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Analysis of carbendazim, benomyl, thiophanate methyl and 2,4-dichlorophenoxyacetic acid in fruits and vegetables after supercritical fluid extraction[1]

Michelangelo Anastassiades[a,*], Wolfgang Schwack[b]

[a]*Chemisches und Veterinäruntersuchungsamt Stuttgart, Sitz Fellbach, Schaflandstrasse 3/2, 70736 Fellbach, Germany*
[b]*Universität Hohenheim, Institut für Lebensmittelchemie, Garbenstrasse 28, 70599 Stuttgart, Germany*

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Abstract

Simple methods for the analysis of carbendazim, benomyl and thiophanate methyl in fruits and vegetables and of 2,4-D in citrus fruits are presented. Sample preparation involves supercritical fluid extraction with carbon dioxide and further analysis is performed without any additional clean-up by GC–MS after derivatisation or directly by HPLC–diode array detection. The SFE methods presented are clearly faster and more cost effective than traditional solvent based approaches. The recoveries, detection limits and repeatabilities achieved, meet the needs of tolerance level monitoring of these compounds in fruits and vegetables.

Keywords: Fruits; Vegetables; Food analysis; Carbendazim; Benomyl; Thiophanate methyl; 2,4-D; Pesticides

1. Introduction

Conventional solvent based methods for the analysis of relatively polar basic and acidic pesticides like carbendazim and 2,4-dichlorophenoxyacetic acid (2,4-D) often involve laborious clean-up steps like acid–base liquid–liquid partitioning and are therefore not very popular in routine analysis [1–8]. In most cases extractability is enhanced by adjusting pH. Aim of this study was to introduce this approach (pH adjustment) in SFE applications and to develop fast and simple analytical methods for the analysis of 2,4-D and the carbendazim group.

The fungicide carbendazim is widely used in crop protection. It is also the principal degradation product of two other compounds benomyl and thiophanate methyl. For this reason benomyl and thiophanate methyl tolerances are generally expressed as carbendazim. During the growing period these agrochemicals are widely applied on various crops like grapes, pome fruits, stone fruits, lettuce and cereals and act systemically. Post-harvest they are usually applied on bananas, citrus fruits, pome fruits, mangoes and potatoes to protect them from decay caused by various fungal pathogens [9,10]. Because of possible health effects, widespread use and insufficient residue data, carbendazim monitoring in food commodities is necessary [1,11].

2,4-D is well known as a selective herbicide used to protect grain crop against broadleaf weed. Less

*Corresponding author.

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known is the fact that 2,4-D is pre- and post-harvestly applied in citrus fruit production. Citrus trees are treated with 2,4-D in order to delay abscission of mature fruits [12]. When applied post-harvest 2,4-D induces healing of injuries, retards senescence and aids in controlling post-harvest decay [10,12]. In countries like Argentina, Uruguay and South Africa citrus fruits destined for export are treated before shipment with water-based wax emulsions containing about 500 ppm 2,4-D. In the USA and Spain however, 2,4-D is only applied on fruits destined for cold-storage. Before the shipping or shining wax is applied, the storage wax containing 2,4-D is washed off.

Supercritical fluid extraction (SFE) offers an environmentally safer extraction, essentially obviates the use of hazardous solvents, generates little waste and reduces time, space and glassware required for extraction [13]. Being an automated procedure SFE significantly reduces manual labour and human intervention in analysis. The high diffusivity and low viscosity of the supercritical CO_2 implies an efficient penetration into matrices and a rapid molecular diffusion of analytes. Thus short extraction times are possible.

Many researchers have investigated the use of SFE in analytical applications in recent years. Early applications have focused on the extraction of relatively non-polar compounds from environmental samples like soil, sludge, and solid-waste [14]. Extraction of pesticides from fruits and vegetables has been more difficult due to the relatively polar nature of most analytes and the high water content of the samples. Water must be controlled in SFE to keep it from damaging the restrictor or affecting the trapping process. Several approaches to remove or control water have been described in literature like lyophilisation [15], use of chemical desiccants (like $MgSO_4$ and Na_2SO_4) [16–18], and addition of water-adsorbing materials which, having a large surface, can physically bind high amounts of water and can help to disperse the sample material (e.g., Hydromatrix/Varian) [13,19–21].

Jimènez et al. [15] developed a method for the extraction of carbendazim from lyophilised lettuce by SFE using CO_2 modified with methanol. However, lyophilisation is time-consuming and not suitable for all matrices. Aharonsson et al. [21] reported a method for the analysis of benzimidazole fungicides including carbendazim and thiophanate methyl involving SFE and high-performance liquid chromatography–UV absorbance detection (HPLC–UV). Hydromatrix was used to bind water. Good recoveries were achieved for bananas, potatoes and apples. However, our experience has shown that especially for commodities with low maximum residue levels (MRLs) the determination limits achieved for carbendazim are not low enough for monitoring purposes when SFE–HPLC–diode array detection (DAD) is used. Furthermore, we have noticed that the extraction of carbendazim from samples with low natural pH is insufficient and that it can be enhanced significantly by alkalising the samples before extraction.

2. Experimental

2.1. Apparatus

The following equipment was used: food processor (Büchi B400); water bath; supercritical fluid extractor: Model 7680T (Hewlett-Packard) equipped with a variable restrictor and a collection system filled with 1 ml ODS 30 μm, extraction thimble 7 ml for HP 7680T with caps, glass-fibre paper 10 mm diameter; block heater for gas chromatography (GC) vials equipped with nitrogen gas evaporation manifold; 1.5 ml screw thread glass vials and crew caps with PFTE/rubber septa; 20 ml screw thread glass tubes with crew caps, leakproof.

For GC the following equipment was used: HP 5890 Serie II, autosampler 7673, Injector Gerstel CIS, solvent vent modus, Detector HP 5970 MSD or HP 6890 with autosampler, pulsed splitless mode, Detector: HP 5973 MSD, both equipped with uncoated pre-columns: 3 m×0.25 mm, pressfit connection to analytical column: HP5MS, 30 m×0.25 mm, 0.25 μm, carrier gas: helium.

For HPLC the following equipment was used: HP1090 Serie II, DAD, Merck LiChrochart 250-4 RP18. 5 μm, column oven 40°C, DAD wavelengths 285 nm and 267 nm, flow: 1.2 ml/min, injection volume 20 μl, buffer pH 8 (4.77 g $Na_2B_4O_7 \cdot 10H_2O$ and 20.5 ml 1 *M* HCl ad 1 l) mobile phase: buffer–acetonitrile (70:30).

2.2. Reagents and materials

The following materials were used: SFE/SFC grade CO_2 contained in gas tanks with dip tube and cryogenic CO_2 coolant grade contained in gas tanks with dip tube (both by Messer Griesheim). Organic solvents: acetone, cyclohexane, ethyl acetate, acetonitrile, methanol, isooctane, all pesticide residue analysis grade, acetonitrile, LC grade, Hydromatrix (Varian), pH indicator strips, 100 ml beaker, polypropylene powder funnel. Sulphuric acid, analytical-reagent grade, sulphuric acid solution 25% (v/v). Potassium carbonate, analytical-reagent grade, potassium carbonate solution 25 and 50% (w/w). PFB-Br (pentafluorobenzyl bromide) 99+% (Aldrich), PFB-Br derivatisation agent: 15% (v/v) solution of PFB-Br in acetonitrile (caution: reagent is a strong lachrymator). TCE (2,2,2-trichloroethanol) 99+%, TFAA (trifluoroacetic anhydride) 99+% (both by Aldrich). TCE derivatisation agent: mix 1 volume part TCE with 4 volume parts TFAA in a 50-ml Erlenmeyer flask with stopper and leave the mixture closed for 20 min until the phases are completely mixed. Potassium hydroxide, analytical-reagent grade potassium hydroxide solution 5% (w/w). Polychlorinated biphenyl (PCB) IUPAC No. 101 as internal standard, 1 μg/ml in isooctane.

2.3. Preparation of standard solutions

Stock solutions were prepared by weighing accurately 100 mg of pesticide in a 100-ml volumetric flask and diluting to volume with acetone–DMF (1:1) for carbendazim, with acetonitrile for thiophanate methyl and with acetone–isooctane (1:1) for 2,4-D.

Working solutions were prepared as follows: for calibrations: 10 μg/ml, 3 μg/ml: prepared by diluting stock solutions with acetonitrile, for fortifications: 30 μg/ml, 10 μg/ml, 3 μg/ml: prepared by diluting stock solutions with acetone.

2.4. Procedure

2.4.1. Sample preparation

Good homogeneity is achieved when samples are comminuted in frozen condition. Small fruits like grapes and soft fruit can be frozen (ca. −15°C) uncut. Bigger fruits like citrus fruits and apples have to be cut coarsely (ca. 2×2 cm) before freezing in order to facilitate chopping afterwards.

For 2,4-D in citrus fruits: a 12.0 g homogenised sample is weighed into a 100 ml beaker (note mass M_1). pH is brought to 9–10 by adding potassium carbonate solution 50% (w/w) (for lemons ca. 1 ml and for other citrus fruits ca. 0.5 ml). The mixture is stirred carefully and the beaker is covered with Parafilm or aluminium foil and placed over a boiling water bath for 20 min. After the sample has cooled down, pH is brought to ~2.5 by adding sulphuric acid 25% (v/v) (for lemons ca. 1 ml and for other citrus fruits ca. 0.5 ml is needed). By adding Hydromatrix beaker mass is brought to M_1+10 g. The mixture is stirred intensively with a spatula until homogeneity is achieved. An aliquot e.g., 5 g of the sample–Hydromatrix mixture is loaded into the extraction thimble. In order to prevent sample from being extruded during extraction a fine grade glass paper is put at both ends of the extraction thimble.

For the carbendazim group in fruits and vegetables: 12.0 g homogenised sample is weighed into a 100 ml beaker (note mass M_2). After pH is brought to 8–9 by adding potassium carbonate solution 50% (w/w) the mixture is shaken carefully. By adding Hydromatrix beaker mass is brought to M_2+8 g. Further procedure as described above. Thimbles are kept frozen until extraction.

Notice: Due to the high conversion rate of benomyl to carbendazim in acidic aqueous solutions it is assumed that most of the benomyl in or on crops is converted to carbendazim in the samples after comminution. Nevertheless, if a considerable amount of intact benomyl is still expected in the homogenised samples, conversion to carbendazim has to be performed artificially prior to alkalisation:

Procedure: 12.0 g homogenised sample is weighed into a 100 ml beaker (note mass M_3). If needed pH is adjusted between 3 and 4. The beaker is covered with Parafilm or aluminium foil and placed in a water bath at ~65°C for 20 min. After the sample has cooled down, pH is brought to 8–9 by adding potassium carbonate solution 50% (w/w). By adding Hydromatrix beaker mass is brought to M_3+8 g. Further procedure see above. Thimbles are kept frozen until extraction.

2.4.2. Extraction

SFE is performed by a HP 7680T Model equipped with automated variable restrictor and solid sorbent collection system.

Instrumental parameters: extraction pressure 329 atm (1 atm=101325 Pa), extraction temperature 55°C (CO_2 density 0.89 g/ml), static extraction time 2.5 min, dynamic extraction time 25 min, flow-rate 1.8 ml/min, restrictor temperature during extraction 50°C, sorbent trap ODS (1 ml), trap temperature during trapping 10°C, elution of the precipitated extract from restrictor and trap to vials with 1.3 ml acetonitrile at 0.4 ml/min and 50°C. The trap is cleaned and regenerated between extractions by rinsing to waste with 3 ml cyclohexane–ethyl acetate (1:1) and 3 ml acetonitrile at 2 ml/min.

2.4.3. Derivatisation with PFB-Br/K_2CO_3

Both, 2,4-D and carbendazim can be derivatised by this agent. Prior to derivatisation, residual thiophanate methyl is artificially converted to carbendazim. Conversion and derivatisation are performed in the 1.5-ml GC vials in which the SFE extracts are collected.

The acetonitrile extract is brought to ~1 ml (if necessary gentle nitrogen stream is used to evaporate). After 100 μl of K_2CO_3 solution 25% (w/w) is added as catalyst, the vials are closed and shaken well. In order to convert thiophanate methyl that may be present in the extract to carbendazim the vials are put into a heating block for ~20 min at 60°C. After adding 200 μl PFB-Br derivatisation agent the vials are closed again and put into the heating block for another 3 h. The vials are opened and the solvent is allowed to evaporate (~50 μl dodecane is added as keeper if nitrogen stream is used to evaporate). One ml isooctane containing the internal standard is added and the vials are shaken well. In order to bind residual water some Na_2SO_4 (about 100 mg) is added and the vials are shaken again. GC–MS analysis is performed without any additional clean-up.

Classical calibration approach: five calibration levels ranging from 0.1 ppm (0.3 μg per vial) to 2 ppm (6 μg per vial) are prepared using the working solutions (see Section 2.3). Volumes are brought to ~1 ml with acetonitrile and the derivatisation is performed as described above.

Standard addition method: the SFE extract is transferred into a volume flask (e.g., 4 ml) and diluted to volume with acetonitrile. Four equal aliquots of this solution are transferred into GC vials and three of them are fortified with approximately $x/2$, x and $2x$ μg of the expected amount (x, μg) of the pesticide to be analysed. Before starting derivatisation volumes are equalised.

2.4.4. Derivatisation with TCE/TFAA (only for 2,4-D)

Alternatively 2,4-D can be converted to its 2,2,2-trichloroethyl derivative. This reaction is considerably faster than the derivatisation with PFB-Br and was found to be very robust and reliable [22–24]. Unfortunately, due to capacity problems, this reaction cannot be performed in an 1.5-ml SFE collection vial. SFE extracts are transferred quantitatively into leakproof 20-ml screw thread glass tubes. One to two drops (~50 μl) dodecane are added as keeper and the acetonitrile is evaporated using a gentle nitrogen stream. After adding 20 μl 25% (v/v) sulphuric acid as catalyst and 1 ml derivatisation agent the tubes are closed immediately, shaken well, and put it into an oven for 35 min at 70°C. After the reaction mixture has cooled down, the tubes are opened and 2 ml isooctane containing the internal standard are added. With caution 6 ml 5% KOH solution is added and the tubes are closed and shaken well. A part of the upper isooctane layer is transferred into a GC vial. This solution can be analysed directly by GC–MS.

Calibration: five calibration levels ranging from 0.1 ppm (0.3 μg per vial) to 2 ppm (6 μg per vial) are prepared using the working solutions (see Section 2.3). The derivatisation is performed as described above after adding dodecane as keeper and evaporating the solvent.

2.4.5. Determination

The extracts obtained by SFE do not need any further clean-up. Carbendazim and thiophanate methyl can be analysed by HPLC–DAD as such or after conversion of thiophanate methyl summarily as carbendazim. However determination by GC–MS after derivatisation is by far more sensitive. Instrumental parameters are shown in Section 2.1.

No serious mass spectral interferences were ob-

served, when the following target and qualifier masses were used for MS determination: 2,4-D–PFB derivative: 400/175/402; 2,4-D–TCE derivative: 352/175/350; and carbendazim-bis–PFB derivative: 551/492/292.

3. Results and discussion

3.1. Preliminary studies

3.1.1. Sample preparation

Due to the relatively small sample size used for SFE a great degree of homogeneity is necessary to obtain statistically representative subsamples. Thus, an intensive sample comminution is required. This step has to be performed carefully in order to minimise analyte degradation. Very good comminution of various commodities is accomplished by chopping frozen coarse cut subsamples. The effect of comminution on analytical accuracy was demonstrated on grapes treated with carbendazim during the growing period. At first the sample was comminuted thoroughly at room temperature. Part of this sample was frozen and comminuted again. Each subsample was analysed seven times by the proposed SFE method. Although the average carbendazim levels found were almost identical (1.76 versus 1.73 ppm), the results obtained from the frozen comminuted sample had a significantly lower variation (R.S.D. 10.9% versus 4.4%).

3.1.2. Release of bound 2,4-D residues

Post-harvestly, 2,4-D is applied on citrus fruits mostly as isopropyl ester or as dimethylamine salt. Ester formulations hydrolyse quickly after application to the free acid form [9,25,26]. The acid can partially bind to components of the matrix like commercial coating waxes or natural components on the fruit surface, forming esters. 2,4-D residues bound in such a way can not be extracted using solvents. Extractable 2,4-D increases significantly when samples with incurred 2,4-D residues are heated at alkaline conditions prior to extraction, An increase of extractable 2,4-D was also noticed when acidified samples were heated, but alkaline hydrolysis is by far more efficient in releasing 2,4-D conjugates. The conditions of hydrolysis (pH, heating time) were optimised in order to minimise the formation of undesired artifacts which can affect GC analysis. It was shown that relatively gentle conditions (100°C/15 min at pH ~9.5) are sufficient to release most of the conjugated 2,4-D giving the free acid. No further 2,4-D release, but darker extracts instead, resulted when more drastic conditions were chosen for hydrolysis.

The effect of alkaline treatment was studied on real samples. The results achieved when alkaline hydrolysis was performed before extraction were two- to six-times higher than those obtained when samples were extracted directly.

3.1.3. Impact of pH on extraction efficiency

The extraction efficiencies of 2,4-D, carbendazim and thiophanate methyl at different pH levels were investigated (see Fig. 1). It was shown that carbendazim, which reacts weakly basic (pK_b=9.52), can be extracted more efficiently at higher pH values. On the other hand, 2,4-D, being an acid (pK_a=2.73), is much better extractable at lower pH. No significant influence of pH on extraction efficiency was noticed for thiophanate methyl between pH 4–9. In order to study the effect of pH on the extraction of incurred carbendazim residues, various commercial samples were extracted with and without prior pH adjustment. It was noticed that for samples with low natural pH like grapes and strawberries, carbendazim results were two- to three-times higher when pH was adjusted. On the other hand for samples with higher natural pH (about 6) like lettuce and potatoes pH adjustment had little effect.

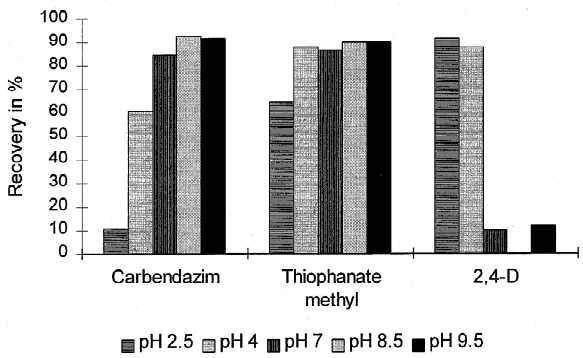

Fig. 1. Impact of pH on SFE recoveries of 2,4-D, carbendazim and thiophanate methyl from fortified orange juice.

3.1.4. Influence of water

Generally, fruits and vegetables contain a high percentage of water. Water can be effectively controlled by mixing the samples with adsorbing materials like silica gel or Hydromatrix (Varian) [19]. The effect of moisture on extraction efficiency was studied by mixing samples with different amounts of Hydromatrix. It was shown that carbendazim recoveries from grapes were essentially the same at sample: Hydromatrix ratios between 0.75:1 and 1.75:1. 2,4-D recoveries from fortified orange juice were also almost identical at ratios between 0.75:1 and 1.65:1.

3.1.5. Conversion of benomyl to carbendazim

Benomyl is known to be a very unstable compound. Various analysts have studied the kinetics of benomyl degradation in organic solvents [27–29] and aqueous solutions [4,29–33]. Until intact benomyl was detected on certain plants several weeks after application [34,35], it was believed that benomyl does not persist to any great extent on or in crops [36]. Due to its low solubility in water 3.8 ppm at 20°C [32], benomyl applied on crops as a suspension does not dissolve immediately and will therefore not degrade rapidly [28]. Baude et al. [37] demonstrated that benomyl has a relative good stability as a residue on treated plants. The conversion rate of benomyl to carbendazim in water is lower than in organic solvents [29,37] and increases as acidity and alkalinity increase [30,31,38]. However, in alkaline aqueous media a competitive benomyl degradation path occurs [31,33,38–40]. Therefore, benomyl should be converted to carbendazim prior to alkalisation and SFE extraction, otherwise undesired degradation will occur.

In this study benomyl hydrolysis to carbendazim was performed at pH 3 by heating the sample at 65°C for 20 min. Mean recoveries of over 90% (as carbendazim) were achieved when samples fortified with benomyl were pre-treated like that (before extraction pH was adjusted at 8, see Section 2.4.1). Further it was shown that both thiophanate methyl and carbendazim are practically not affected by this treatment. Benomyl recovery was significantly lower (73% as carbendazim) when fortified samples were adjusted at pH 8 and extracted without prior conversion. Due to undesired degradation the recoveries became even lower when these samples were stored at room temperature before extraction (e.g., 23% after 8 h).

Notice: The presence of carbendazim as impurity in benomyl standards and the high degradation rate of benomyl in organic solvents, makes it practically impossible to obtain carbendazim-free solutions [33]. Benomyl stock solution (500 μg/ml) was prepared in cold acetonitrile. The HPLC–DAD analysis of this solution immediately after preparing has shown that approximately 12% of the benomyl was already converted to carbendazim. This solution was relatively stable when stored at −18°C. At room temperature, however, a fast conversion of benomyl to carbendazim was noticed. The conversion factor 1.52 was used for calculating benomyl recoveries.

3.1.6. Conversion of thiophanate methyl to carbendazim

Thiophanate methyl is more stable than benomyl and generally persists over long periods in treated crops [2,40]. As it is shown in Fig. 2 thiophanate methyl conversion to carbendazim during the extraction process is negligible even under alkaline conditions. As a consequence determination of thiophanate methyl side by side with carbendazim is possible (HPLC–DAD). When the alkalised samples are stored in extraction vessels at room temperature a conversion of thiophanate methyl to carbendazim occurs. However, due to competitive reactions, the conversion rate is low (maximally 60%). No serious degradation of thiophanate methyl was noticed within three days when samples were stored at −18°C (see Fig. 2). Furthermore, we have noticed that thiophanate methyl converts to carbendazim even in the SFE extracts solved in acetonitrile. Therefore, if thiophanate methyl is to be determined as such, both, extraction and HPLC analysis have to be performed without any delay.

3.1.7. Artificial conversion of thiophanate methyl to carbendazim and derivatisation

Carbendazim and thiophanate methyl have a common MRL calculated as carbendazim. Carbendazim can be determined very sensitive as PFB derivative using GC–MS [22]. Therefore it is reasonable to determine both compounds summarily as carbendazim. We have developed a method which allows

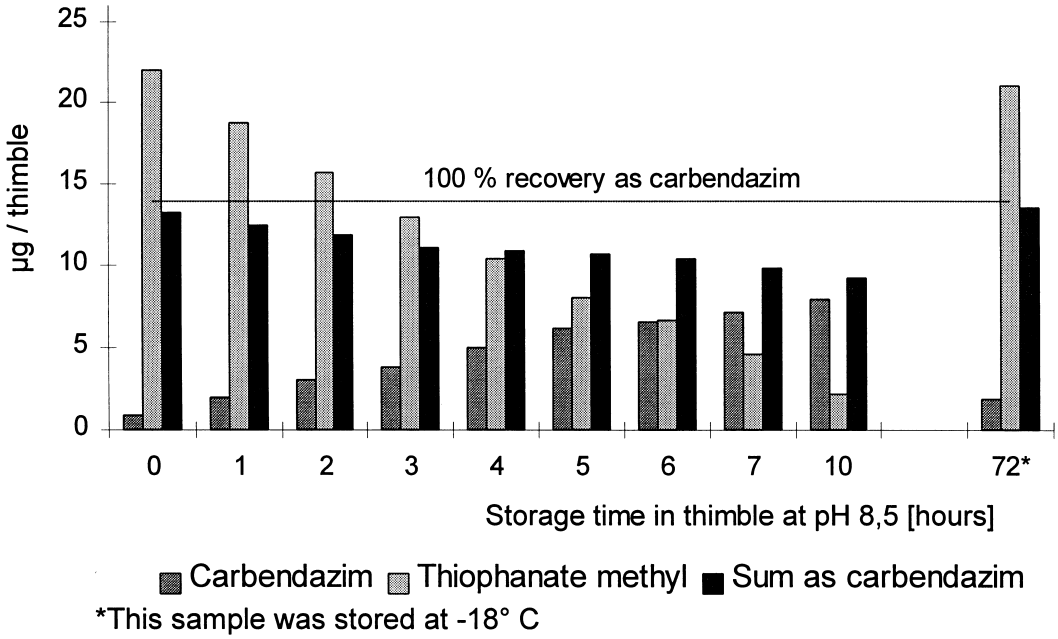

Fig. 2. Undesired degradation of thiophanate methyl in the extraction thimble during storage at room temperature.

both, the conversion of thiophanate methyl to carbendazim and the subsequent derivatisation with PFB-Br in the vials in which the SFE extracts are collected. Both reactions are catalysed by potassium carbonate solution.

In order to optimise the conditions of the conversion a simple kinetic study was performed. The progress of this reaction was observed using RP-HPLC–DAD. The first step of this base catalysed reaction, which is reflected by the progressive decrease in thiophanate methyl peak area, seems to follow first-order kinetics. This is indicated from the linearity of the plots of the logarithm of the peak area of thiophanate methyl against time. Corresponding to thiophanate methyl decrease intermediate products, which were not further investigated, appear (see Fig. 3). The formation of carbendazim can be accelerated by increasing the temperature. However temperature should be chosen below 70°C in order to minimise carbendazim degradation to 2-aminobenzimidazole (see Fig. 4). The formation of intermediate products from thiophanate methyl appears to be fast. Significantly slower and thus rate limiting is the subsequent formation of carbendazim (see Fig. 3). This has consequences in analysis: derivatisation agent should be added after the conversion is completed (at 60°C, after ~20 min see Fig. 4) otherwise it will react with intact thiophanate methyl or with intermediate products forming other derivatives than the desired one and too low results will be achieved.

Notice: It is also possible to perform the determination of total carbendazim by HPLC–DAD.

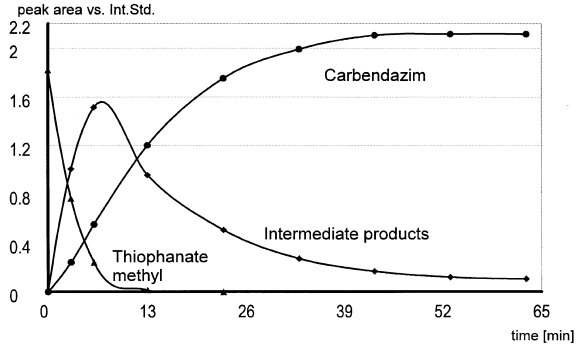

Fig. 3. Kinetics of thiophanate methyl conversion to carbendazim in acetonitrile–K_2CO_3, t=50°C.

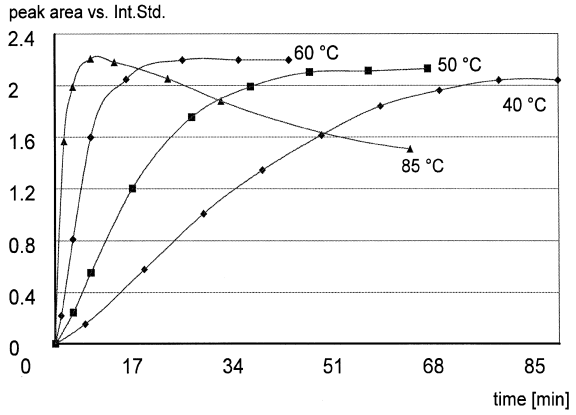

Fig. 4. Formation of carbendazim during conversion of thiophanate methyl in acetonitrile–K_2CO_3 at different temperatures.

3.2. Validation

For recovery studies several blank matrices were fortified with spiking solutions in acetone. After stirring with a thin spatula the samples were left for a few minutes at room temperature to evaporate solvent. Calibrations for carbendazim and thiophanate methyl were made by the method of standard addition using carbendazim standard solution. To determine 2,4-D recovery, calibration was made using standards prepared by spiking SFE extracts of blank grapefruit matrix. The recoveries for carbendazim achieved from grapefruit, grapes and iceberg lettuce (fortification level 0.33 ppm) lay between 91 and 93%, for thiophanate methyl in grapefruit and apples (0.66 ppm) recoveries were 85 and 90%, respectively and for 2,4-D in grapefruit (0.25 ppm) 93%.

For comparison purposes several commercial citrus samples with incurred 2,4-D residues were analysed by the proposed SFE method and by a solvent-based extraction method [24]. An excellent correlation between the two methods was achieved.

The limits of detection and determination were calculated following guidelines which were elaborated by the Deutsche Forschungsgemeinschaft (DFG) (calibration curve procedure) [41]. To obtain the calibration curve a serie of fortification experiments with four chosen levels laying close to the expected detection limit was run. Four replicate determinations of each fortification level were made. The calculated limits of detection/determination were: for 2,4-D as TCE derivative 6 ppb/14 ppb and for carbendazim as bis-PFB derivative 3 ppb/5 ppb.

The proposed SFE method for the determination of carbendazim was checked by analysing a chopped apple sample fortified with an unknown amount of this compound. This sample was supplied by the European Union in March 1997 within the scope of a proficiency test [42]. The reported spiking level was 0.653 ppm. The result obtained by the proposed SFE method was 0.66 ppm while the mean result of all the 58 participating laboratories was 0.507 ppm.

3.3. Examination of commercial samples

Various crop samples were analysed since 1996 within the scope of food surveillance by the SFE methods presented in this paper. Since, most of the existing solvent based methods for the analysis of 2,4-D and carbendazim in food are very labour intensive, few laboratories include the analysis of these compounds in their routine program. Thus, limited residue data is available.

The 2,4-D results of the analysis of 62 citrus samples from various countries are listed in Table 1.

The analysis of over 250 commercial samples in 1997 has demonstrated that the fungicides of the carbendazim group are widely used in agriculture and that MRL violations occur (see Table 2).

Table 1
Residues of 2,4-D in commercial citrus fruit samples (analysed in 1996 and 1997)

Origin	No. of samples	Samples with residues	Residues in mg/kg
South Africa and Zimbabwe	17	14 (82%)	0.01–1.6
South- and central-America	9	9 (100%)	0.04–0.35
Mediterranean countries	36	0	–

Table 2
Results of carbendazim[a] residue analysis in commercial samples (1997)

	No. of samples	Samples with residues	German MRL (mg/kg)	>MRL	Residues in mg/kg
Lettuce	131	21 (16%)	1.0	2	<0.01–11
Stone fruit	20	10 (50%)	0.1	2	<0.01–0.3
Mango	15	6 (40%)	0.1	2	<0.01–0.8
Papaya	10	3 (30%)	0.1	1	0.04–0.48
Champignon	15	6 (40%)	1.0	–	<0.01–0.13
Citrus fruit	24	10 (42%)	5.0	–	<0.01–0.12
Strawberry	46	22 (48%)	1.5	–	<0.01–0.8

[a] Carbendazim/benomyl/thiophanate methyl as a total.

4. Conclusions

It is shown that SFE is a good alternative technique for the extraction of carbendazim, thiophanate methyl and 2,4-D from various fruits and vegetables. Better extraction efficiencies were achieved by adjusting pH before extraction.

The fungicides carbendazim, benomyl and thiophanate methyl are widely used in agriculture for pre- and post-harvest protection of many crops. Having a common MRL, calculated as carbendazim, these three compounds are determined as a total. Carbendazim is extracted more efficiently at alkaline conditions. Before extraction benomyl can be artificially converted to carbendazim. Thiophanate methyl is analysed as such by HPLC–DAD or after conversion as carbendazim. Analysis of total carbendazim can also be performed by HPLC–DAD. Unfortunately, this approach cannot be used to monitor low MRLs. Considerably better determination limits are achieved by GC–MS after derivatisation with PFB-Br.

2,4-D is used to treat citrus fruits after harvest in order to maintain the natural resistance against fungal decay. Being an acid 2,4-D can be considerably better extracted at lower pH levels. A significant increase of extractable 2,4-D from incurred citrus samples was achieved by performing alkaline hydrolysis prior to SFE extraction. Determination of 2,4-D is carried out by GC–MS after derivatisation with 2,2,2-trichloroethanol or PFB-Br.

Reproducibility studies indicate that 3 g subsample is sufficient to represent a larger sample. Standard deviations for incurred samples usually lay between 3 and 10% depending on residue level. The recoveries from fortified samples (low fortification levels) ranged between 90 and 93% for carbendazim and 2,4-D and between 85 and 90% for thiophanate methyl.

Various commercial samples were analysed in 1996 and 1997 using the proposed SFE methods. 2,4-D residues were found in 33% of the analysed citrus fruits. Carbendazim was found in 40–50% of the analysed strawberries, citrus fruits, champignons, mangoes and stone fruit. Seven samples had violative carbendazim residues.

The results of this study demonstrate that the proposed SFE methods are clearly faster, less expensive and environmentally safer than traditional solvent based approaches and that they can be successfully introduced in the routine analysis of these compounds.

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