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Chromatographic analysis of imazalil and carbendazim in fruits Method validation and residue monitoring program 1995

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Abstract

A single and simple procedure for the determination of both carbendazim and imazalil is described. The proposed analytical methodology is based on a liquid–liquid extraction with ethyl acetate and further analysis with HPLC–UV in the case of carbendazim, and GC–nitrogen–phosphorus detection in the case of imazalil. Detection levels have been 0.01 mg/kg for carbendazim and 0.005 mg/kg for imazalil. Recoveries have been no less than 77% for carbendazim and 92% for imazalil. The method has been validated with fortified samples at different concentration levels. Different confirmation criteria have been studied and applied in routine analysis. The analytical procedure proposed has been applied to the analysis of 200 fruit samples belonging to the Residue Monitoring of Hygiene Food Program 1995 of the Spanish Ministry of Health, which has been performed in our laboratory. The results obtained have confirmed the viability of the method in routine analysis for these pesticides. A first evaluation of the presence of residues of both fungicides in fruits produced in Spain has been made.

Keywords: Fruits; Food analysis; Carbendazim; Imazalil; Pesticides

1. Introduction

Carbendazim and imazalil are two wide spectrum systemic fungicides, with a wide use in Spain in the treatment of fruits and vegetables. Therefore, an important goal of the different pesticide residue monitoring programs is the analysis of these fungicides. Two different sample preparation methodologies are usually required to analyze both compounds, and consequently, a great deal of time and money are spent in their analysis. Existing chromatographic methods involve different liquid–liquid extraction followed by preconcentration and GC or HPLC analysis.

Different extraction procedures using benzene [1], *n*-hexane [2] or ethyl acetate [3], followed by GC–electron-capture detection [1], HPLC–UV [2] or GC–nitrogen–phosphorus detection (NPD) [3], have been described for imazalil analysis. Methods using acetone [4], methanol [5–7], ethyl acetate [8,9] and chloroform [10] as extractive solvents followed by a solid-phase extraction [4–6,8] or a liquid–liquid partitioning [7,9], as well as a supercritical fluid extraction [11], analyzing in all cases by HPLC with UV and/or fluorescence detection have been developed for carbendazim analysis. The present work describes a simple and reliable procedure for the determination of both fungicides using liquid–liquid extraction followed by HPLC with UV detection for carbendazim and GC–NPD for imazalil better than HPLC–UV because of its lower detection limit. We

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also present confirmation studies which assure us of the presence of these pesticides in fruit samples.

2. Experimental

2.1. Chemicals

Ethyl acetate, *n*-hexane and sodium sulfate (residue analysis grade), acetonitrile (HPLC grade), sodium hydrogencarbonate, sodium hydroxide and sulfuric acid (analysis grade), were purchased from Merck.

2.2. Standards

Carbendazim 99% purity and imazalil 97% purity, were obtained from Dr. Ehrenstorfer (Germany).

2.2.1. Imazalil

Stock solution was prepared by weighing 10 mg and dissolving in *n*-hexane to 100 ml (0.1 mg/ml). Working solutions used in validation studies were prepared as follows: Solution A was obtained by diluting 1 ml of stock solution in *n*-hexane to 100 ml (1 μg/ml); other solutions were prepared by diluting 1, 2, 4, 6 and 8 ml of solution A in *n*-hexane to 10 ml respectively.

2.2.2. Carbendazim

Stock solution was prepared by weighing 10 mg and dissolving in acetonitrile to 100 ml (0.1 mg/ml). Working solutions used in validation studies were prepared as follows: Solution B was obtained by diluting 1 ml of stock solution in acetonitrile–water (1:1) to 25 ml (4 μg/ml); Solution C was obtained by diluting 1 ml of stock solution in acetonitrile–water (1:1) to 50 ml (2 μg/ml); other solutions were prepared by diluting 1, 3, 5 and 6 ml of solution C in acetonitrile–water (1:1) to 10 ml.

2.3. Apparatus

As food chopper, a robot coupe R-10 cutter mixer (Zanussi) was used. The centrifuge was a Macrotronic (P Selecta).

2.3.1. HPLC

A modular HPLC system was used consisting of a LKB 2150 pump, a Waters 484 UV variable-wavelength detector, a Waters U6K injector and a Waters 745 data module.

Column: 125×4.6 mm I.D. LiChrospher 100 RP-C_{18} 5 μm (Merck) and a Nova-Pak C_{18} pre-column.

2.3.2. Operating conditions

Flow-rate, 1 ml/min; chart speed, 0.5 cm/min; column at ambient temperature; wavelength, 280 nm; injection volume, 20 μl.

2.3.3. GC

A Hewlett-Packard 5890 A gas chromatograph equipped with NPD and with a split–splitless capillary injection port was used.

Column: 30 m×0.25 mm I.D., 0.25 μm capillary SPB-608 (Supelco).

Operating conditions: temperature programmed from 150°C (1 min) to 285°C (15 min) at 8°C/min; flow-rate of carrier gas (helium) 1.5 ml/min; injection in splitless; chart speed 0.3 cm/min; injection volume 1 μl.

2.4. Sample preparation

A representative fruit sample of 2 kg was homogenized with cutting equipment and 10 g were weighed in a centrifuge tube. 15 ml of ethyl acetate were added, shaking for 10 min and centrifuging at 2500 *g* (4500 rpm) for 15 min. The ethyl acetate fraction was transferred to a 100 ml separatory funnel. Solid residue was extracted again with 15 ml of ethyl acetate, shaking and centrifuging, and the organic fraction was transferred to the same separatory funnel. This fraction was rinsed with 10 ml sodium hydrogencarbonate (50 g/l) and again, with 10 ml distilled water, discarding washings. Organic fraction was extracted with two 10 ml portions of 0.025 *M* sulfuric acid, transferring aqueous acidic phases to 100 ml separatory funnel. Then the aqueous phase was rinsed with 15 ml ethyl acetate. Organic phase was discarded and aqueous phase was adjusted to pH 8 with 1 *M* sodium hydroxide and transferred to 100 ml separatory funnel. Aqueous phase was extracted twice with 10 ml portions of ethyl acetate, combining organic phases and discard-

Table 1
Detection and quantification limits for imazalil and carbendazim in fruit samples

	LD (mg/kg)	LC (mg/kg)
Imazalil	0.005	0.010
Carbendazim	0.010	0.020

LD, Detection limit; LC, Quantification limit.

ing aqueous phases. Organic extract was filtered through bed of anhydrous sodium sulfate, rinsing with 10 ml ethyl acetate and collecting in a graduated cylinder. Ethyl acetate was added to a 30 ml final volume.

2.4.1. HPLC

15 ml of the final volume were taken and transferred to a 25 ml bottom flask, concentrating to total dryness under a stream of nitrogen in a rotary evaporator at 45°C. Dry extract was redissolved in 0.5 ml mobile phase acetonitrile–water–ammonium hydroxide (15:85:0.6). 20 μl were injected for carbendazim analysis.

2.4.2. GC

15 ml of the final volume were taken and transferred to a 25 ml bottom flask, concentrating to total dryness under stream of nitrogen in rotary evaporator

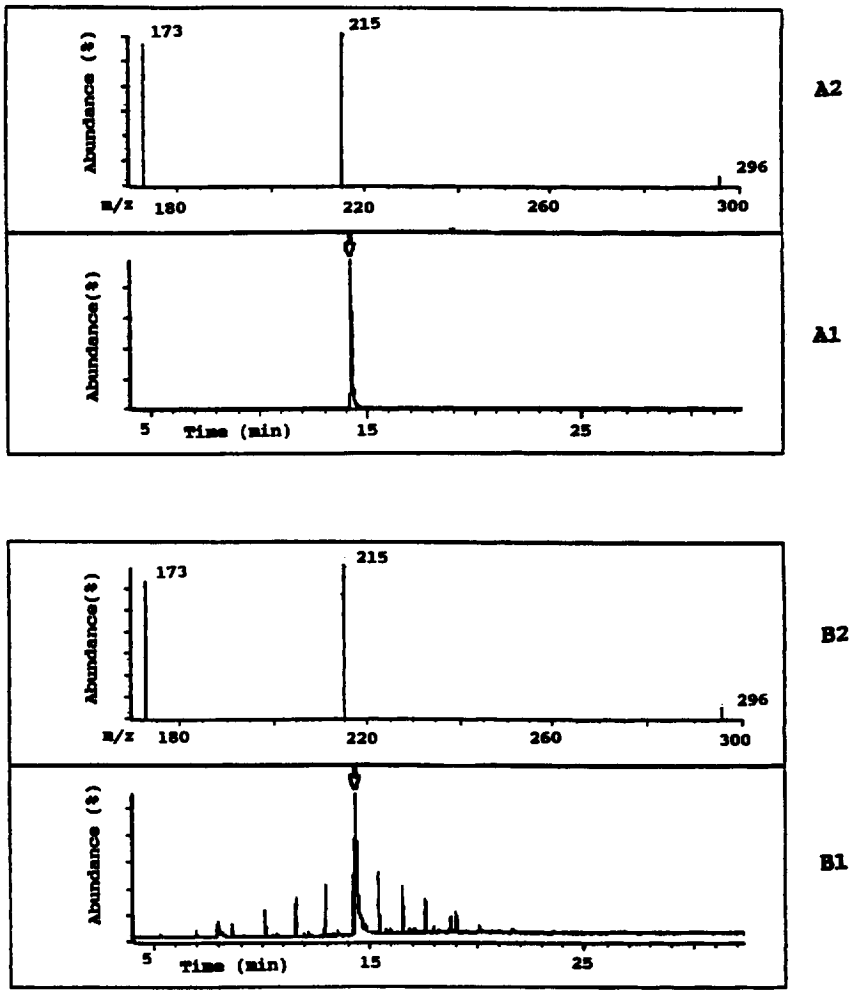

Fig. 1. Total ion current (TIC) and mass spectra in selected-ion monitoring (SIM) mode of imazalil. Mass of selected ions: 173, 215 and 296. (A1) TIC of imazalil standard solution (t_R of imazalil 14.22 min). (A2) Mass spectrum imazalil peak. (B1) TIC of real sample. (B2) Mass spectrum of $t_R = 14.22$ min peak.

at 45°C. Dry extract was redissolved in 0.5 ml *n*-hexane. 1 μl was injected for imazalil analysis.

3. Results and discussion

3.1. GC and HPLC studies

The linearity of response ranged from 0.2 to 4 μg/ml for carbendazim and from 0.1 to 1 μg/ml for imazalil. Equations of calibration curves were: $y=16.24x-2.86$ with a coefficient of regression $r=0.998$ for carbendazim and $y=43.97x-2.21$ with a coefficient of regression $r=0.990$ for imazalil.

Detection limit values were calculated using a 5:1 signal-to-baseline-noise ratio. Quantification limit values were calculated using a 10:1 signal-to-baseline-noise ratio. Detection and quantification limits are shown in Table 1.

A further clean-up of samples were not necessary to obtain chromatograms without interferences.

3.2. Validation studies

Repeatability and reproducibility as well as recoveries, were studied at two different fortification levels in ten fruit samples (apples) (Table 2).

All samples were analyzed consecutively in the same day, for the same analyst to study repeatability and for different analysts on different days to study reproducibility.

In all cases every sample was injected in duplicate.

Relative standard deviations were not higher than 6% for imazalil and 5% for carbendazim in repeatability studies, and were lower than 7% for both compounds in reproducibility studies. Recoveries were no less than 92% for imazalil and 77% for carbendazim, and significant differences were not found at either of the two different fortification levels. The results of repeatability, reproducibility and recoveries were considered adequate to the validation of the method.

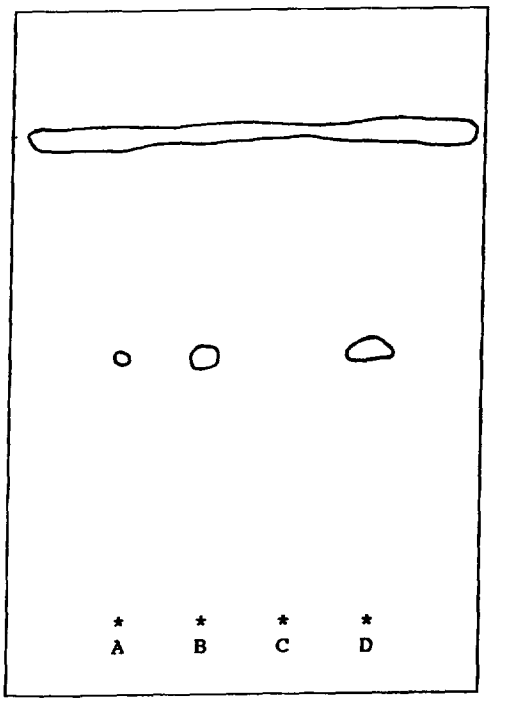

Fig. 2. Thin-layer chromatography of carbendazim; (A) standard containing 0.1 μg; (B) standard containing 0.5 μg; (C) real negative sample; (D) confirmation of presence of carbendazim in real sample.

3.3. Identification studies

Gas chromatography–mass spectrometry in case of imazalil, and thin-layer chromatography in case of carbendazim, were used to confirm the presence of such fungicides. The mass of the tentative selected ions for imazalil were: 173, 215 and 296, corre-

Table 2
Method validation studies

Fortification levels (mg/kg)	Mean of recoveries (%)	Repeatability R.S.D. (%) ($n=5$)	Reproducibility R.S.D. (%) ($n=5$)
Validation studies for imazalil in fortified fruit samples			
0.04	100	4.5	6.5
1.00	92	6.8	7.7
Validation studies for carbendazim in fortified fruit samples			
0.06	77	2.5	6.4
1.00	82	5.7	7.6

Table 3
Results of the Spanish Residue Monitoring of Hygiene Food Program 1995

Commodity	Number of samples analyzed	Total findings		Findings>50% MLR		Range found (mg/kg)		Spanish MLRs (mg/kg)	
		IMZ	CBZ	IMZ	CBZ	IMZ	CBZ	IMZ	CBZ
Grape	25	–	10	–	2	–	0.05–2.80	0.02	5.00
Strawberry	25	–	11	–	3	–	0.08–3.60	0.02	5.00
Tomato	25	–	1	–	–	–	0.06	0.02	2.00
Orange	25	8	–	2	–	0.04–3.00	–	5.00	2.00
Lemon	25	9	–	–	–	0.03–0.08	–	5.00	2.00
Potato	25	–	–	–	–	–	–	0.02	0.10
Apple	25	15	4	3	–	0.03–3.20	0.04–0.10	5.00	2.00
Pear	25	16	5	2	–	0.04–3.20	0.05–0.09	5.00	2.00
Total	200	48	31	7	5				

IMZ, Imazalil; CMB, Carbendazim.

sponding to the ions $[Cl_2PhC_2H_4^+]$, $[Cl_2PhOC_4H_6^+]$ and M^+, respectively (Fig. 1). Coincidence in R_F values has been used to confirm the presence of carbendazim in samples by thin-layer chromatography (Fig. 2).

3.4. Analysis of real samples

Personnel from The Ministry of Health in charge of the Monitoring Program 1995 collected 200 fruit samples from 10 different supermarkets from

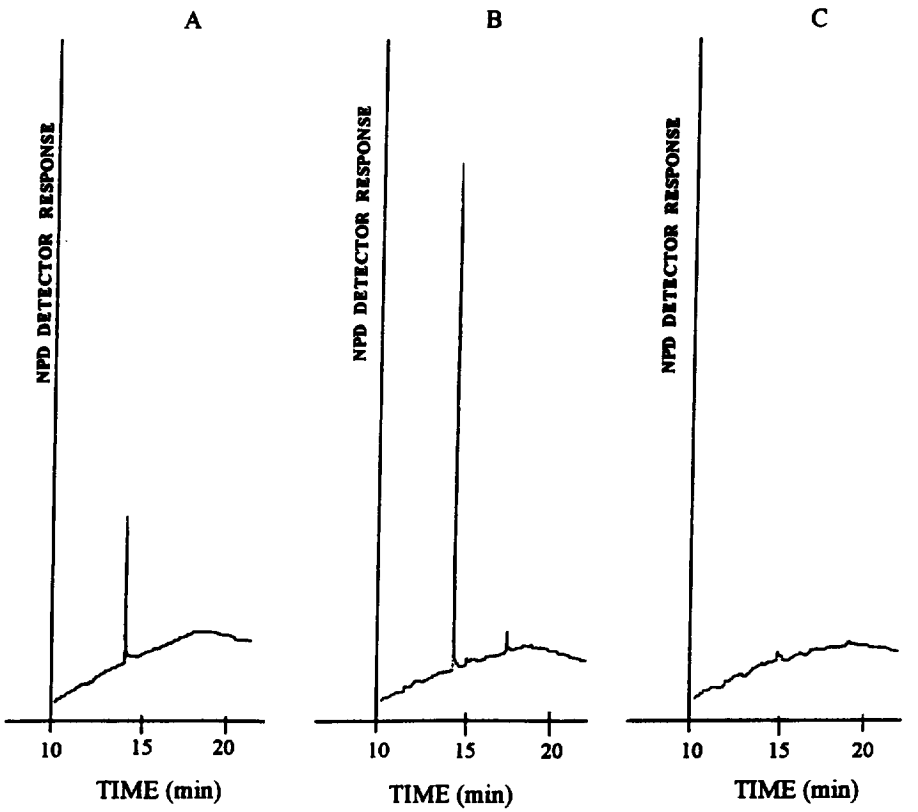

Fig. 3. GC–NPD of 1 μl injections of imazalil on SPB-608 capillary column (t_R = 14.29 min). (A) Standard solution containing 0.4 μg/ml; (B) real sample containing 0.12 mg/kg; (C) real negative sample.

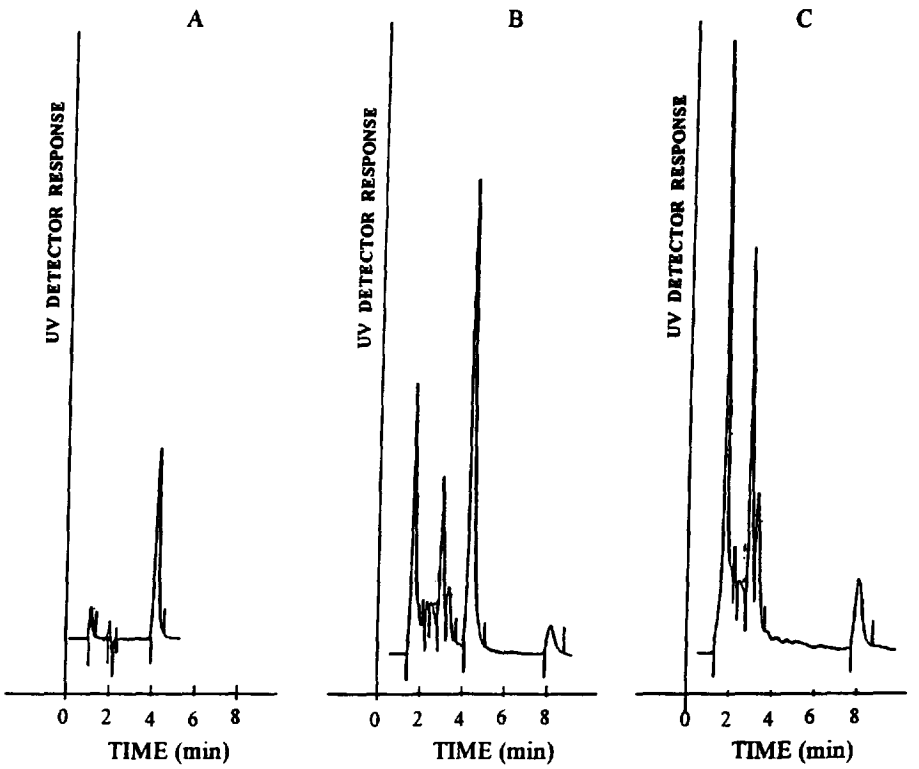

Fig. 4. HPLC–UV of 20 μl injections of carbendazim on LiChrospher-C_{18} RP 5 μm column (t_R=4.15 min). (A) Standard solution containing 1 μg/ml; (B) real sample containing 0.35 mg/kg; (C) real negative sample.

January to June. A 5 kg sample was sent to the National Food Center for the subsequent analysis, within the Residue Monitoring of Hygiene Food Program 1995. 2.5 kg were frozen to use in case repetition would be necessary. 2.5 kg were homogenized to be analyzed following the proposed method.

4. Conclusions

Validation studies show the viability of the procedure in routine analysis for carbendazim and imazalil. On the other hand, a single method to analyze both fungicides, offered a great saving of time and money in relation to other methods. The simplicity of the method allows 12 samples of each pesticide to be analyzed simultaneously every 24 h.

The results obtained in the analysis of the samples included in the Monitoring Program (Table 3), demonstrate the presence of imazalil and carbendazim residues at 16% and 24%, respectively, in relation to the total finding, but only at 3.5% and 2.5% respectively in relation to finding higher than 50% of maximum residue levels (MRLs). No samples violating Spanish legislation were found.

Apples and pears in the case of imazalil and grapes and strawberries in the case of carbendazim were the commodities with a higher presence of residues due to Spanish agricultural usages.

Standard and sample chromatograms are shown in Figs. 3 and 4.

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