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Short communication

Decay study of pesticide residues in apple samples

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Abstract

A method for monitoring pesticides in apple samples, including Soxhlet extraction, an evaporation step and capillary gas chromatography with nitrogen–phosphorus detection, is applied to a decay study of the carbamate pesticide ethiofencarb. The evaporation step is carried out by a surface nitrogen flow and the recoveries of ethiofencarb measured in standard solutions of ethyl acetate and apple extracts. The results of the decay studies show that ethiofencarb is degraded in the apple peel faster than in the interior of the fruit. The methodology is then applied to decay studies in the laboratory of the organophosphorus pesticides, diclofluanid, fenitrothion and malathion.

Keywords: Apples; Food analysis; Pesticides; Carbamates; Organophosphorus compounds

1. Introduction

Widespread use of pesticides produces a very important problem, since their persistence in the environment is normally high. Safety intervals following the application of pesticides on crops have been established by the authorities in all countries, but their persistence may be superior to that interval.

Organophosphorus (OP) pesticides are widely used, but carbamates are increasingly important, since they present a low persistence and act as reversible inhibitors of acetylcholinesterase, much safer than the irreversible OP inhibitors. Since carbamates are the third largest group of synthetic organic pesticides and have become widely used in foliar applications to field and vegetable crops, a method for monitoring their residues in agricultural products is required.

Chromatographic methods have been used to determine carbamate pesticides in fruits and vegetables. Thin-layer chromatographic methods have been reviewed [1]. Capillary gas chromatography is a well-established technique for the analysis of pesticide residues in food and environmental samples [2–4]. Liquid chromatographic techniques for the multiresidue analysis of carbamates have been applied; Moye et al. [5] introduced an HPLC method for post-column derivatization and fluorescence detection of these compounds, which was further optimised for application to food samples [6–8].

Decay studies that monitor pesticides in crops provide the necessary information about their degradation and fate in agricultural environments. Several studies have reported on crops treated with carbamate [2,4] and OP pesticides [9–12]. In our previous work [11,12], a sample pretreatment was developed to monitor OP pesticide residues in several parts of the fruit, from the peel to the pulp. This paper

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describes the application of this methodology to the monitoring of the carbamate pesticide, ethiofencarb, in apple samples. The method has been improved with the addition of an evaporation step before the GC determination. The new methodology was also applied to a decay study, under laboratory conditions, of the OP pesticides, diclofluanid, fenitrothion and malathion; they have been studied before under these conditions [12], but without the evaporation step.

2. Experimental

2.1. Materials and methods

2.1.1. Apparatus

A Hewlett-Packard HP 5890 gas chromatograph, equipped with a nitrogen–phosphorus detection (NPD) system and an HP Ultra 2 fused-silica capillary column (crosslinked 5% phenylmethyl silicone gum phase of 0.33 μm film thickness, 25 m×0.2 mm I.D.) was used to determine the pesticides. The operating conditions were as follows; injector temperature, 250°C; detector temperature, 275°C; oven temperature, 205°C. The nitrogen carrier gas flow-rate was 0.6 ml/min and the NPD flow-rates were 70 ml/min (air) and 4 ml/min (hydrogen); the auxiliary gas flow-rate (nitrogen) was 30 ml/min.

Sample processing was carried out using six Soxhlet units, equipped with 250-ml flasks and 125-ml condensers and with individual temperature regulation in each, a small volume evaporator system with four inputs of nitrogen, one to each solution and a centrifuge (ORTO).

2.1.2. Chemicals

Pesticide standards (diclofluanid, ethiofencarb, fenitrothion, malathion and parathion methyl), at 99.9% purity, were obtained from Riedel-de Häen (Seelze, Germany). The ethyl acetate was of HPLC quality from Lab-Scan (Dublin, Ireland) and the isobutyl methyl ketone (IBMK) and xylene, both of PRS quality, were obtained from Panreac (Barcelona, Spain). The anhydrous calcium chloride came from Merck (Darmstadt, Germany). The commercial pesticides, euparen (diclofluanid 50%, w/w), folithion (fenitrothion 50%, w/v) and croneton (ethiofencarb 50%, w/v) came from Bayer (Germany) and keythion (malathion 50%, w/v) was from Industrial Chemical Key (Spain).

2.1.3. Solutions

Pesticide solutions (1000 μg/ml) were prepared by dissolving the standards in ethyl acetate. The more dilute solutions were prepared by dilution with the solvent required. Aqueous pesticide suspensions for treating the apples were prepared by dissolving the commercial products in distilled water. Dilution was carried out according to commercial recommendations as follows: diclofluanid, 1.5 ml/l; ethiofencarb, 2.50 ml/l; fenitrothion, 1 ml/l and malathion, 2 ml/l. Parathion methyl (8.8 μg/ml) was used as the internal standard throughout the study.

2.1.4. Quantification

Quantification of the pesticides was carried out using the internal standard method (parathion methyl was chosen as the internal standard). The heights of the chromatographic peaks were chosen as the quantification parameter. The chromatographic response was calibrated separately for each pesticide.

Ethiofencarb showed a linear response range from 5.74 to 40 μg/ml. Repeatability value, expressed as the relative standard deviation (R.S.D.) of ten determinations, was 2.7%. For diclofluanid, fenitrothion and malathion, the linear response ranges and the repeatability values have been reported previously [12].

2.1.5. Methodology

The sample treatment procedure has been described previously by Sanz Asensio et al. [12] for its application to OP pesticides. An evaporation step to concentrate pesticides in the organic extracts was carried out. The volume evaporated in all cases was 10 ml and the final volume was 1 ml.

Different solvents for the Soxhlet extraction of ethiofencarb from the apple matrix were tested. Apple samples were fortified with pesticide and then Soxhlet extracted for 90 min with: (1) ethyl acetate, (2) xylene, (3) IBMK and (4) a mixture of ethyl acetate–xylene (1:1, v/v). The best recoveries (106%) were obtained when ethyl acetate was used.

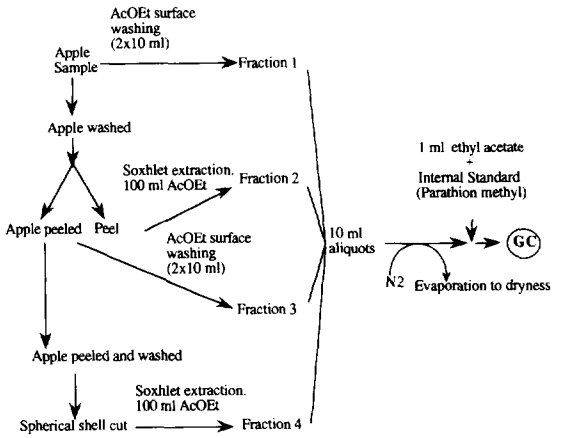

Fig. 1. Sample treatment scheme, including evaporation step.

The overall sample treatment applied for the decay studies on apple samples is shown in Fig. 1.

2.2. Results and discussion

2.2.1. Standard recoveries

Aliquots containing 10 ml from a standard solution of ethiofencarb in ethyl acetate (1.65 μg/ml, in order to obtain a final concentration within the linear response range of the method) were evaporated to dryness.

The evaporation system allows for evaporation of four aliquots at the same time and recoveries of four series were therefore carried out, each one involving four evaporated aliquots. The analysis of variance (ANOVA) statistical treatment was applied to compare the overall results obtained in the recoveries for the fourth evaporation series. The results indicate that there is no significant difference among the four series of data collected (P=95%).

2.2.2. Standard recoveries from apple extracts

Five untreated apples, without any pesticide, were processed as described in Section 2.1, to obtain the four extracts. The aliquots were fortified with ethiofencarb to a level of 1.65 μg/ml and each aliquot was evaporated to dryness. As apple extracts were then involved, a solid residue appeared and it was necessary to centrifuge the final solutions.

For each of the apple fractions, two series of four evaporations each were performed and the recoveries were measured. ANOVA was applied to compare the two series of each fraction; no significant differences were found within any fraction. Ethiofencarb recoveries, for a standard solution and for the apple extracts, are shown in Table 1.

2.2.3. Decay study

A decay study was carried out under laboratory conditions, applying an evaporation step. To carry out the study, 20 apples were each sprayed with 50 ml of an aqueous suspension of croneton (commercial dose: 2.5 ml/l). Samples were taken at different times, each in duplicate, from the first day after the treatment to a total time of 36 days.

Ethiofencarb was not detected in the first and second fractions of the apple samples, apart from the sample taken the day after treatment, at a concentration of 0.73 μg/g of apple and 0.34 μg/g of apple, respectively.

The third and fourth fractions of the apple were combined together and named the internal fraction, since only apple pulp is involved in them and the pesticide behaviour was similar in both. Ethiofencarb was detected until eleven days after the treatment, and no pesticide was detected afterwards. Fig. 2 shows the results obtained from these two fractions, called the "internal fraction". Results are expressed as the pesticide percentage within the apple fraction related to the initial pesticide concentration in the whole apple.

2.2.4. Organophosphorus decay studies

The methodology was applied to a decay study of diclofluanid, fenitrothion and malathion pesticides

Table 1
Average recoveries of pesticides after the evaporation of 10-ml aliquots of standard solution of ethyl acetate and apple extracts fortified with ethiofencarb, diclofluanid, fenitrothion and malathion (1.63 μg/ml, 0.830 μg/ml, 0.600 μg/ml and 1.10 μg/ml, respectively)

	Average recoveries (%)±R.S.D.			
	Ethiofencarb	Diclofluanid	Fenitrothion	Malathion
Standard	43±0.93	99±6.3	79±0.81	65±1.1
Fraction 1	94±0.90	115±3.2	90±1.6	100±1.8
Fraction 2	95±1.9	103±1.3	83±1.3	95±1.6
Fraction 3	81±0.76	104±1.9	81±0.67	94±1.5
Fraction 4	97±1.1	104±1.0	69±0.91	88±0.95

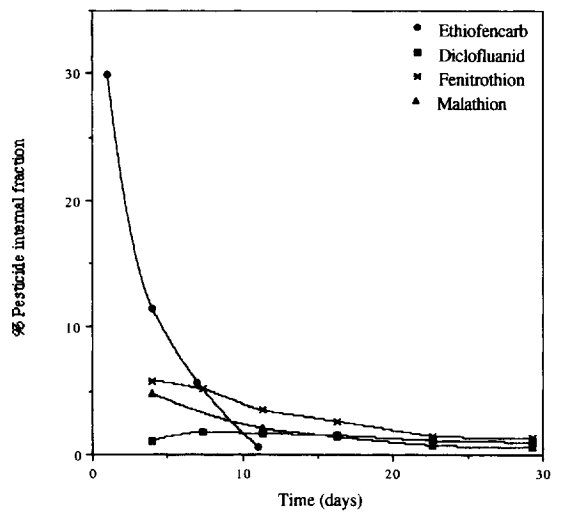

Fig. 2. Degradation of pesticides in the internal fraction of the apple.

under the same conditions. Decay studies of these pesticides have been reported previously [12] and the aim of the new study was to confirm the applicability of the improved method.

2.2.5. Evaporation step recoveries

The same procedure that was used for ethiofencarb was followed. Pesticide concentrations in the evaporated solutions were the following; diclofluanid (0.830 μg/ml), fenitrothion (0.600 μg/ml) and malathion (1.10 μg/ml). ANOVA was applied to the data obtained from the different evaporation series and no significant differences were found in any case.

The total average results for each fraction (Table 1) showed that the matrix effect was different for each pesticide. Malathion recoveries were the only ones affected by matrix influence, in the same way as the ethiofencarb recoveries.

2.2.6. Decay studies

Three sets of 20 apples, one set for each pesticide, were sprayed with aqueous suspensions of the commercial pesticides at the recommended doses. Two samples of each set were taken at different time intervals, up to a total time of 40 days.

Pesticides were found in all the apple fractions. The degradation of the pesticides in the exterior of the fruit (fraction 1) agreed with that obtained in previous studies. The degradation of malathion was faster than that obtained for diclofluanid and fenitrothion. Final percentages of the pesticides in this fraction were 43% for malathion, 54% for diclofluanid and 71% for fenitrothion.

All the pesticides penetrated into the apple. Diclofluanid penetrated least, about 2% of the total initial pesticide in the second and internal fraction. Malathion and fenitrothion, on the other hand, showed around 12% maximum penetration in the second fraction and 5% and 6%, respectively, in the internal fraction.

The behaviour of the pesticides in fraction 2 is shown in Fig. 3. Behaviour over time was different for each pesticide. Pesticide apparently penetrates into the fruit, where it is exposed to an interaction with biological agents; thus two processes are involved in the pesticide behaviour, i.e. penetration and degradation. Fenitrothion had a rising trend followed by a stabilisation, while malathion showed an increasing concentration followed by a decreasing behaviour.

Within the apple (Fig. 2), the pesticides are also affected by the two processes mentioned above. Their evolution indicates that biological agents within the fruit have greater degradation potential than those in the peel. Diclofluanid concentration was

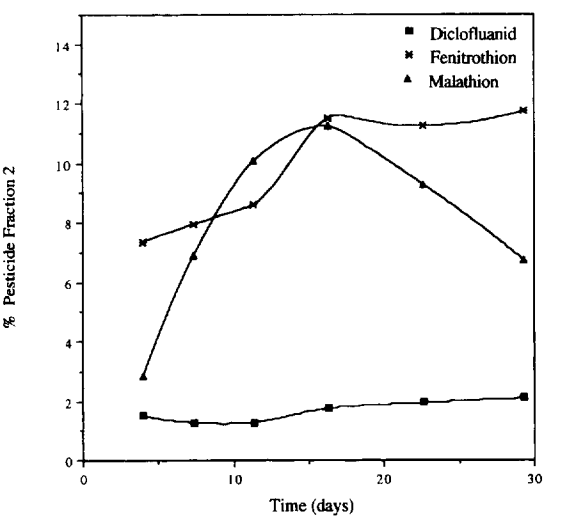

Fig. 3. Behaviour of diclofluanid, fenitrothion and malathion in fraction 2, over time.

constant within the peel and the pulp and no degradation was observed over the total time of the study.

3. Conclusions

The methodology proposed allows monitoring of pesticide residues in apple samples and the evaluation of their behaviour in the different parts of the fruit, with different characteristics. By applying an evaporation step, it is possible to measure low pesticide concentrations and to study their internal behaviour, completing the previous method proposed by Sanz Asensio et al. [12].

As there are no physical environmental agents involved in the elimination of pesticides under laboratory conditions, it can be deduced that ethiofencarb is easily eliminated from the apple surface, due to quick chemical and biological degradation. Penetration into the fruit occurs from the first day after the treatment (about 35% of total ethiofencarb in the whole apple), where it is degraded more slowly, due to biological degrading agents.

OP pesticides have greater persistence, and their elimination from the exterior of the apple takes more time than the established safety intervals. All pesticides penetrate into the fruit, except diclofluanid, whose systemic behaviour is too low. Malathion and fenitrothion show about 20% of the total pesticide inside the apple. Taking into account the fact that these studies were performed under laboratory conditions, where environmental agents (wind, rain, sunlight) were not involved, the decay is higher within the fruit, where biological degradation occurs.

Acknowledgments

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