ELSEVIER

Journal of Chromatography A, 858 (1999) 123–131

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Inclusion of the fungicide chlozolinate in a multiresidue method

Ch. Lentza-Rizos*, E.J. Avramides

National Agricultural Research Foundation (N.AG.RE.F.), 1 S. Venizelou St., 14123 Lycovrissi, Athens, Greece

Received 11 May 1999; received in revised form 8 July 1999; accepted 9 July 1999

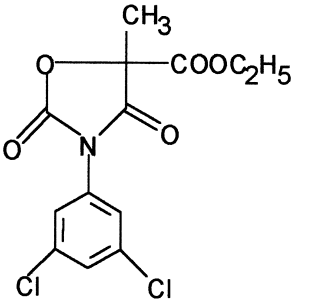
Abstract

A multiresidue GC method for the monitoring of electron-capturing compounds has been validated for the fungicide chlozolinate in the four crops with uses to be supported during re-evaluation procedures within the European Union. The method was also tested for a further eight crops. Matrix-matching of standards was found to be desirable to avoid the random occurrence of artificially high recoveries. The high accuracy and precision of the method, with a mean recovery of 94% and an RSD of ±5%, shows it to be suitable for routine residue control in a wide range of crops.

Keywords: Fruits; Vegetables; Food analysis; Pesticides; Chlozolinate; Dicarboximides

1. Introduction

Chlozolinate [structure and chemical names in Fig. 1, log octanol–water partition coefficient (log $P_{o/w}$)=3.15, solubility in water=32 mg/l at 25°C] is a fungicide belonging to the dicarboximide group which is used on a variety of crops: fruiting vegetables, stone fruits, artichokes, carrots, grapes, potatoes and strawberries. It was included in the first list of compounds re-evaluated within the European Union in the framework of directive 91/414/EEC [1,2]. Although its use on edible crops to be supported in the future will be restricted to stone fruits and grapes, the necessity to monitor all produce for residues remains. Methods published in the literature are high-performance liquid chromatography (HPLC) methods based on that of Cabras et al. [3],



chlozolinate

ethyl (±)-3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxo-= oxazolidine-5-carboxylate (IUPAC)
(±)-ethyl 3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxo-= 5-oxazolidine-carboxylate (C.A.)

Fig. 1. Structure and chemical names of chlozolinate.

*Corresponding author. Fax: +30-1-2818-735.
E-mail address: rizos@internet.gr (C. Lentza-Rizos)

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00801-8

which enables the simultaneous determination of dicarboximide fungicides and their degradation product 3,5-dichloroaniline, and are, therefore, group specific. Although chlozolinate degrades to a number of metabolites, the residue definition proposed includes only the parent compound. Two gas chromatography (GC) methods specific for chlozolinate which use thermionic specific detection (TSD) have been developed by the manufacturing company ISAGRO, Milan, Italy, and validated satisfactorily for peaches, apricots and grapes [4] and for grapes and wine [5]. However, for routine residue control it is desirable to be able to analyse residues of as many compounds as possible simultaneously and it is, therefore, important to examine the possibility of including chlozolinate in multiresidue methods.

The aim of this work was to investigate the possibility of determining residues of chlozolinate in several crops using a routinely applied multiresidue method and to validate this method for the fungicide according to the relevant international guidelines [6–9].

2. Experimental

2.1. Analytical method

Since chlozolinate contains two chlorine atoms compared to only one nitrogen atom, greater sensitivity would be expected with electron-capture detection (ECD) than with TSD. The method selected for this study was a simple, low-cost, multiresidue method for electron-capturing compounds [10] which is already used routinely in our laboratory for monitoring purposes. All solvents and reagents used were of high purity and suitable for pesticide residue analysis. A 25-g homogenized sample (reduced from 50 g in the original method) is extracted with a mixture of 50 ml toluene and 25 ml propan-2-ol using an Ultra-Turrax homogenizer for 3 min. The upper toluene phase is decanted through a funnel with a plug of quartz-wool into a separatory funnel and clean-up is effected in two stages through (a) liquid–liquid partitioning with two 125-ml portions of an aqueous solution of 2% Na_2SO_4 and (b) vigorous shaking for 1–2 min of a 10-ml aliquot of the toluene phase with 1 g of a mixture of adsorbents (activated charcoal Altec 6/16 granular–Celite 545; 3:1, w/w) followed by filtration through a fluted filter paper to obtain the final extract.

2.2. Matrices and fortification levels

The four most important crops, grapes, peaches, apricots and plums, were fortified at four concentrations (1, 0.4, 0.1 and 0.04 mg/kg) with five replicate samples at each level. Two additional levels, 5 and 0.004 mg/kg, were included for grapes in order to test the method at residue concentrations around the proposed maximum residue level (MRL) of 5 mg/kg and at the estimated limit of quantitation (LOQ). Plums, as representative of stone fruits, were also fortified at 0.004 mg/kg.

A second set of measurements was carried out on seven crops for which the use of chlozolinate is still authorised but is likely to be withdrawn in the future, and on one further crop, apples, for which there is no approved use. Fortification was at the expected residue level of 1 mg/kg (8 to 12 replicates). Extension of the study to include these crops covers the need to ensure that monitoring programmes will detect illegal use of the pesticide. In addition, the method was tested for grape samples with incurred residues, using grapes of varieties Cardinal and Victoria from plots of vineyards of table grapes which had been treated with a commercial formulation of chlozolinate, as described elsewhere [11].

2.3. GC determination

The GC determinations were carried out using a Hewlett-Packard 5890 Series II gas chromatograph fitted with an ECD system operated at 300°C, a Hewlett-Packard 7673 autosampler and a split–splitless injector operated in the splitless mode (250°C, 60 s, 1 μl). The carrier and make-up gases were helium and nitrogen (50 ml/min), respectively, and two fused-silica chromatographic columns were employed: an Rtx-5 column (5% diphenyl–95% dimethylpolysiloxane stationary phase, 30 m×0.250 mm I.D., 0.25 μm film thickness) with a He pressure of 95 kPa and an HP608 column (30 m×0.530 mm I.D., 0.5 μm film thickness) with a He pressure of 37 kPa. The following oven temperature programme

was used: 80°C, hold 1 min; 15°C/min to 190°C; 3°C/min to 280°C, hold 5 min.

Quantification was carried out using calibration curves with three to five levels which covered relatively small concentration ranges (typically a factor of 10). The lowest calibrated level and highest calibrated level were chosen in each case so as to ensure that the range of pesticide concentration in the samples of the batch was covered, and bracketing injections of standards were used, i.e., standard injections were made both preceding and following each batch of samples. Recoveries for grapes, plums, apricots and peaches at all fortification levels and for apples and aubergines at 1 mg/kg were determined with matrix-matched standards. The blank commodity used for matrix-matching was identical to that used for preparation of the spiked samples. Recoveries at 1 mg/kg for these matrices and for the other crops included in the study were determined with non matrix-matched standards since, at the time these measurements were made, matrix-matching of GC standards was not routine practice in our laboratory.

2.4. Method validation

The validation of an analytical method in individual laboratories, i.e., in-laboratory validation, generally includes testing it for accuracy, precision, the limits of detection (LOD) and quantitation (LOQ), specificity, sensitivity, linearity, ruggedness and practicability [6–9]. More recent requirements involve the examination of matrix effects and verification of the stability of an analyte in both calibration solutions and sample extracts [6,7].

The basic criteria which must be met in order for a method to be considered quantitative are satisfactory accuracy and precision, i.e., a recovery in the range 70 to 110% (mean recovery 80–100%) and a relative standard deviation (RSD) within ±10%. Precision is characterised through data on (a) repeatability, which estimates within-batch variation (same analyst, test material and equipment over a short time interval), and (b) internal reproducibility, (preferably different analysts, batches of reagents, and possibly the use of different equipment over a longer time interval).

Most series of replicates for a given fortification level and crop were carried out on the same day by the same analyst, thus providing extensive data for testing the repeatability of the method. Internal reproducibility is indicated by the RSD values of the mean recoveries for different crops fortified at 1 mg/kg. These were carried out over a period of many months, and extracts were prepared by several different laboratory assistants, some of whom were short-term temporary staff with no previous training in the field. These data, therefore, also provide a test of the ruggedness and practicability of the method.

The calculation of the LOD was made based on three-times the standard deviation of the chlozolinate concentration found for ten repeat injections of the extract of a sample fortified at the estimated LOQ. Measurements were made for both grape and plum extracts. The LOQ was confirmed by calculation of the RSD of the recoveries for five replicate samples each of grapes and plums fortified at this level [7].

For specificity, blanks of at least two samples of each crop were checked for interfering peaks on the two chromatographic columns used, and the retention times of a wide range of other electron-capturing compounds were checked against that of chlozolinate. Sensitivity and the linearity of the detector response to the analyte were examined by the injection of matrix-matched standard solutions of chlozolinate at 12 concentrations in the range 0.002 to 10 μg/ml.

The effect of matrix-matching on recoveries was evaluated by repeating some of the earlier analyses for which matrix-matched standards had not been used. Measurements were made for six different crops at the 1 mg/kg fortification level.

In order to test the stability of chlozolinate, the extracts from samples of grapes obtained from field trials with the pesticide were stored at −20°C and reanalysed after six months. The stability of the matrix-matched calibration standards during GC analysis was assessed by comparison of the detector response to standards left on the instrument for three days with that of freshly-prepared standards.

3. Results and discussion

Table 1 gives the percentage recoveries for the four main crops (grapes, plums, apricots and peaches) at four to six fortification levels and for

Table 1
Percentage recoveries of chlozolinate from grapes, stone fruits, aubergines and apples, determined using matrix-matched standards for GC analysis

Crop	Level (mg/kg)	Recovery for five replicate samples		Overall recovery per crop	
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
Grape	5	86	3	93	6
	1	98	2		
	0.4	86	4		
	0.1	96	3		
	0.04	96	4		
	0.004	93	1		
Plum	1	97	5	94	6
	0.4	94	6		
	0.1	96	2		
	0.04	98	2		
	0.004	88	8		
Apricot	1	97	6	97	5
	0.4	94	5		
	0.1	96	6		
	0.04	99	2		
Peach	1	90	3	92	4
	0.4	92	3		
	0.1	90	2		
	0.04	96	2		
Aubergine	1	93	2		
Apple	1	95	1		
Recovery over all substrates				94	5

apples and aubergines fortified at 1 mg/kg. All the mean recoveries lie within the range 86 to 99%, with an overall recovery of 94% and RSD of 5%. The repeatability of the measurements is good, with values for the RSD of within-batch measurements lying between 1 and 8%. Table 2 presents recoveries for analyses carried out without matrix-matching. These include measurements for the above crops fortified only at 1 mg/kg in addition to data for the other crops included in the study. The range of RSD values for within-batch analyses is comparable to that obtained for the equivalent matrix-matched determinations. However, recoveries for individual samples are sometimes high, reaching a maximum of 119% compared to 102% when matrix-matched standards are used. Where a direct comparison can be made (for six crops fortified at 1 mg/kg), it can be seen that an increased recovery is sometimes, but not always, observed when standards are not matrix-matched. Internal reproducibility is indicated by the RSD values for the mean recoveries for each crop in Table 2. These range from 4 to 12%, with all except one value below 10%.

With the procedures described above, values for the LOD and LOQ of the method were 0.001 mg/kg and 0.004 mg/kg, respectively. The lowest calibrated level routinely achieved was 0.002 μg/ml, corresponding to a concentration in the crop of 0.004 mg/kg.

No coextractives from the crops tested were found to interfere with the quantification of chlozolinate. Fig. 2 shows chromatograms of (A) a typical grape

Table 2
Percentage recoveries of chlozolinate from various crops fortified at 1 mg/kg and determined using non matrix-matched standards for GC analysis (recoveries in bold type were determined at a different time from the preceding measurements of the same series)

Crop	Analyst	Recovery of replicate samples (%)						Overall recovery per crop	
		1	2	3	4	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Apples	a	96	108	108	99	103	6	101	5
	b	102	97	104	108	103	4		
	c	95	98	100	98	98	2		
Apricots	a	101	87	100	97	96	7	97	4
	b	97	102	101	101	100	2		
	e	97	92	95	99	96	3		
Aubergines	a	104	99	104	103	102	2	103	7
	b	105	98	110	119	108	8		
	d	111	91	97	100	100	8		
Courgettes	a	119	116	104	104	111	7	108	6
	d	103	106	105	104	104	1		
Cucumbers	b/f	104	104	**95**	**80**	96	12	93	12
	c/g	101	**100**	**79**	**83**	90	12		
Grapes	b	103	101	99	101	101	2	95	7
	g	91	93	87	85	89	4		
Melons	a	97	104	104	100	101	3	99	4
	b	103	97	94	95	97	4		
	d	94	99	102	94	97	4		
Peaches	a	93	105	104	105	102	6	103	7
	b	110	114	108	106	110	3		
	b/f	91	105	**101**	**96**	98	3		
Peppers	a	94	107	104	109	104	6	101	6
	b	104	97	97	**103**	100	4		
	d	99	107	103	**86**	99	9		
Plums	a	91	95	93	101	95	4	99	5
	b	102	102	106	110	105	4		
	c	97	100	97	98	98	1		
Strawberries	b	101	104	91	**90**	96	7	96	8
	f	107	110	**97**	**96**	102	7		
	g	89	90	**91**	**89**	90	1		
Tomatoes	a	99	101	91	96	97	4	99	5
	b	107	101	99	102	102	3		
	c	98	102	92	104	99	5		
Overall mean recovery								100	7

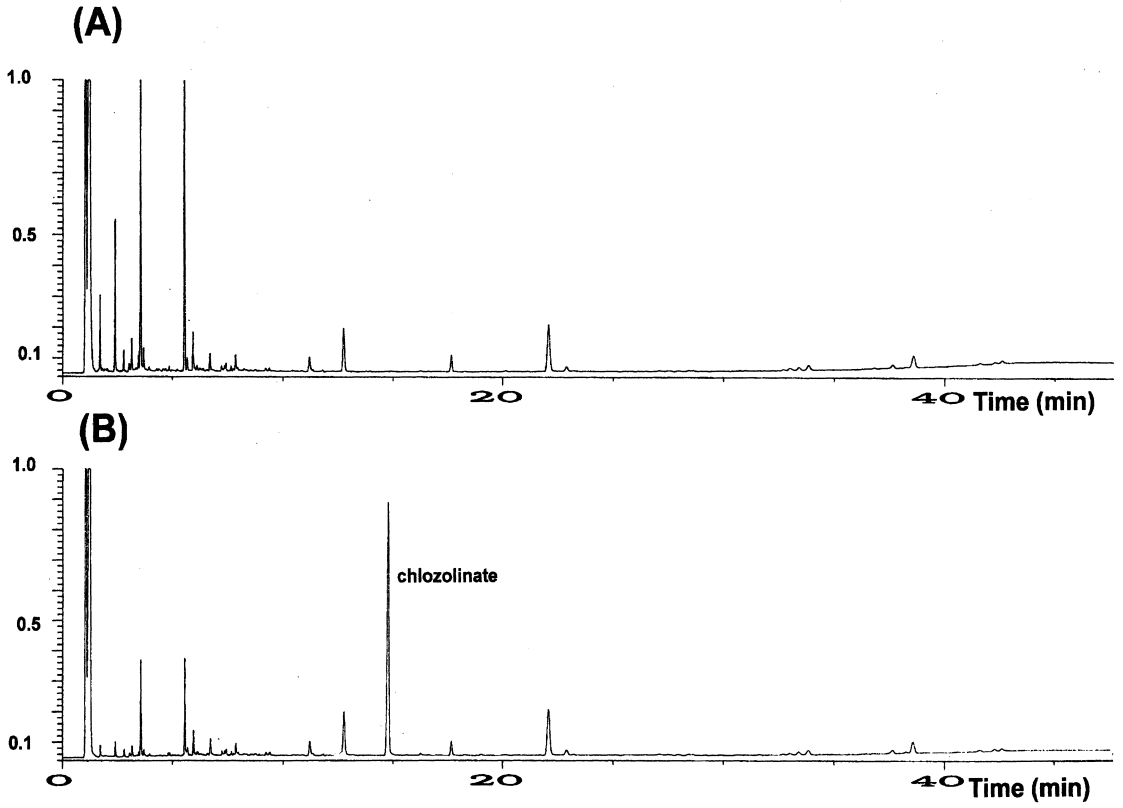


Fig. 2. Chromatograms of (A) a typical grape control sample extract and (B) 0.1 μg/ml of chlozolinate in grape extract.

control sample and (B) chlozolinate standard in grape extract. Of the 50 pesticides routinely sought using this multiresidue method in our laboratory, those giving a retention time within 0.2 min of the chlozolinate peak on either of the analytical columns used are given in Table 3. Only two compounds, penconazole and heptachlor epoxide, give peaks which are only partially resolved from that of chlozolinate on both columns. Heptachlor epoxide is a superseded compound, which is most unlikely to occur in samples monitored for chlozolinate. Although the partial resolution of the penconazole and

Table 3
Retention times and recoveries of compounds eluting close to chlozolinate

Pesticide	Retention time (min)		Recovery (%)	Recommended for use on vines or stone fruit
	Rtx-5 column	HP608 column		
Captan	16.585	16.831	93	Yes
Chlorfenvinphos I (minor peak)	16.136	14.371	88	
Chlorfenvinphos II	16.579	15.206	87	
Chlozolinate	16.413	14.214	93	Yes
Heptachlor epoxide	16.461	14.345	96	
Penconazole	16.308	14.359	46	Yes
Phorate sulphoxide	14.482	14.199	50	
Pyrifenox (one of two peaks)	16.418	15.303	25	Yes
Tolylfluanid	16.485	15.327	95	Yes

chlozolinate peaks should be sufficient to prevent confusion of the two compounds, the identity of any residue would be better confirmed, in the absence of GC–MS capabilities, with a different GC column. For example, with an Rtx-50 column (50% diphenyl–50% dimethylpolysiloxane stationary phase, 30 m×0.250 mm I.D., 0.25 μm film thickness) retention times are 22.68 and 23.03 min for chlozolinate and penconazole, respectively.

Excellent linearity was observed over a wide concentration range, as shown in Fig. 3A. The detector response began to fall off only above 5 μg/ml. Despite the regression line correlation of $r^2=1.000$, it is desirable to carry out quantification with a calibration curve of limited range because of very slight curvature observed when concentrations of more than a factor of ten are covered. This is particularly important for low concentrations, since a small change in the intercept of the regression line may have a relatively large effect on the concentration calculated. Fig. 3B gives the regression line for the concentration range 0.002 to 0.02 μg/ml for comparison with that for the full concentration range.

The results obtained for the study of the stability

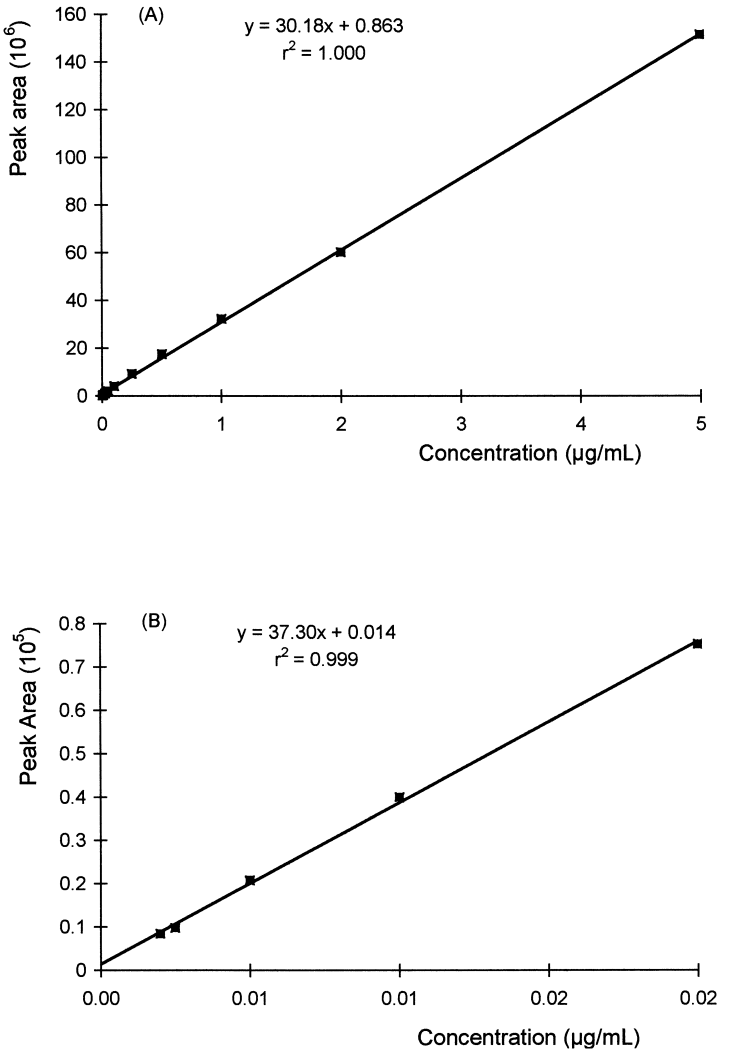


Fig. 3. Plots of detector response against chlozolinate concentration over the range (A) 0.002–5 μg/ml and (B) 0.002–0.02 μg/ml.

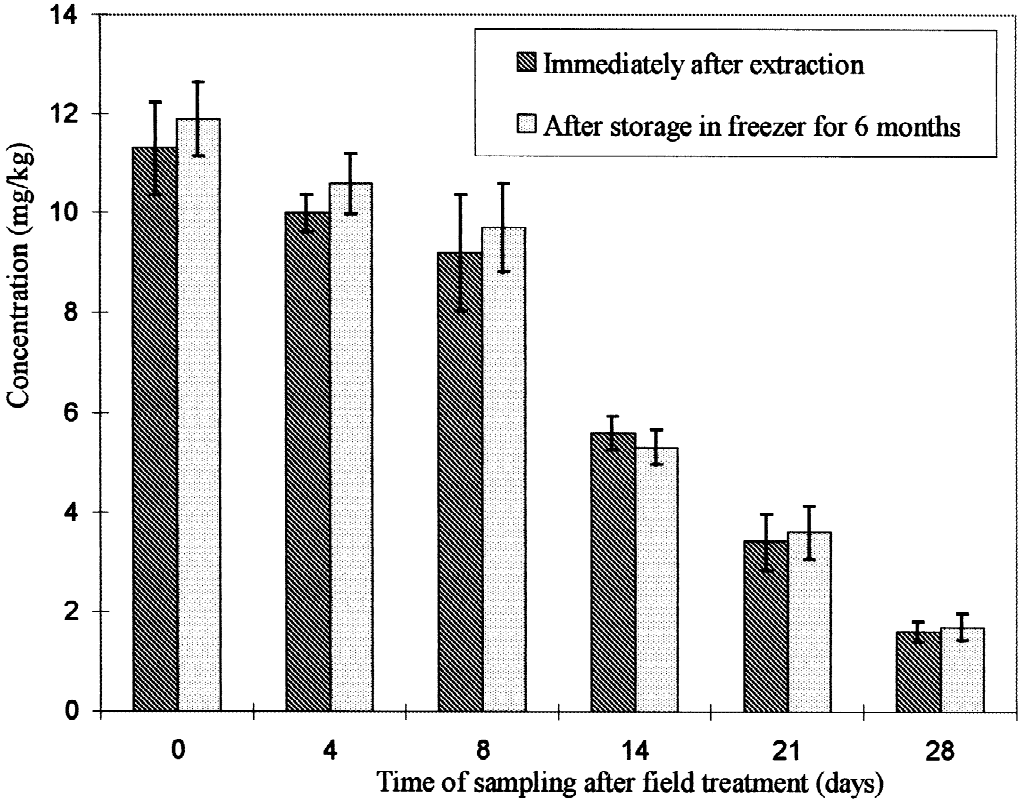


Fig. 4. Chlozolinate in grape samples from field trials (mean of measurements for three subsamples, each analysed in duplicate). GC analysis was carried out immediately after sample extraction and again after storage of the extracts for six months at −20°C.

of chlozolinate in the extracts of grape samples with incurred residues are shown in Fig. 4. There is no indication of degradation in sample extracts stored at −20°C over six months. Similarly, no instances of degradation of chlozolinate in calibration solutions were observed.

4. Conclusions

The multiresidue method tested gives good accuracy and precision for the analysis of chlozolinate residues in all the crops tested. In addition, it shows good sensitivity and specificity and the sample extracts are stable under the conditions required for GC analysis. Although the effect of crop enhancement is not large, the use of non matrix-matched calibration standards tends to lead to randomly higher recoveries. The analytical method is, therefore, shown to be suitable for the monitoring of chlozolinate residues in fruits and vegetables.

Acknowledgements

This work was supported by the National Agricultural Research Foundation (N.AG.RE.F.) within the framework of the ''DIMITRA'' project. The authors are grateful to Kalliopi Kokkinaki for assistance with preparation of the manuscript.

References

[1] EEC Council Directive 91/414/EEC of 15 July 1991, Official Journal of the European Communities No. L 230/1-32, European Commission, Brussels, 1991.

[2] Commission Regulation (EEC) No. 3600/92 of 11 December 1992, Official Journal of the European Communities No. L 360/10-16, European Commission, Brussels, 1992.
[3] P. Cabras, M. Meloni, F.M. Pirisi, R. Pirisi, Pestic. Sci. 15 (1983) 247.
[4] G. Zini, ISAGRO, Company File No. 67, Milan, 1996.
[5] G. Zini, ISAGRO, Company File No. 43/1, Milan, 1990.
[6] European Commission, Quality Control Procedures for Pesticide Residues Analysis – Guidelines for Residue Monitoring in the European Union, European Commission, Brussels, 1997, Commission Working Document 7826/VI/97.
[7] Codex Alimentarius Commission, In-House Method Validation, CX/MAS 98/8, Food and Agriculture Organisation of the United Nations, Rome, 1998.
[8] A.R.C. Hill, S.L. Reynolds, Analyst. 124 (1999) 953.
[9] EC Commission Directive 96/46/EC of 16 July 1996, Official Journal of the European Communities No. L 214/18-24, European Commission, Brussels, 1996.
[10] P.A. Greve (Ed.), Analytical Methods for Residues of Pesticides in Foodstuffs, 5th ed., Ministry of Welfare, Health and Cultural Affairs, The Netherlands, 1988, p. 3, Part I.
[11] Ch. Lentza-Rizos, E.J. Avramides, A. Argyropoulou, V. Papadimitriou, K. Kokkinaki, J. Agric. Food Chem., submitted for publication.