ELSEVIER

Food Chemistry 52 (1995) 57–61

0308-8146/95/$9.50

Investigation into contamination of processed fruit products by carbendazim, methyl thiophanate and thiabendazole

Anna Sannino

Stazione Sperimentale per l'Industria delle Conserve Alimentari, viale Tanara 31/A 43100 Parma, Italy

(Received 27 October 1993; revised version received and accepted 24 March 1994)

A reversed phase high-performance liquid chromatographic (HPLC) method was used for determining benomyl and its metabolites carbendazim, thiabendazole and methyl thiophanate in fruit products (nectars, purees, concentrates and jams). The residues were extracted with HCl/methanol and partitioned into dichloromethane on an Extrelut 20 cartridge column. The fungicides were then separated on a reversed phase HPLC column using an ion-pairing mobile phase and finally determined by UV and fluorescence detection. Eighty-three samples of commercial fruit products were analysed by this method. Positive samples were confirmed by normal phase chromatography using a Diol column. The determination limit was 0·010 mg kg^{-1} for carbendazim and methyl thiophanate, and 0·001 mg kg^{-1} for thiabendazole.

INTRODUCTION

Benomyl, carbendazim (MBC), methyl thiophanate (TM) and thiabendazole (TBZ) are systemic fungicides used as either preharvest or postharvest treatment for the control of a wide range of fruit and vegetable pathogens (Monico-Pifarrè & Xirau-Vaireda, 1987; Papadoupoulou-Mourkidou, 1991). MBC is the major metabolite and fungitoxic principle of benomyl and TM. Benomyl is almost completely converted into MBC both directly on fruits and during the analysis (Chiba & Cherniak, 1986). On the other hand, TM is only partially degraded to MBC under the same conditions (Bicchi *et al.*, 1989). As a consequence of this, in Italy, benomyl and TM tolerance limits must be expressed as MBC.

Because of possible health effects, widespread use and insufficient residue data, there is an increasing need to monitor these fungicides in processed food and in particular fruit products. In this paper, fruit nectars and purees (apple, pear, apricot and peach), citrus juices and strawberry jams were analysed. The simultaneous determination of benomyl (as total MBC), TM and TBZ residues was carried out by reversed phase high-performance liquid chromatography (HPLC), using an ion-pairing mobile phase with UV and fluorescence detectors coupled in tandem.

Confirmation tests were performed by normal phase HPLC.

MATERIALS AND METHODS

Reagents and standards

Solvents suitable for residue and HPLC analysis were obtained from Merck (Darmstadt, Germany).

Fungicide standards (99·0%) were obtained from S.I Ehrenstorfer (Augsburg, Germany). TM and TBZ stock solutions for calibrating UV and fluorescence detectors and for sample fortification were prepared by dissolving the standards in acetone, whereas MBC was dissolved in methanol. Standard solutions for the analytical determinations were obtained by evaporating aliquots of stock solutions to dryness under a stream of nitrogen and taking up the residue in the mobile phase.

Sample extraction and purification

Nectars, jams and juices were purchased from supermarkets in Parma. Fruit purees and citrus juice concentrates were semiprocessed products directly supplied from manufacturers. The clean-up method was based on the procedure described by Bicchi *et al.* (1989). Fifty grams of product taken from a homogeneous sample (fruit nectars and purees, strawberry jams and citrus juices) were homogenised with 100 ml of 0·02 N HCl/methanol (80:20) by a Waring blender for at least 5 min, centrifuged at 5000 rpm for 15 min and filtered through a glass microfibre filter under vacuum. The procedure was repeated with 50 ml of HCl/methanol solution. The extract was diluted to 200 ml and filtered

through two Minisart NML filters (Sartorius Göttingen, Germany) in series (1·2 and 0·5 μm). An aliquot of this solution (20 ml) was brought to pH 7·5 with diluted NaOH and loaded on to an Extrelut 20 column (Merck). After adsorption for 20 min, elution was performed with 80 ml of dichloromethane. The eluate was evaporated to dryness with a rotary evaporator at a bath temperature of 30°C and the residue was finally taken up in the mobile phase (1 ml). An aliquot (25 μl) was injected into the chromatograph. For samples of citrus juice of 50° Brix, the preparation was the same after fivefold dilution.

Analyses of individual samples, including spiked ones, were done in duplicate and mean values were presented.

To obtain data on residue levels in unspiked and spiked samples the same extract was injected once.

Quantitation was carried out by comparison of the peak areas, using external standardisation.

HPLC analysis

The HPLC apparatus (Waters Associates, Milford, MA, USA) consisted of a Model 600E solvent delivery system with an Ultra Wisp 715 autosampler, a Model 490 UV programmable multiwavelength detector connected in tandem with a model 470 scanning florescence detector and a Waters 820 Maxima chromatography workstation for data handling.

UV detection conditions were: simultaneous detection at 280 nm (maximum response for MBC and TM) and 305nm (TBZ maximum response). Detector sensitivity was set at 0·01 AUFS. The fluorimetric detector operated at an excitation wavelength of 280 nm and at an emission wavelength of 310 nm for MBC maximum response (attenuation ×1, gain ×1000). After MBC elution, wavelengths were adjusted to 305 and 345 nm (TBZ maximum response) and detector sensitivity was set at attenuation ×32 and gain ×100 by the 'program mode'.

The column used was a Supelcosil LC-18-DB (25 cm × 0·4 cm i.d.) with 5 μm particles. The mobile phase was prepared according to Gilvydis & Walters (1990) and consisted of 35% methanol in ion-pairing solution. The HPLC column temperature was maintained at 40 ± 0·1°C by means of a 'column heater' controlled by a temperature control module (Waters Associates). The flow rate was 1·5 ml min^{-1}.

Confirmation analysis

The HPLC column used was a Lichrosorb DIOL 5 μm (15 cm × 3 mm) (Merck). The mobile phase consisted of an isopropanol/*n*-hexane (15:85) mixture with some 32% NH_4OH drops added. The flow rate was 1·5 ml min^{-1} An aliquot (25 μl) of solution obtained by dissolving the residue from the clean-up procedure with the mobile phase was injected. UV and fluorometric detector conditions were the same as for ion-pairing HPLC.

RESULTS AND DISCUSSION

Because of complete hydrolysis of benomyl to MBC during analysis, only the determinations of carbendazim residues were considered.

Figure 1 shows the HPLC chromatograms of apricot puree before and after fortification with 0·1 mg kg^{-1} of MBC and TM, and 0·01 mg kg^{-1} of TBZ. As can be seen, there are no peaks interfering with the active compounds analysed.

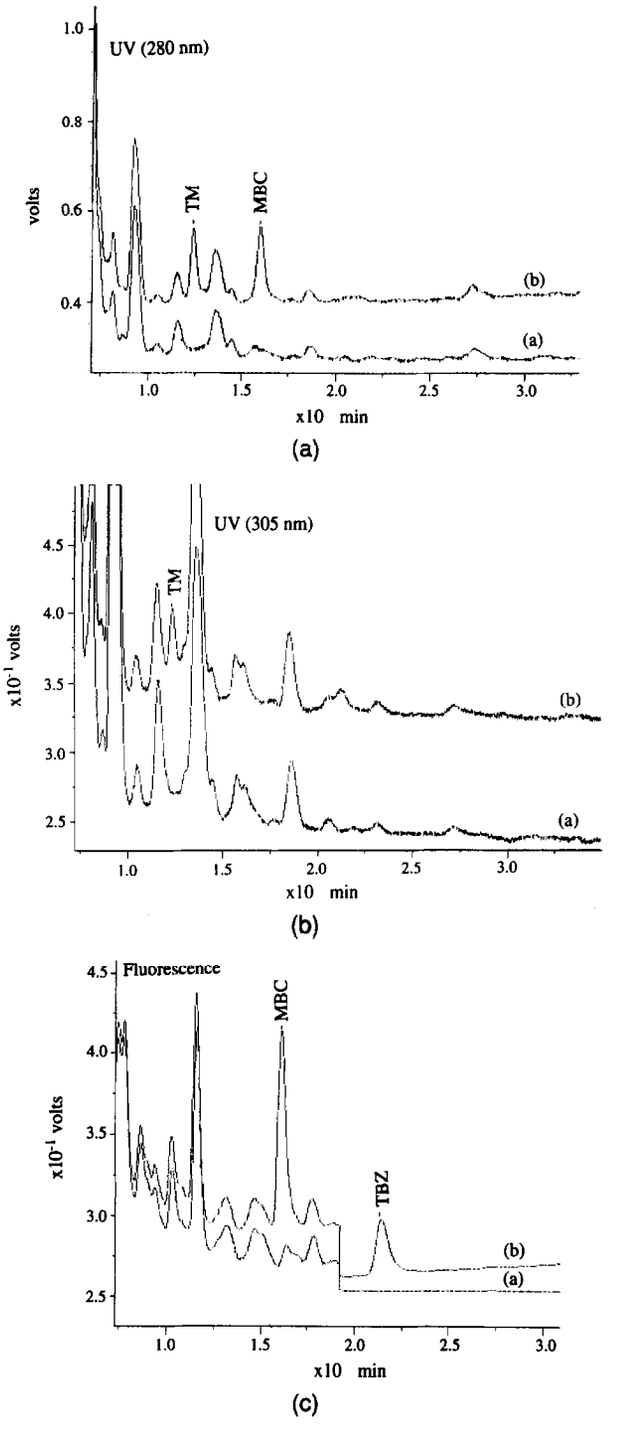

Fig. 1. Chromatograms of (a) apricot puree free from MBC, TM and TBZ, and (b) apricot puree spiked with 0·1 mg kg^{-1} of MBC and TM and 0·010 mg kg^{-1} of TBZ obtained by UV (at 280 and 305 nm), and (c) fluorescence detection at excitation/emission wavelengths of 280/310 nm, immediately following MBC elution at 305/345 nm. Column: 5 μm Supelcosil LC-18-DB; injection volume, 25 μl; mobile phase: 35° methanol in ion-pairing solution (1 g sodium decanesulphonate dissolved in a mixture of 200 ml of water, 7 ml of phosphoric acid and 10 ml of triethylamine and diluted to 1 litre with water). Column temperature: 40°C; flow rate: 1·5 ml min^{-1}.

Table 1. Recoveries (%) of MBC, TM and TBZ from spiked commercial fruit products[a]

	MBC added (mg kg^{-1})			TM added (mg kg^{-1})			TBZ added (mg kg^{-1})		
	0·025	0·1	0·25	0·025	0·1	0·25	0·025	0·1	0·25
Peach puree	93·1(90·0–95·5)	92·0(89·7–93·8)	94·2(92·2–95·8)	71·0(68·5–72·0)	72·0(70·4–73·4)	70·0(68·7–71·8)	93·3(91·8–94·9)	91·4(89·7–92·8)	95·3(93·7–97·0)
Pear puree	86·4(85·0–89·0)	90·0(88·0–91·0)	87·0(85·8–88·0)	67·8(66·3–69·1)	67·0(65·1–68·9)	69·0(67·4–70·7)	90·1(88·8–91·8)	81·4(79·7–82·9)	87·9(85·8–89·2)
Apricot puree	92·0(89·1–93·5)	93·0(91·7–94·5)	91·0(89–92)	66·8(65·2–68·6)	67·9(66·0–70·1)	71·0(69·4–72·2)	85·0(83·7–87·0)	92·0(90·7–94·0)	89·2(87·5–91·0)
Apple puree	90·0(88·5–91)	89·7(88·0–91·5)	90·0(88·2–91·7)	67·0(65·2–68·6)	68·0(67·8–70·0)	67·4(66·0–68·5)	83·0(82·0–85·0)	93·0(91·2–94·5)	90·4(88·8–92·1)
Strawberry jam	86·3(85·0–88·1)	87·0(86·2–89·0)	92·9(91·5–94·5)	66·2(64·8–67·9)	68·6(67·0–71·0)	68·0(67·5–70·0)	87·0(85·7–89·0)	89·0(87·3–91·8)	88·5(86·5–89·9)
Orange juice	89·0(87·8–90·8)	89·6(87·8–91·8)	90·0(88·0–92·0)	—	—	—	90·4(87·8–92·0)	91·0(88·9–92·7)	89·0(87·8–90·9)
Average	89·5	90·2	90·8	67·8	68·7	69·1	88·1	89·6	90·1
SD	2·8	2·1	2·5	1·9	1·9	1·4	3·8	4·2	2·7
CV%	3·1	2·3	2·8	2·7	2·8	2·1	4·3	4·7	3·0

[a]Means of three determinations. Ranges in parentheses.

Preliminary tests conducted using the clean-up method described by Gilvydis & Walters (1990) proved unsatisfactory for TM and MBC recoveries at fortification levels lower than 0·5 mg kg^{-1}. In contrast, the results obtained by the purification procedure based on the method of Bicchi *et al.* (1989) were excellent, as shown in Table 1. Recovery tests were performed by fortifying strawberry jams, fruit purees and juices, which were found negative when analysed for the same active substances. As Table 1 shows, the average TM recoveries were lower than 70% for all levels, in accordance with the results obtained by Bicchi *et al.* (1989) for apple and pear products. In fact, under the acidic conditions used in the extraction of active principles, 15% of TM was converted into MBC.

Because of peak interfering with the active compound, TM quantification was precluded in citrus samples. Moreover, when a citrus extract was injected, interferences were observed due to matrix substances retained by the column for a long time. These interfering substances eluted giving very large peaks, thus making it necessary to clean the system for 1 h. UV detection chromatograms of citrus samples were particularly complex with peaks overlapping MBC. However, less sample background was seen in flourescence chromatograms, and MBC quantification was thus possible (Fig. 2).

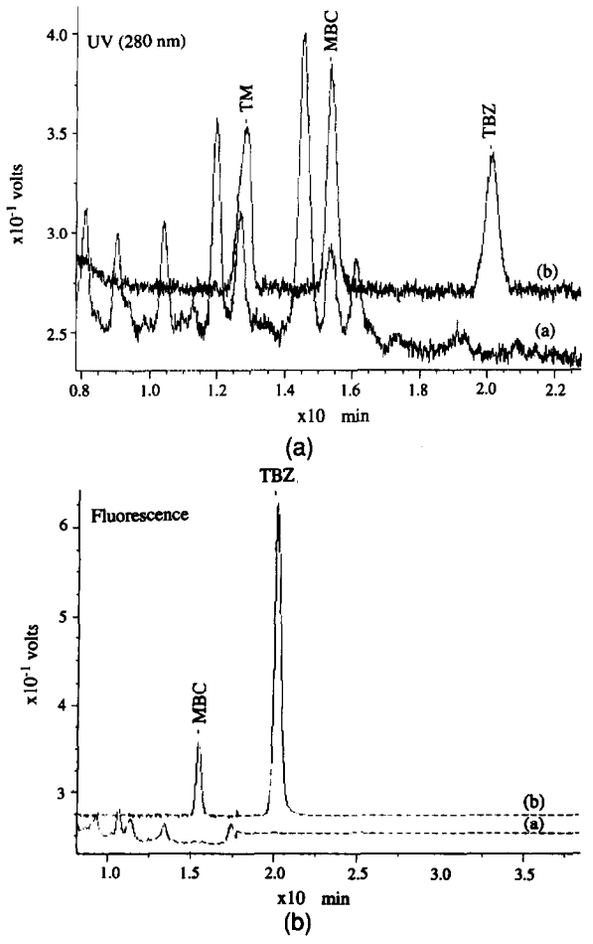

Fig. 2. Chromatograms of (a) orange juice and (b) standard mixture of MBC, TM and TBZ (0·25 mg kg^{-1}) obtained by UV (at 280 nm) and fluorescence detection. Conditions same as for Fig. 1.

TBZ determination was always carried out by fluorimetric detection since the response obtained was about 10 times greater than with UV detection (at 305 nm). When possible, MBC was also quantitatively measured by fluorescence detection. However, owing to coextractive interference in the fluorescence chromatograms, the MBC residue in pear extract was determined by UV detection at 280 nm.

In all cases, UV and fluorimetric detectors connected in series allowed the simultaneous determination of the nonfluorecent TM and fluorescent MBC and TBZ and proved suitable as a confirmatory method for the latter fungicides.

The minimum detectable limit of MBC and TM was 1·25 ng (with a signal-to-noise ratio of 3:1) equivalent to a 0·010 mg kg^{-1} residue in the sample (limit of determination). The detection limit for TBZ was 0·125 ng (with a signal-to-noise ratio of 3:1) equivalent to a 0·001 mg kg^{-1} residue. These values represent the sensitivity of the proposed analytical method for all the products examined, except for citrus juice concentrates. For 50°Brix products, the method was applied in the same way, but starting, of course, with a smaller sample; as a consequence, method sensitivity was lowered, depending on dilution.

A further confirmatory analysis of positive samples was carried out by means of a Diol column operating in normal phase HPLC. In this case, the elution order of active compounds was reversed compared to that obtained by ion-pairing chromatography.

Table 2 lists results for MBC and TBZ residues in fruit products purchased from the market or supplied by different manufacturers. Results show that, of 83 samples analysed, 27 contained measurable levels of MBC and 23 measurable levels of TBZ. Methyl thiophanate residues were not found, probably because of complete degradation to MBC during fruit processing.

Absence of detectable quantities of fungicides was observed in strawberry jam samples. However, high levels of carbendazim have been found by Baldi *et al.* (1981) and Leone *et al.* (1981) in fresh strawberries, although benzimidazole fungicides are not registered for use on these fruits in Italy (Ministerial Decree, 1990).

As regards citrus products, use of TBZ is permitted for the control of postharvest disease of fruits not intended for juice production. However, the samples of citrus products analysed contained TBZ residues, though in very low amounts.

From Table 2 it can be seen that higher levels of TBZ were present in pome fruit products (nectars and purees) with highest residue values ranging from 0·020 to 0·190 mg kg^{-1}. The use of this fungicide is legally permitted only on apples and pears and residue tolerance limit is 3 mg kg^{-1}.

MBC concentration values obtained for pome and stone fruit products were considerably below the limits that the Italian law establishes for apricot and peach (0·5 mg kg^{-1}) and for pome fruits (1 mg kg^{-1}). Therefore, the results appear to be reassuring as to contami-

Table 2. Results of MBC and TBZ residues analysis in 83 samples of commercial fruit products[a]

Product	Samples analysed	Samples with MBC	MBC residue range (mg kg^{-1})	Samples with TBZ	TBZ residue range (mg kg^{-1})
Peach nectar	9	5	0·012–0·035	2	0·002–0·002
Peach puree	5	2	0·023–0·026	1	0·008
Pear nectar	9	5	0·015–0·070	4	0·030–0·100
Pear puree	4	2	0·016–0·021	1	0·020
Pear baby nectar	2	0	—	0	—
Apricot nectar	9	5	0·018–0·030	0	—
Apricot puree	6	3	0·020–0·095	0	—
Apple nectar	9	3	0·012–0·015	6	0·006–0·020
Apple puree	4	2	0·020–0·038	2	0·050–0·190
Apple baby nectar	2	0	—	0	—
Strawberry jam	8	0	—	0	—
Orange juice	3	0	—	2	0·008–0·010
Concentrated orange juice (50°Bx)	4	0	—	3	0·010–0·015
Lemon juice	3	0	—	2	0·010–0·010
Grapefruit juice	2	0	—	1	0·008
Concentrated grapefruit juice (50°Bx)	2	0	—	1	0·015
Total	83	27		25	

[a]Determinations made in duplicate.

nation of commercial fruit products, probably because of systematic control of pesticide residues by the processing industry. Moreover, the fruits destined for juice production usually come from orchards with controlled pesticide treatments.

REFERENCES

Baldi, M., Pietrogrande, M. C., Zanoni L., Bovolenta A., Maietti, S. & Penanazzi L. (1981). Indagine sulla contaminazione da fungicidi a nucleo benzimidazolico su mele, pere, fragole prodotte nella provincia di Ferrara. *Ind. Aliment.*, (Sept.), 594–5.

Bicchi, C., Belliardo, F., Cantamessa, L., Gasparini, G., Iscardi, M. & Sesia, E. (1989). Simultaneous determination of benzimidazole fungicides by HPLC on apples, pears and their pulps. *Pestic. Sci.*, **25**(4), 355–60.

Chiba, M. & Cherniak, E. A. (1986). High performance liquid chromatographic method for simultaneous determination of benomyl carbendazim in aqueous media. *J. Agric. Food Chem.*, **34**, 108–12.

Gilvydis, D. M. & Walters, S. M. (1990). Ion-pairing liquid chromatographic determination of benzimidazole fungicides in foods. *J. Assoc. Off. Anal. Chem.*, **75**(59), 753–61.

Leone, G., Conti, G. G. & Martinelli, D. (1981). Determinazione dei residui di BMC mediante spettrometria UV e di vinclozolin mediante GLC in fragole del mercato. *Riv. Soc. Ital. Sci. Aliment.*, **6**, 383–88.

Ministerial Decree (July 18) (1990). Ordinary Supplement of Italian Official Journal (G:U), No. 202, 30 Aug. 1990.

Monico-Pifarre, A. & Xirau-Vayreda, M. (1987). Monitoring residues of carbendazim (applied as benomyl) and thiabendazole in wellspur apples. *J. Assoc. Anal. Chem.*, **70**(5), 596–8.

Papadoupoulou-Mourkidou, E. (1991). Postharvest-applied agrichemicals and their residues in fresh fruits and vegetables. *J. Assoc. Off. Chem.*, **74**, 745–65.