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# Application of ion-exchange cartridge clean-up in food analysis I. Simultaneous determination of thiabendazole and imazalil in citrus fruit and banana using high-performance liquid chromatography with ultraviolet detection

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**Abstract**

A simple, rapid and reproducible analytical method for thiabendazole (TBZ) and imazalil (IMA) in citrus fruit and banana has been developed. The method involves the use of an ion-exchange cartridge for sample clean-up followed by ion-pair high-performance liquid chromatography with ultraviolet detection. The recoveries of TBZ and IMA from citrus fruits spiked at levels of 10 μg/g and 5 μg/g were in the range of 94–98% and 93–98% with coefficients of variation of 0.5–2.2% and 1.6–2.7%, respectively. The recoveries of TBZ and IMA from banana spiked at levels of 3 μg/g and 2 μg/g were 94% and 94% with coefficients of variation 1.1% and 4.9%, respectively. The detection limits for TBZ and IMA were 0.1 μg/g in citrus fruit and 0.05 μg/g in banana. 

*Keywords:* Citrus fruit; Bananas; Clean-up methods; Thiabendazole; Imazalil

## 1. Introduction

In order to prevent citrus fruit and banana from deteriorating during storage and transportation, thiabendazole (TBZ), imazalil (IMA), *o*-phenylphenol and diphenyl have been widely used as fungicides. In Japan, these compounds have been approved for use with citrus fruit and banana and their tolerance levels are listed in Table 1 [1]. Among them, the most frequently found fungicides in citrus fruit and banana are TBZ and IMA [2,3]. One of the major roles as public health agencies is to

Table 1
Tolerances of thiabendazole, imazalil, *o*-phenylphenol and diphenyl in food in Japan

| Fungicide | Objective food | Tolerance (μg/g) |
|---|---|---|
| Thiabendazole | Citrus fruit | 10 |
| | Banana | 0.3 |
| | Banana (flesh) | 0.4 |
| Imazalil | Citrus fruit (without mandarin orange) | 5 |
| | Banana | 2 |
| *o*-Phenylphenol | Citrus fruit | 10 |
| Diphenyl | Grapefruit | |
| | Lemon | 70 |
| | A sort of orange | |

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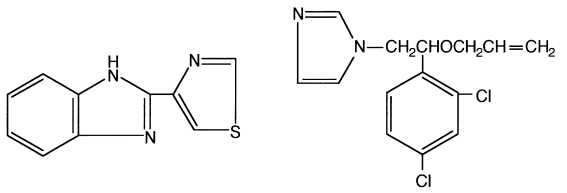


Fig. 1. Chemical structures of thiabendazole and imazalil.

provide safe products for consumers through quantification of these fungicides in citrus fruit and banana. However, these compounds are usually analyzed separately which is inefficient in analysis time and materials.

Several high-performance liquid chromatographic (HPLC) methods have been reported for the simultaneous analysis of TBZ and IMA [4–10]. Nevertheless, most of these methods involve labour-intensive sample clean-up and a combination of ultraviolet (UV) and fluorescence (FL) detectors, and yet they still suffer from low recoveries of TBZ and IMA from samples. Consequently, there is a great need for a simple analytical method which permits the simultaneous determination of TBZ and IMA.

We have reported in our previous studies [11–14], on the applicability of sample clean-up by the ion-exchange cartridge in combination with ion-pair HPLC for the analysis of ionizable compounds. Our objective was to develop an analytical method for the quantitative determination of TBZ and IMA, which are ionizable substances with a chromophore (Fig. 1).

This paper describes a sample clean-up using an ion-exchange cartridge followed by ion-pairing LC determination with UV detection for the simultaneous analysis of TBZ and IMA in citrus fruit and banana.

## 2. Experimental

### 2.1. Chemicals and reagents

Ethyl acetate, methanol, acetonitrile, phosphoric acid, anhydrous disodium hydrogen phosphate ($Na_2HPO_4$), anhydrous sodium sulfate ($Na_2SO_4$), sodium chloride (NaCl) and other chemical reagents were of analytical reagent grade. Sodium 1-tridecane sulfonate, sodium 1-laurylsulfate and sodium 1-dodecanesulfonate were ion-pairing reagents from Tokyo Kasei Kogyo (Tokyo, Japan).

Bond Elut PRS (Lot No. 163407), Bond Elut CBA (Lot No. 151676), Bond Elut SCX (Lot No. 171277), Bond Elut PSA (Lot No. 120598), Bond Elut SAX (Lot No. 183537) and Bond Elut C18 (Lot No. 073234) were purchased from Varian (Harbor City, USA). BAKERBOND SPE Carboxylic Acid (Lot No. 421087) was obtained from J.T. Baker (Phillipsburg, USA). Sep-Pak Vac Silica (Lot No. T6109G1) and Sep-Pak Vac NH2 (Lot No. T5276G1) were purchased from Waters (Milford, USA). All cartridges were of 3 ml capacity packed with 500 mg of solid-phase.

TBZ and IMA were purchased from Sigma (St. Louis, USA) and from Riedel-de Haën (Hannover, Germany), respectively. Separate stock solutions of TBZ and IMA were prepared by dissolving 50 mg in 50 ml of methanol. Subsequent dilutions were made up with the HPLC mobile phase. All the working standards were stored in 10-ml light resistant vials at 5°C and were stable for up to 1 week.

### 2.2. Apparatus

The HPLC system consisted of a LC-10AD pump, a SIL-10AXL auto injector, a SCL-10A system controller, a SPD-10A UV–Vis absorbance detector, and a CR-6A recorder (Shimadzu, Kyoto, Japan).

### 2.3. Chromatographic conditions

The separation was performed on a TSKgel ODS-80Ts column (5 μm, 150×4.6 mm, I.D.; TOSOH, Japan) at ambient temperature. The mobile phase consisted of acetonitrile–methanol–water (4:3:3, v/v) containing 10 m*M* of sodium 1-tridecanesulfonate, adjusted to pH 2.5 with phosphoric acid. The flow-rate of the mobile phase was 1 ml/min and detection was carried out at 225 nm.

### 2.4. Analytical procedure for TBZ and IMA in citrus fruit and banana

#### 2.4.1. Preparation of crude extracts of citrus fruit and banana

Citrus fruit was sliced and homogenized with a mixer. A 5-g aliquot of representative sample was weighed into 250-ml centrifuge tube and blended with 20 g of anhydrous disodium sulfate, 1.5 g of anhydrous disodium hydrophosphate and 30 ml of ethyl acetate using a high-speed blender. After centrifugation (3100 rpm, 8 min), the supernatant was transferred to an 100-ml Erlenmeyer flask. The residual plug was reextracted with another 20 ml of ethyl acetate, combining the supernatants in the Erlenmeyer flask.

Banana was homogenized and extracted as described above using a 10-g sample with 40 g of anhydrous disodium sulfate and 50 ml of ethyl acetate. The banana sample was reextracted with 30 ml of ethyl acetate.

#### 2.4.2. Purification of crude extract by a double cartridge (Sep-Pak Vac Silica/Bond Elut PRS)

A Sep-Pak Vac Silica cartridge was preconditioned with 10 ml of methanol followed by 10 ml of ethyl acetate. Similarly, a Bond Elut PRS cartridge was conditioned with 10 ml of water followed by 10 ml of methanol. A Sep-Pak Vac silica cartridge was mounted on top of the Bond Elut PRS cartridge with an adapter and this double cartridge was placed on top of a vacuum block.

The crude extract collected in the Erlenmeyer flask was passed through the double cartridge at a flow-rate of ca. 1 ml/min. The double cartridge was washed with 5 ml of ethyl acetate and the top Sep-Pak Vac Silica cartridge was removed. The Bond Elut PRS cartridge was washed with 10 ml of methanol followed by 10 ml of 0.1 *M* NaCl aqueous solution, and the retained analytes were eluted with 10 ml of HPLC mobile phase into a 10-ml volumetric flask. A 20-μl aliquot of eluate was injected into the HPLC.

### 2.5. Quantitation

Calibration curves were obtained by plotting the absolute peak heights of analytes versus the concentration of standard solutions, and were linear over the range of 0.01–100 μg/ml (for both TBZ and IMA) with correlation coefficients over 0.999. Quantification of analytes in unknown samples were calculated from the calibration curves and reported in grams of sample weight. Recoveries were calculated as the ratio of the peak heights of the analytes from the fortified samples to the peak heights of standard solutions.

## 3. Results and discussion

### 3.1. Optimization of HPLC conditions

It is difficult to determine TBZ and IMA on the same chromatogram with isocratic HPLC due to the significant difference in their polarities. For this reason, the ion-pair technique was applied in this method.

The most suitable ion-pair reagent and the pH value of the mobile phase were investigated in the following sections for optimization of the HPLC conditions.

#### 3.1.1. Effect of ion-pair reagent

The polarity of TBZ is much higher than that of IMA. In order to increase the retention of TBZ on the HPLC column, the following ion-pair reagents were added to the mobile phase; sodium 1-laurylsulfate, sodium 1-dodecanesulfonate and sodium 1-tridecanesulfonate. Using sodium 1-tridecanesulfonate, TBZ showed strong retention on the HPLC column. Therefore, sodium 1-tridecanesulfonate was chosen as an ion-pair reagent.

In addition, the various concentrations of the ion-pair reagent (sodium 1-tridecanesulfonate) ranging from 0 m*M* to 12 m*M* were added to the mobile phase to obtain the optimal separation of TBZ and IMA. As the concentration of the ion-pair reagent increased, the retention of each TBZ and IMA was improved and the best result was achieved at 6–12 m*M*. However, at 6–8 m*M*, the separation between TBZ and the coexistant substance from the samples was not sufficient and sodium 1-tridecanesulfonate was slightly insoluble at 12 m*M*. Therefore, 10 m*M* was selected for the concentration of the ion-pair reagent.

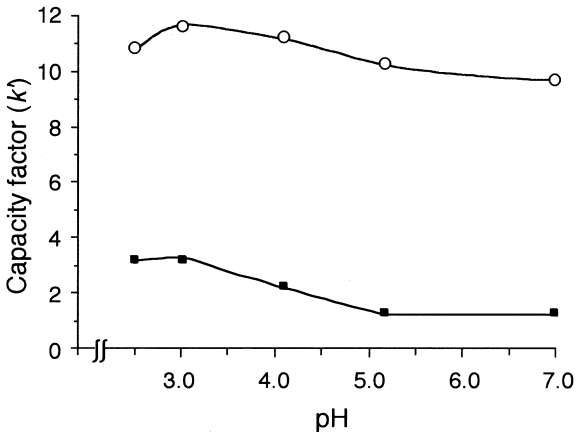


Fig. 2. Influence of the pH of the mobile phase on the capacity factor ($k'$) of TBZ and IMA. Operating conditions: column, TSKgel ODS-80Ts (150×4.6 mm I.D.); mobile phase: acetonitrile–methanol–water (4:3:3, pH 2.5–7.0) containing 10 m*M* sodium 1-tridecanesulfonate; flow-rate: 1.0 ml/min; detector: UV 225 nm. (■) TBZ, (○) IMA.

### *3.1.2. The pH of the mobile phase*

The pH of the mobile phase was varied from 2.5 to 7.0 using phosphoric acid (shown in Fig. 2). The capacity factor of IMA was always between 10 and 12 at a given pH, whereas that of TBZ was less than 2.0 when the pH value was higher than 4.0. A pH of 2.5 provided a good capacity factor ($k'>3.0$) for both TBZ and IMA, hence, the pH value of the mobile phase was adjusted to 2.5.

### *3.2. Purification of crude extracts by double cartridge*

It is desirable to use HPLC mobile phase as an elution solvent from the cartridges for a good reproducibility in HPLC. We investigated the suitability of various ion-exchange cartridges (Bond Elut PRS, Bond Elut CBA, Bond Elut SCX, and BAKERBOND SPE Carboxylic Acid) using the mobile phase as an elution solvent. After 40 ml of ethyl acetate spiked with TBZ and IMA (25 μg each) was passed through the cartridge, the analytes were eluted with 10 ml of HPLC mobile phase from each cartridge. Bond Elut PRS was found to provide the best results; TBA and IMA were completely eluted out. Therefore, Bond Elut PRS was used as a clean-up cartridge in the subsequent studies.

Next, the optimal volume of elution solvent was investigated. After retention of TBZ and IMA (25 μg each) on the PRS cartridge, eluate was collected every 1 ml for determination by HPLC. As shown in Fig. 3, nearly all of TBZ and IMA was recovered with 5 ml of elution solvent (TBZ: 98%, IMA: 100%). However, a trace amount of TBZ (2%) was still recovered in the fractions of 6–9-ml. Therefore, we used 10 ml of an elution solvent from the PRS cartridge.

It was apparent that the PRS cartridge alone gave unsatisfactory results for clean-up of citrus fruit and banana extracts. The PRS cartridge loaded with orange extract was then washed with 10 ml of various solvents (*n*-hexane, methanol, ethyl acetate, acetonitrile, water, and various aqueous salt solutions), and the analytes were eluted with 10 ml of mobile phase. Although most of the interfering peaks at shorter retention time than that of TBZ were eliminated with methanol and aqueous NaCl washing, there were still small amounts of the interfering substances overlapping the peak of TBZ. In search of a more effective sample clean-up method, a combination clean-up consisting of a PRS cartridge and a preclean-up cartridge of different packing material

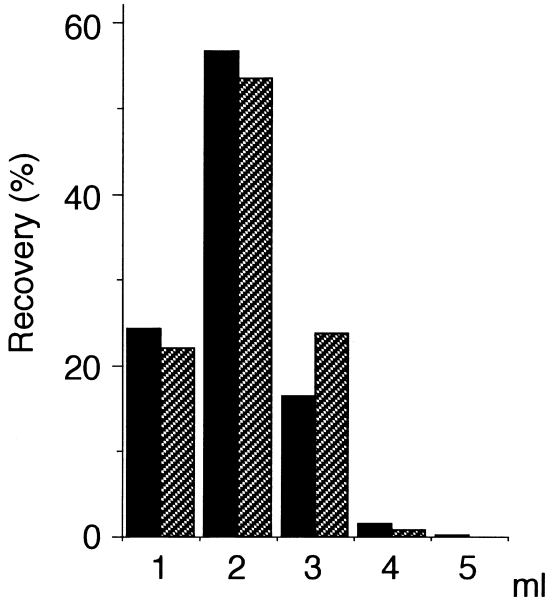


Fig. 3. Elution of TBZ and IMA with the mobile phase from Bond Elut PRS. (black bars) TBZ, (grey bars) IMA.

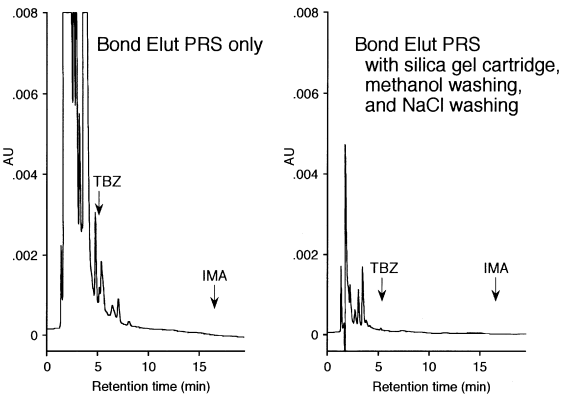


Fig. 4. Effects of silica-gel cartridge, methanol washing and NaCl washing.

(Bond Elut PSA, Bond Elut SAX, Bond Elut C18, Sep-Pak Vac NH2, Sep-Pak Vac Silica) was tested using orange extract. The top preclean-up cartridge was removed and then the bottom PRS cartridge was washed with 10 ml of methanol followed by 10 ml of aqueous NaCl solution. Among the cartridges tested, only Sep-Pak Vac Silica cartridge gave satisfactory results; no interferences at the retention time of TBZ were observed. Chromatograms in Fig. 4 demonstrate the total elimination of interfering substances after using this clean-up method.

NaCl solution is a one of the popular solvents for the mobile phase in ion-exchange chromatography because it is easy to control the ion strength. The influence of the concentration of NaCl on the recoveries of TBZ and IMA from PRS cartridge was examined. The orange extracts spiked to the levels of 10 μg/g and 5 μg/g each of TBZ and IMA were analysed according to the procedure in Section 2.4, except for the concentrations of NaCl solution. The recoveries (results of three replicates) of TBZ and IMA are shown in Fig. 5. As the concentration of NaCl was lowered, the recoveries of TBZ and IMA increased because of decreasing the ion strength, however, some interfering peaks appeared on the chromatogram when 0.05 *M* NaCl was used. The concentration of 0.1 *M* NaCl was selected as a washing solution.

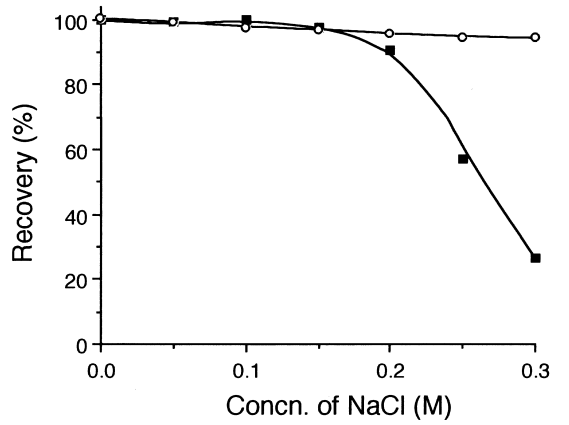


Fig. 5. Influence of the concentration of NaCl washing solution on recoveries of TBZ and IMA. Results of three replicates. For symbols, see Fig. 2.

### *3.3. Recoveries*

Samples were fortified with TBZ (10 and 1 μg/g for citrus fruits and 3 and 0.3 μg/g for banana) and IMA (5 and 0.5 μg/g for citrus fruits and 2 and 0.2 μg/g for banana), and analysed according to the procedure in Section 2.4. The recoveries and corresponding coefficients of variation (C.V.) are listed in Table 2. The average recoveries for TBZ ranged from 92 to 103% with the coefficients of variation (C.V.) ranging from 0.5 to 2.5%. For IMA, the average recoveries ranged from 93 to 100% with the coefficients of variation ranging from 0.8 to 4.9%. Typical chromatograms of the fortified orange extracts are shown in Fig. 6 and of the fortified banana extracts, in Fig. 7. The detection limits for TBZ and IMA were 0.1 μg/g in citrus fruit, and were 0.05 μg/g in banana ($S/N$ ratio=6:1, TBZ; 3:1, IMA).

These results clearly indicated that the reproducibility and accuracy suited the purpose of the proposed method for the simultaneous analysis of TBZ and IMA in citrus fruit and banana.

## 4. Conclusions

A high-performance ion-pair chromatographic method with UV detection for the separation and determination of TBZ and IMA in citrus fruit and banana in a single assay was developed. The results obtained indicate that the combination of an ion-pair HPLC and an ion-exchange cartridge clean-up can provide sufficient sensitivity and the proposed meth-

Table 2
Recoveries of TBZ and IMA from citrus fruits and banana

| Sample | TBZ | | | IMA | | |
|---|---|---|---|---|---|---|
| | Added (μg/g) | Recovery[a] (%) | C.V.[b] (%) | Added (μg/g) | Recovery[a] (%) | C.V.[b] (%) |
| Orange | 10 | 97 | 0.5 | 5 | 98 | 1.7 |
| | 1 | 100 | 1.9 | 0.5 | 95 | 1.7 |
| Grapefruit | 10 | 98 | 1.6 | 5 | 98 | 1.6 |
| | 1 | 98 | 0.8 | 0.5 | 100 | 1.1 |
| Lemon | 10 | 94 | 2.2 | 5 | 93 | 2.7 |
| | 1 | 94 | 2.5 | 0.5 | 93 | 1.0 |
| Banana | 3 | 94 | 1.1 | 2 | 94 | 4.9 |
| | 0.3 | 98 | 1.2 | 0.2 | 95 | 0.8 |

[a] Average of 5 trials.
[b] Coefficient of variation.

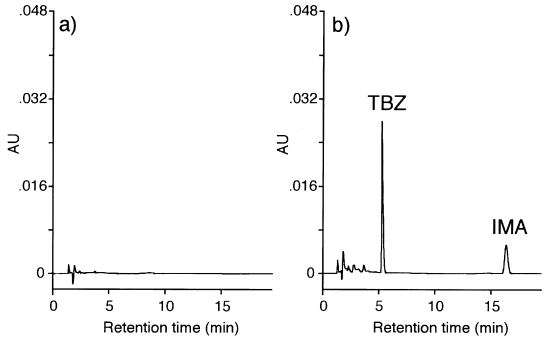


Fig. 6. Typical HPLC chromatograms of orange extracts. (a) Orange (control); (b) added at levels of 10 ppm (TBZ) and 5 ppm (IMA).

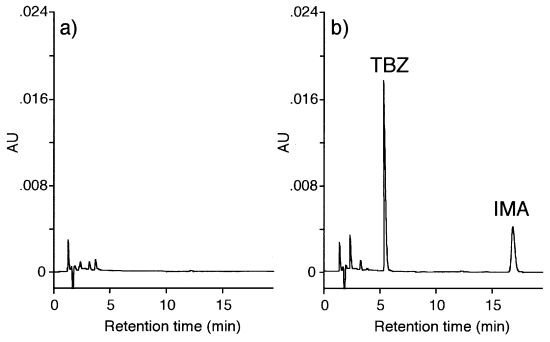


Fig. 7. Typical HPLC chromatograms of banana extracts. (a) Banana (control); (b) added at levels of 3 ppm (TBZ) and 2 ppm (IMA).

od is applicable to the practical analysis of TBZ and IMA in citrus fruit and banana. Therefore, we recommend the present analytical method for the simultaneous determination of TBZ and IMA in citrus fruit and banana.

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