Behaviour of Acephate and its Metabolite Methamidophos in Apple Samples

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Key Words

Gas chromatography
Acephate, methamidophos
Pesticide residues
Apples

Summary

A study of the decay of acephate in apple samples was carried out, including penetration studies and the transformation of acephate in to its main metabolite, methamidophos. Sample treatment involved extraction with ethyl acetate and determination by gas chromatography with nitrogen – phosphorus detection (GC-NPD). Three different parts of the fruit were studied separately: apple surface, peel and pulp. Recoveries were measured at three spiked levels, ranging from 0.050 to 0.504 μg g^{-1} for acephate and 0.049 to 0.492 μg g^{-1} for methamidophos. Mean acephate recoveries were 93.0 to 115.5 % from peel and 99.2 to 110.2 % from pulp, while methamidophos recoveries were 77.2 to 104.2 % and 77.5 to 98.6 % from peel and pulp, respectively (n = 6). Results showed that acephate penetrates into the fruit, where it is transformed to methamidophos. This transformation was not seen on the external apple surface.

Introduction

The use of synthetic pesticides is claimed to be the most effective method of controlling vegetable diseases and insects. Organophosphorus pesticides (OP) are widely used as insecticides for different types of cultivation. However, with regard to food and environmental pollution, it is well known that pesticides cause many problems.

Interest in the safety of food products has recently increased, and pesticide residues in food crops are the subject of increasingly strict regulations. The breakdown of pesticides in food, air and soil may be due to biological, physical, chemical or photochemical degradation. Pesticide residues in fruit and vegetables have impelled governments to set up monitoring programs.

Decay studies which monitor pesticides in crops provide the necessary information about their degradation and fate in agricultural environments. The environmental impact of an agrochemical on plants is primarily determined by two parameters: its dissipation from the surface and its penetration into plant tissues. Our previous decay studies on OP pesticides [1–3] showed not only their persistence on fruits, but also that, due to their systemic action, residues can be found within the fruit for several days after application.

Determination of pesticide residues over such a wide range of thresholds can require different clean-up and analysis procedures because, in particular with the lower limits, matrix effects can strongly interfere. The widely increased demand for pesticide residue control, however, requires multi-residue analysis particularly in connection with integrated pest management.

Acephate (O,S-dimethyl acetylphosphoramidothioate) is a broad-spectrum systemic insecticide widely used for the control of pest insects such as aphids, thrips, sawflies, leaf miners and lepidopterus larvae. It is partially metabolised in plants to yield the highly toxic methamidophos (O,S-dimethyl phosphoramidothioate), an excellent insecticide in its own right. This is also widely used all over the world, and is one of the most commonly found by regulatory agencies in residue monitoring [4].

Due to the polarity of both compounds (the water solubility is 818 g L^{-1} (20 °C) for acephate and 1000 g L^{-1} for methamidophos [5]), and from our experience, the multiresidue methods (MRM) usually employed for less polar compounds are not efficient enough for these pesticides [6, 7]. Several methods have been described in the literature to monitor acephate and methamidophos in plants. A gas chromatographic (GC) method for their analysis has been described by Leary [8], obtaining excellent recoveries with an ethyl acetate-sodium sulfate-based extraction. Today the use of ethyl acetate for multiresidue solvent extraction is replacing other MRM procedures [9–10] with good results.

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In this work, a semi-micro method is used to follow changes in acephate and methamidophos residues in apples. The proposed methology is based on the ethyl acetate extraction method, but it consumes less time and reagents. In order to perform further degradation and penetration studies of acephate, the sample treatment involves three different parts of the fruit, as was done in previous work [1–3]. The overall methodology has been applied to a degradation, penetration and transformation study of acephate in apple trees.

Experimental

Apparatus

A Hewlett-Packard HP 5890 gas chromatograph equipped with a nitrogen phosphorus detector (NPD) and an HP Ultra 2 fused silica capillary column (crosslinked 5 % phenyl methyl silicone gum phase of 0.33 μm film thickness, 0.2 mm internal diameter and 25 m length) was used for pesticide determination.

The chromatographic conditions were: injector temperature 200 °C and detector temperature 300 °C; the oven temperature programme had an initial temperature of 50 °C for 1 min then a rise at a rate of 30 °C min^{-1} to a temperature of 225 °C held for 7 min, followed by a second rise at the rate of 60 °C min^{-1} to a temperature of 275 °C and then held for 5 min. The nitrogen carrier gas flow rate was 0.6 mL min^{-1} and the flow rates for the nitrogen-phosphorus detector were 70 mL min^{-1} (air) and 4 mL min^{-1} (hydrogen); the auxiliary gas flow rate (nitrogen) was 30 mL min^{-1}. Injection was by splitless mode with a purge time of 1 min and the sample volume injected was 1 μL. With these chromatographic conditions, retention times were 7,3 ($t_{R\text{-Acephate-}}$) and 6,6 ($t_{R\text{-Methamidophos-}}$)

To homogenate the samples, Trituradora Moulinex; Polytron PT 3000, Kinemática; Centrifuge Heraeus, Labofuge 400; Ultracentrífuge Heraeus; Rotary vacuum evaporator Heidolph OB 2000, were used for this work.

Reagents

Pesticide standards, certified and 99.9 % pure, were obtained from Riedel-de-Häen (Seelze, Germany). Ethyl acetate used was HPLC or PRS grade quality from Lab-Scan. The anhydrous sodium sulfate was from Carlo Erba. The commercial pesticide Orthene (acephate 50 %, m/m) came from the Chevron Chemical Company.

Pesticide solutions: Stock solutions of 1.00 mg mL^{-1} of acephate, 1.00 mg mL^{-1} of methamidophos and 1.00 mg mL^{-1} of diazinon, used as internal standard throughout the study, were prepared in acetone. More dilute solutions were obtained by the dilution of stock solutions in ethyl acetate.

Aqueous pesticide suspensions for treating the apples were prepared by dissolving the commercial product (Orthene) in water. Dilution was according to commercial recommendations of 1 g L^{-1} . A previous analysis of the commercial product did not show the presence of methamidophos in the aqueous pesticide suspension.

Sampling and Processing

Ten apples were taken per day, several days a week for a period of six weeks. The apples were chosen in a random way from different parts of the tree. The apples were weighed and processed as follows:

The apples were washed in 50 mL of ethyl acetate, to dissolve the pesticide residue remaining on the exterior of the fruit. The wash-liquid was dried with anhydrous sodium sulfate and aliquots of 800 μL were made up to 1600 μL. Internal standard (diazinon) was added at a final concentration of 0.448 μg mL^{-1}. The solutions obtained were labelled as "*apple surface*"; 1 μL of this solution was injected in the GC/NPD system. As the acephate concentration in the apple surface decreased with time, it was necessary to concentrate the washing instead of diluting it, for the samples taken from the 14th day onwards. In these samples, the apple surface solution was obtained as follows: 1200 μL of the solution was evaporated to dryness and 400 μL of diazinon in ethyl acetate (0.448 μg mL^{-1}) was added.

The washed apples were then peeled and the peel and the pulp were chopped separately. From each mixture, 4 aliquots of 5 g each were extracted by following the methodology described below. The extracts obtained were labelled as "*apple peel*" and "*apple pulp*", respectively.

Quantification of the pesticides, acephate and methamidophos, was carried out using the internal standard method (diazinon was chosen as the internal standard), and were determined by GC-NPD. Both areas and heights were used for the quantification. It has been found that acephate and methamidophos display the called matrix-induced GC response enhancement [11]. Therefore, standard solutions for the calibration were prepared in the three different matrices; standard solutions of "*apple surface*" were made up in ethyl acetate, while those of "*apple peel*" and "*apple pulp*" were obtained by addition of standard solutions in ethyl acetate to the residue obtained after treating apples without acephate and methamidophos in the same way as described below.

Extraction Method

The method applied for the extraction of the pesticide and its metabolite from the apple was based on the ethyl acetate-sodium sulfate extraction method described by Leary [8]. The quantity of sample extracted was 5 g and the solvent volume for the extraction was 30 mL. The method was applied separately to the peel and the pulp, and extraction recoveries for acephate and methamidophos were calculated for each of the fruit parts. The procedure was as follows:

Table I. Values obtained for Acephate and Methamidophos.

Matrix/Pesticide	Linear Range	r	LOD	LOQ
"apple surface"				
Area				
Acephate	$A = 0.4960 + 1.480\ C_{Ac}$	0.9993	0.0897	0.1019
Methamidophos	$A = -0.0540 + 0.988\ C_{Mt}$	0.9980	0.2008	0.2054
Height				
Acephate	$A = -0.1430 + 0.988\ C_{Ac}$	0.9993	0.0120	0.0165
Methamidophos	$A = -0.3794 + 2.095\ C_{Mt}$	0.9980	0.2001	0.2063
"apple peel"				
Area				
Acephate	$A = 83.3 + 7.38\times10^{4}\ C_{Ac}$	0.990	0.0039	0.0158
Methamidophos	$A = -1.98\times10^{3} + 7.65\times10^{4}\ C_{Mt}$	0.97	0.0308	0.0422
Height				
Acephate	$A = -5.54\times10^{-14} + 3.21\times10^{4}\ C_{Ac}$	1	0.0057	0.0191
Methamidophos	$A = 3.06\times10^{-14} + 1.59\times10^{4}\ C_{Mt}$	1	0.0116	0.0386
"apple pulp"				
Area				
Acephate	$A = -38.8 + 5.91\times10^{4}\ C_{Ac}$	0.995	0.0063	0.0195
Methamidophos	$A = -823 + 6.99\times10^{4}\ C_{Mt}$	0.98	0.0166	0.0277
Height				
Acephate	$A = 485 + 2.39\times10^{4}\ C_{Ac}$	0.995	0.0000	0.0006
Methamidophos	$A = 138 + 2.35\times10^{4}\ C_{Mt}$	0.98	0.0005	0.0153

C_{Ac} (Acephate concentration): $\mu g\ mL^{-1}$
C_{Mt} (Methamidophos concentration): $\mu g\ mL^{-1}$
LOD and LOQ : $\mu g\ mL^{-1}$

Aliquots of 5 g of peel and pulp were extracted twice with ethyl acetate (15 + 15 mL) with a Polytron PT20; the sodium sulfate (5 g) was added after the first extraction. The mixture was centrifuged at 4000 rpm for 10 minutes. 15 mL of the extract was evaporated to dryness in the rotary vacuum evaporator at 40 °C. The residue was dissolved in 1 mL of 0.448 $\mu g\ mL^{-1}$ diazinon solution in ethyl acetate. In the pulp extracts, acephate and methamidophos were determined directly by GC-NPD. The peel extracts, however, showed a solid residue that remained insoluble in ethyl acetate and it was necessary to centrifuge them before the determination. This centrifuging was carried out at 12000 rpm for 10 minutes.

Response and LOD and LOQ determinations were carried out for each pesticide in the different apple fractions.

Recovery Studies

Ten apples that had not been treated with any pesticide were washed with 50 mL of ethyl acetate. The apples were then peeled and the peel and the pulp were chopped in a Moulinex food processor to obtain two separate, homogeneous mixtures. This step was necessary because it was to be further applied to the degradation study, in order to obtain pesticide behaviour on the apple surface.

To determine extraction efficiency, samples of peel and pulp were spiked with acephate and methamidophos at three levels with analytical standards dissolved in acetone, 0.050; 0.126; 0.504 and 0.049; 0.123; 0.492 $\mu g\ g^{-1}$ of apple, respectively. These concentrations ranged from slightly higher than the LOQ obtained to the LMR allowed by the EU (0.5 $\mu g\ g^{-1}$ for acephate and 0.2 $\mu g\ g^{-1}$ for methamidophos). The samples obtained were then submitted for extraction and analysis as described above. Each recovery test was repeated six times. Each pesticide was quantified by the internal standard method.

Results and Discussion

Analytical Characteristics of the Method

Due to the matrix effect on the chromatographic response, a study of the response obtained in the apple matrix was carried out, also obtaining the LOD and LOQ values. Internal standard methods were used in all cases. The criteria established by Parker [12] to find the method's limit of detection (LOD) and the limit of quantification (LOQ) were used in this study. Briefly, LOD and LOQ are defined as 3 and 10 times the background signal contributed by the matrix blank at the analyte retention time. Table I shows the parameter values obtained. It also shows the importance of the peel and pulp matrix effect on the chromatographic response of acephate and methamidophos.

Recovery studies carried out for the different concentrations levels gave results ranging from 93 to 116 %

Table II. Extraction recoveries.

Pesticide	Fortified $\mu g\ g^{-1}$	Peel		Pulp	
		Mean rec. (%)	RSD	Mean rec. (%)	RSD
Acephate	0.0504	100.25	4.8	110.25	8.3
	0.1260	115.50	3.1	108.75	2.6
	0.5040	93.00	5.0	99.25	2.6
Methamidophos	0.0492	77.25	4.8	77.50	5.9
	0.1230	104.25	8.2	79.25	5.1
	0.4920	91.10	3.6	98.62	3.2

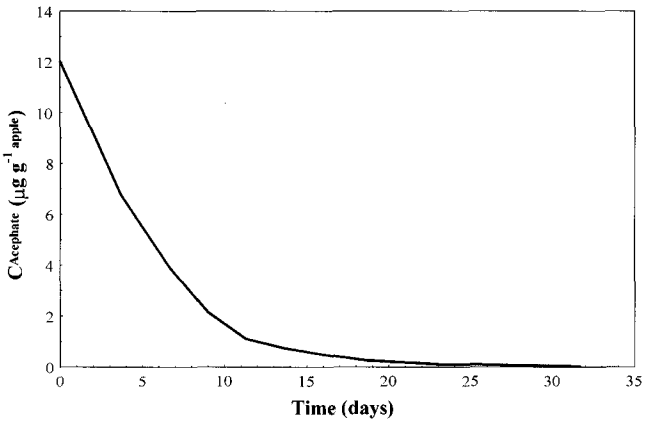


Figure 1
Acephate concentration behaviour on the apple surface.

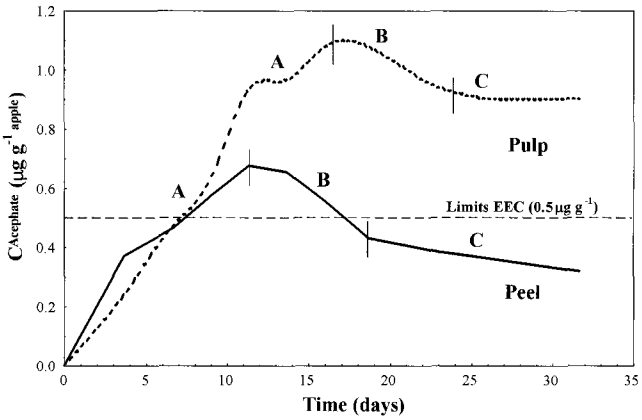


Figure 2
Acephate concentration behaviour within the apple (peel and pulp).

(acephate) and 77 to 104 % (methamidophos). The results obtained expressed as percentages of pesticide recovered, are indicated in Table II.

Decay Study

The aims of this study were to find experimentally the decay time of acephate while checking the pesticide penetration inside the fruit and the transformation of acephate to methamidophos on the apple surface and within it.

A degradation study of acephate in apple trees was carried out under normal environmental conditions. To carry out the study, an apple tree (which had not been treated with any other pesticide) was sprayed with the aqueous suspension of acephate. Samples were taken at different times from the day of the treatment to a total time of 42 days.

Evolution of the Acephate in the Apple Surface

Figure 1 shows the acephate behaviour on the external surface of the fruit, in accordance with the results obtained from "*apple surface*" fractions. A continuous decay of the pesticide over time was observed, to a concentration level below the LOQ.

No other compound was detected as a possible degradation product of acephate. The elimination of the pesticide from the fruit surface could be caused more by environmental agents or penetration processes than by degradation.

Evolution of the Acephate in the Apple Peel and Apple Pulp

Figure 2 shows the pesticide behaviour in the peel and pulp. It is possible to define three different zones in each one:

Zone A: From the beginning of the study, there was a rise of pesticide concentration with time, shown in both peel and pulp.

Zone B: An intermediate zone in which acephate concentration decreases rapidly.

Zone C: Final zone in which the concentration continues decreasing but at a very slow rate.

Figure 2 shows that in the peel there is an initial tendency for the concentration to rise. This is due to the penetration process from the surface. The maximum concentration in the peel occurs when a maximum slope of decrease is shown on the surface (see Figure 1). This maximum value could remain constant if there were no

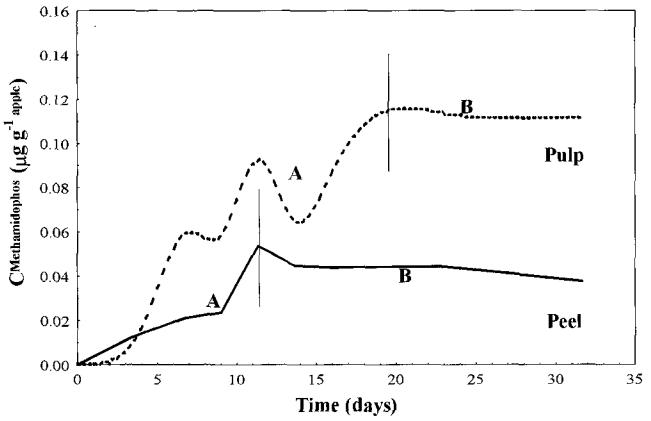
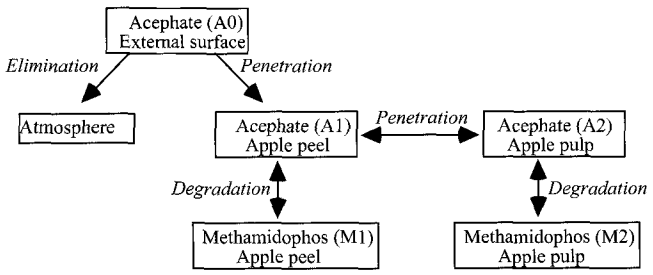


Figure 3

Methamidophos concentration behaviour within the apple (peel and pulp).

Scheme I. Equilibria in which the pesticide is involved in the apple.



other proccesses involved other than penetration. Instead of this, once the maximum had been reached (beginning of B zone), the acephate concentration decreased gradually in the B zone and very slowly in the C zone. This behaviour can be explained by taking into account other processes that are involved in the acephate behaviour inside the apple peel.

The equilibria are:

I) Equilibrium of penetration into the pulp

II) Equilibrium of degradation in the peel

It is obvious from Figure 2 that acephate penetrates from the peel to the pulp. Acephate was detected in the apple pulp and showed a similar behaviour as in the peel. The maximum concentration in the pulp was reached at a later time than that obtained in the peel, as expected. Once the acephate peel contibution to the pulp decreases, the degradation process is more important, entering the B zone.

The appearance of degradation products shows clearly the degradation equilibrium process (II). Methamidophos was the only degradation product detected and its presence justifies the equilibria in both fractions, peel and pulp.

Figure 3 shows the methamidophos concentration behaviour in peel and pulp and, as for acephate, different zones are defined:

Zone A: From the beginning of the study a zone of increasing concentration to a maximum value.

Zone B: From the maximum value to the end of the study. In this zone the methamidophos concentration remains constant.

The presence of the methamidophos is due to the acephate degradation in both fractions. The continuous increase in the metabolite in zone A is a consequence of the increasing acephate concentration and its continuous degradation. Equilibrium was reached in zone B and methamidophos concentration remains constant.

Scheme I shows equilibria in which acephate is involved in the whole apple.

Conclusions

The method 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. On the basis of the recoveries obtained, the method could be applied to other kinds of samples.

Elimination of acephate from the surface is due to environmental action. No degradation products were detected on the surface.

Penetration into the fruit occurs from the first day. Acephate is degraded inside the apple to its major metabolite, methamidophos. Penetration from the peel to the pulp also occurs.

The highest concentration of acephate inside the fruit is obtained between the 11th and 12th days after treatment. OP pesticides have greater persistence, and their elimination from the interior of the apple takes more time than the established safety intervals. At the applied dose and after the safety interval given (21 days), acephate remained inside the apple at a higher level than the EU established LMR, while methamidophos concentration is below this value.

Acephate and methamidophos behaviour for the last day of the study shows a slow decrease in concentration. This fact suggests that the equilibria stabilises.

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