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Short communication

# Selective clean-up applicable to aqueous acetone extracts for the determination of carbendazim and thiabendazole in fruits and vegetables by high-performance liquid chromatography with UV detection

Alfonso Di Muccio*, Silvana Girolimetti, Danilo Attard Barbini, Patrizia Pelosi, Tiziana Generali, Luciano Vergori, Giovanni De Merulis, Antonio Leonelli, Patrizia Stefanelli

*ISS – Istituto Superiore di Sanità (National Institute of Health), Lab. Tossicologia Applicata, Viale Regina Elena 299, 00161 Rome, Italy*

**Abstract**

Fungicide residues in vegetables (benomyl, carbendazim, thiabendazole) are analyzed through a clean-up procedure that uses a portion of the aqueous acetone extract prepared for multiresidue methodology. A portion of the aqueous acetone extract (equivalent to 5 g of vegetables) is loaded onto an Extrelut-20 cartridge (the cartridge is filled with a coarse, large-pore diatomaceous material). Then, acetone is partially removed by an upward stream of nitrogen at 2 l/min for 30 min. Benzimidazolic fungicides are recovered by percolating the cartridge with 100 ml of 0.1 *M* phosphoric acid solution, which also serves to convert benomyl to carbendazim. The percolating acid solution is drained on-line through a strong cation-exchange (SCX) solid-phase extraction cartridge with the aid of a slight vacuum. Benzimidazolic fungicides are retained on the SCX cartridge. The phosphoric acid solution is discarded together with the washings of the SCX cartridge, i.e., water followed by methanol–water (75:25), that remove unwanted coextractives. Finally, benzimidazolic fungicides are recovered by eluting the SCX cartridge with methanol–ammonium formate buffer (75:25). The final extract is then analyzed by reversed-phase HPLC with UV detection. Recoveries from crops such as apples, lettuce, strawberries and citrus fruits are generally greater than 80% and no interferences were observed. The clean-up is simple and straightforward, requires only disposable items, water solutions and a few milliliters of solvent and a minimum number of manipulations, and does not require concentration steps or electrical equipment. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Fruits; Vegetables; Food analysis; Sample handling; Carbendazim; Thiobendazole; Pesticides

## 1. Introduction

Benzimidazolic compounds [(benomyl, carbendazim (MBC), thiabendazole (TBZ)] are important fungicides for the protection of several crops both in field and post-harvest treatments. Typical extraction solvents include ethyl acetate [1,2], acetone–dichloromethane–light petroleum (1:1:1, v/v/v) [3], and methanol–0.02 *M* HCl (80:20, v/v) [4,5] and supercritical fluid extraction (SFE) [6].

For the clean-up, apart from the conventional separatory funnel acid–base partition [1], SFE requires expensive equipment, and careful selection of

*Corresponding author. Tel.: +39-6-4990-2615, Fax: +39-6-4440-112, E-mail: admuccio@sun.iss.it

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extraction conditions, modifier, and vegetable-to-solid hydromatrix ratio to get appropriate recovery. Size-exclusion chromatography in our experience does not give a selective clean-up and requires care of a liquid chromatographic system. Selective automated clean-up on diol-cartridges has been reported by Hiemstra et al. [3], but it appears to have one major drawback because it only works with a specific brand of diol cartridges. For laboratories that are accustomed to ethyl acetate extraction, we have described [2] a clean-up based on an on-column treatment of the raw concentrated ethyl acetate extract distributed over a macroporous material with dilute hydrochloric acid, followed by an on-line retention of MBC and TBZ on a strong cation-exchange (SCX) cartridge.

As many laboratories use acetone for multiresidue methodology [7], we deemed it useful to try to set up a simplified selective clean-up based on a portion of the initial aqueous acetone extract of vegetables normally prepared for the multiresidue determination of pesticides in fruits and vegetables.

## 2. Experimental

### 2.1. Reagents and chemicals

Ammonium hydroxide (20% $NH_3$); formic acid 99%; phosphoric acid concentrated, 85% purity, density 1.834 g/ml were used. Acetonitrile, methanol, water were of HPLC-grade. 0.1 *M* phosphoric acid was prepared by diluting 0.6 ml conc. phosphoric acid to 100 ml with water. 5 *M* ammonium formate buffer (pH 6.8) was prepared from ammonium hydroxide and formic acid. Extrelut-20 cartridges (Merck, cat. No. 1.15096) were obtained from Bracco Italia, Milan, Italy. Bakerbond SPE cartridges, 500 mg, 3 ml, aromatic sulfonic acid (phenyl-SCX) (code 7090-03) were purchased from Baker Italia, Milan, Italy.

Pesticide reference standards from the collection in this laboratory were kindly supplied from the main manufacturer and were 99% pure. Carbofuran, 1 mg/ml in methanol, was used as internal standard.

MBC stock standard solution was prepared at 19.01 μg/ml by dissolving 1.901 mg in 100 ml acetonitrile. TBZ stock standard solution was prepared at 19.77 μg/ml by dissolving 1.977 mg in 100 ml.

A mixed standard solution was prepared by diluting stock standard solutions: 10 ml MBC+10 ml TBZ to 50 ml with acetonitrile. Resulting concentrations were 3.80 and 3.95 μg/ml for MBC and TBZ, respectively.

The calibrant solution was prepared by mixing 1 ml of mixed standard solution and 1 ml of methanol–5 *M* ammonium formate buffer (pH 6.8) (1:1, v/v), and adding 20 μl of the internal standard solution. The resulting concentrations in the calibrant solution were: 1.90 μg/ml for MBC; 1.98 μg/ml for TBZ and 10 μg/ml for carbofuran.

### 2.2. Apparatus

High-performance liquid chromatography (HPLC) analyses were carried out on a Hewlett-Packard Model 1050 instrument consisting of an autosampler (set to inject 20 μl), a pumping unit, a UV programmable-wavelength spectrophotometric detector. Chromatograms were recorded on a Hewlett-Packard Model 3396 II integrator.

The HPLC conditions were as follows. HPLC column Ultracarb 5 ODS (20) Phenomenex, obtained from LabService Analytica, Bologna, Italy with a LiChrospher 60-RP-select B (5 μm) pre-column (LiChrocart 4-4, Merck, cat. No. 50963). Eluent composition program: A=water; B=acetonitrile; $t$= 0 min, A–B (70:30); $t$=5 min, A–B (70:30); $t$=35 min, A–B (10:90); $t$=45 min, A–B (10:90); $t$=50 min, A–B (70:30); $t$=60 min, A–B (70:30). Column oven temperature, 40°C. UV detector, wavelength time-programmed at 280 nm for MBC, 305 nm for TBZ, and again at 280 nm for carbofuran.

A Sartorius microbalance, Model XM 1000P, at 0.000001 g, was used.

### 2.3. Procedure

Aqueous acetone extracts of fruits and vegetables were prepared according to Ref. [7].

Take an aliquot of 20 ml of the extract (equivalent to 5.0 g of crop) and transfer it onto the top of an Extrelut-20 cartridge. Allow the liquid to drain and wait 10 min to obtain an even distribution in the filling material. Remove part of the acetone by

passing through the column, from bottom to top, a nitrogen flow of 2 l/min for 30 min.

Activate a phenyl-SCX cartridge by passing 3×2 ml of methanol and 3×2 ml of 0.1 *M* phosphoric acid water solution. Stop the flow when the meniscus is at the top of the phase. Disconnect the Extrelut-20 cartridge from the gas line. Connect the exit of the Extrelut-20 cartridge to the phenyl-SCX cartridge by means of an adapter. Start the elution of the Extrelut cartridge with 5×20-ml portions of 0.1 *M* phosphoric acid. Connect the phenyl-SCX cartridge to a vacuum manifold. Let pass all the 100 ml of the acid solution at ca. 1 ml/min and discard. Disconnect the Extrelut-20 cartridge. Wash the phenyl-SCX cartridge with 2 ml water, followed by 3×2 ml methanol–water (75:25, v/v) and discard. Elute MBC and TBZ from the phenyl-SCX cartridge with 2 ml of acetonitrile–methanol–5 *M* ammonium formate (pH 6.8) buffer (50:25:25, v/v/v), and save for the HPLC analysis. Add 20 μl (20 μg) of the carbofuran internal standard solution and analyse by HPLC comparing the chromatographic parameters (peak retention times and area ratio to I.S.) with those of standard solutions of MBC and TBZ. Calculate the residue amount by the internal standard technique.

## 3. Results and discussion

The main aim of this work was to develop a simplified method composed of steps carried out on disposable items and based on a portion of the aqueous acetone extract usually prepared for the multiresidue determination of pesticide in vegetables [7].

The raw aqueous acetone extract is dispersed on a macroporous diatomaceous material contained in a ready-to-use, disposable cartridge. After dispersing the raw extract solution over the Extrelut material, most of the acetone is removed by passing nitrogen through the cartridge, from bottom to top. In this way, the coextractives are adsorbed to the diatomaceous material and the major part is retained by the cartridge, because the coextracted material is poorly soluble in diluted acid. This step offers substantial advantages over the classical separating funnel partitioning of the extract between ethyl acetate and diluted acid solution [1]. These advantages include a straightforward operation with lack of emulsions, no solvents, reduced handling operations and the use of disposable items.

On the other hand, the high surface area of the material ensures a high degree of dispersion and, hence, good efficiency of transfer of MBC and TBZ into diluted acid. Thus in a single step, the Extrelut-20 cartridge performs the conversion of benomyl into MBC and effects a partial clean-up of the extract without any possibility of emulsions.

Under the conditions selected (2 l/min×30 min) ca. 70% of the acetone was removed from the Extrelut-20 cartridge to avoid an excess of acetone in the acid that could disturb the elution behaviour of the SCX cartridge. The yield of conversion of benomyl into MBC was not determined specifically in this work because we have already proven that under similar conditions it is satisfactory [2].

As both MBC and TBZ are in cationic form at low pH, this offers an opportunity of selectively isolating the analytes of interest. Therefore, we used a SCX cartridge to reconcentrate MBC and TBZ residues from the volume of acid solution used to elute them from the Extrelut-20 cartridge. The behaviour of phenyl-SCX has been studied in our previous work [2]. Its elution scheme has been slightly modified in conjunction with the new Extrelut-20 cartridge system.

At beginning of the method development we tried to isolate MBC and TBZ directly from the raw acetone extract of vegetables by passing 20 ml (equivalent to 5 g of vegetable) through the phenyl-SCX cartridge.

Indeed, standard MBC and TBZ can be recovered from aqueous acetone, but they are then not eluted in the appropriate fraction, because the high concentration of acetone upset the elution behaviour of the phenyl-SCX cartridge. However, with acetone extracts of vegetables, it was quite impossible because the amount of coextractives rapidly clogged the SCX cartridge.

After diluting the aqueous acetone extract (20 ml to 50 ml with water), it was possible to pass it through the SCX cartridge. Compounds of interest were retained along with a significant amount of coextractives, that were subsequently eluted over several fractions including the fraction containing the analytes. So, this approach was abandoned and the

raw aqueous acetone extract was applied to the Extrelut-20 cartridge. Most of coextractives are retained on the Extrelut-20 cartridge or pass through the SCX cartridge and are discarded with the dilute acid solution. Most of the remaining coextractives retained by the phenyl-SCX cartridges are washed by the methanol–water (75:25, v/v) fraction. The clean-up with phenyl-SCX is very selective and the HPLC–UV chromatograms did not show any interfering peaks at the retention times of the analytes and I.S. Figs. 1 and 2 show the chromatograms of lemon and lemon spiked with the standard compounds, respectively.

The complete clean-up takes some 2 h. The Ultracarb 5 ODS column eluted with acetonitrile–0.1 *M* ammonium formate buffer (pH 6.8) gives a satisfactory peak shape for MBC, but less satisfactory for TBZ, as can be seen in Fig. 2. A column as that used by Hiemstra et al. [3] seems a better choice and is being tried. The eluent composition program is the one used in our laboratory for screening also other compounds, but for this particular analysis it can be easily shortened, if needed.

Recovery experiments were carried out with strawberries ($n=2$), lettuce ($n=2$), apples ($n=2$), lemons ($n=2$), grape ($n=2$). Results reported in Table 1 show satisfactory values for the tested crops. Although satisfactory results were achieved, a full validation of the method is needed to calculate accuracy and precision.

Quantitation was carried out by the use of internal standard, as the final extract is not in a defined volume. Under the conditions of the method a limit of quantitation of 0.05 mg/kg and 0.1 mg/kg for MBC and TBZ, respectively, can be roughly estimated from the chromatogram reported in Fig. 2.

The determinations were carried out by HPLC–UV at the appropriate wavelength for each compound. As the aim of this work was to develop a clean-up procedure, for the analyses we just used UV detection. Of course, in the analyses of real samples, as it is usual at residue level, the identity of peaks has to be confirmed by a combination of chromatographic and/or spectroscopic techniques. When MBC and TBZ are determined by HPLC, UV–diode array detection, spectrofluorimetric detection and liquid chromatography–mass spectrometry are the techniques of choice.

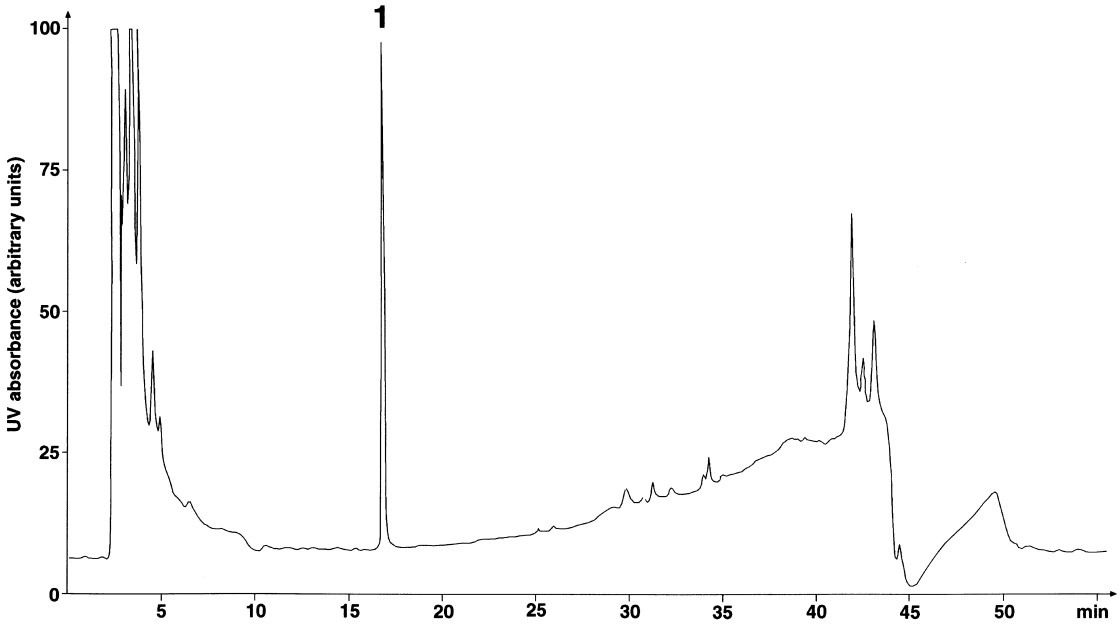


Fig. 1. Chromatogram of unspiked lemon: matrix concentration 5 g/2 ml, 20 μl injected. Peak: 1=retention time 16.78 min, carbofuran 200 ng.

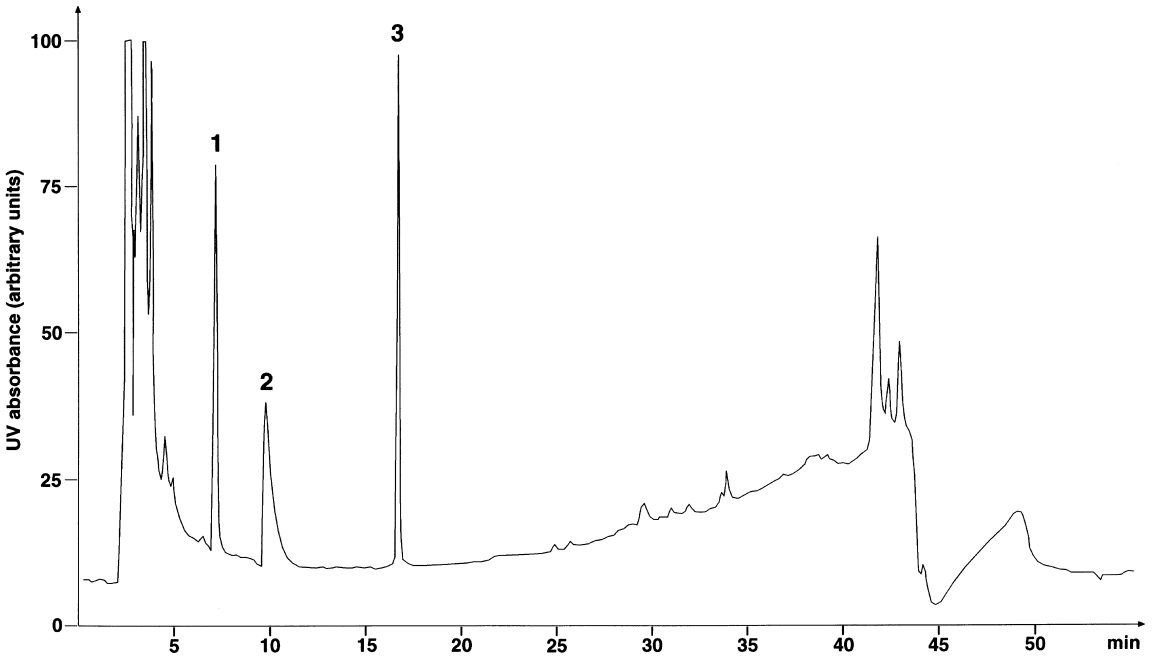


Fig. 2. Chromatogram of lemon spiked with MBC (0.76 mg/kg) and TBZ (0.79 mg/kg): matrix concentration 5 g/2 ml, 20 μl injected. Peaks: 1=retention time 7.19 min, MBC; 2=retention time 9.83 min, TBZ; 3=retention time 16.79 min, carbofuran 200 ng.

Table 1
Recovery values of MBC and TBZ from spiked vegetables analyzed with acetone extraction, Extrelut-20/0.1 *M* $H_3PO_4$ partition and phenyl-SCX clean-up

| Crop | Recovery (%) | |
|---|---|---|
| | Spiking levels (mg/kg) | |
| | MBC=0.76 | TBZ=0.79 |
| Strawberries ($n$=2) | 94, 90 | 80, 72 |
| Lettuce ($n$=2) | 95, 91 | 86, 97 |
| Apples ($n$=2) | 92, 91 | [a] |
| Lemon ($n$=2) | 97, 93 | [a] |
| Grape ($n$=2) | 101, 101 | 84, 82 |

[a] Not analyzed because already present in the sample.

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