observed on the basis of the concentrations calculated on spiked samples (20 μg/l each pesticide). Such concentrations were expressed as recoveries and found to be consistent with the added amounts (recoveries close to 100% with R.S.D.s about 4–7%, $n=7$). Table 2 also shows the dynamic linear range and the regression coefficients (r) of the linear fittings for the standards subjected or not to the sample treatment. The regression coefficient was worse for the standards subjected to the sample treatment.

The performance of calibrations with spiked musts treated as real samples can be used to obviate the quantitation errors caused by the matrix-effect.

3.4. Detection and quantitation limits

Table 3 lists the detection and quantitation limits calculated experimentally as a signal-to-noise ratio of 3 and 10, respectively, on a must extract spiked with the pesticides after extraction, and the detector used in each case. The detection limit ranged between 1 and 40 μg/l while the quantitation limit ranged from 7 to 100 μg/l. Therefore, the detection limit provided by the analysis method varied between ca. 0.04 and 1.6 μg/l. Obviously, the matrix effect decreases such limits as it enhances the chromatographic response of the analytes and reduces the background noise.

4. Conclusions

The so called matrix effect prevents accurate GC quantitation of pesticides in must extracts, higher recoveries being observed when the amount of co-extracted substances increases.

The use of ODS–SPE with acetone as eluent, a clean-up on Florisil, and a matrix-standard calibration with spiked must samples subjected to the same treatment as the samples, is proposed to improve the analyte quantitation.

Table 2
Recovery obtained from must samples by standard-matrix calibration ($n=7$)

	Recovery (%) ±R.S.D. (%)	LDR (mg/l)	r standards	r standards +matrix
Trichlorphon	98.6±7.0	0.050–1.0	0.9997	0.9990
Triadimefon	99.7±6.3	0.025–1.0	0.9999	0.9900
Dichlofluanid	98.3±5.3	0.050–1.1	0.9983	0.9940
Dicofol	101.3±6.5	0.015–1.4	0.9995	0.9948
Triadimenol	95.3±7.3	0.050–1.5	0.9999	0.9854
Procymidone	98.6±6.4	0.050–1.0	0.9996	0.9939
Captan	100.3±7.0	0.100–1.5	0.9989	0.9985
Folpet	101.8±7.3	0.100–1.3	0.9990	0.9782
Nuarimol	103.6±4.9	0.025–1.0	0.9995	0.9952
Oxadixyl	92.2±5.3	0.100–1.5	0.9990	0.9874
Tetradifon	102.3±3.2	0.050–1.1	0.9999	0.9976
Cypermethrin	97.9±3.0	0.100–1.0	0.9995	0.9570

R.S.D.: relative standard deviation.
LDR: linear dynamic range.
r: coefficient of regression.

Table 3
Retention time, detection and quantitation limits obtained for the pesticides ($n=7$)

	Retention time (min)	Detector	Detection limit (μg/l)	Quantitation limit (μg/l)
Trichlorphon	10.20	NPD	1	35
Triadimefon	41.40	NPD	10	40
Dichlofluanid	43.40	ECD	10	40
Dicofol	44.38	ECD	3	10
Triadimenol	47.45	NPD	35	65
Procimidone	49.70	ECD	2	7
Captan	56.08	ECD	25	70
Folpet	57.49	ECD	25	80
Nuarimol	75.66	ECD	7	23
Oxadixyl	78.26	NPD	20	65
Tetradifon	88.01	ECD	3	7
Cypermetrin	109.71[a]	ECD	40	100

[a] retention time of the middle peak.

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