Simple and Rapid Determination of Thiabendazole, Imazalil, and *o*-Phenylphenol in Citrus Fruit Using Flow-Injection Electrospray Ionization Tandem Mass Spectrometry

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A simple and rapid analytical method for thiabendazole (TBZ), imazalil (IMA), and *o*-phenylphenol (OPP) in citrus fruit has been developed by using flow-injection electrospray ionization tandem mass spectrometry for the first time. The method involves the combined use of stable isotopically labeled internal standards (thiabendazole-$^{13}C_6$, imazalil-d_5, and *p*-phenylphenol-d_9) and a multiple reaction monitoring technique. The average recoveries for the fungicides at the tolerance levels (TBZ and OPP, 10 mg/kg; IMA, 5 mg/kg) ranged from 77 to 101%, with the coefficients of variation (CVs) ranging from 0.7 to 4.2% ($n = 5$). At half the tolerance levels (TBZ and OPP, 5 mg/kg; IMA, 2.5 mg/kg), the average recoveries ranged from 62 to 112%, with the CVs ranging from 0.7 to 8.4% ($n = 5$). The CVs of the average recoveries, obtained from lemon samples fortified with three fungicides at the tolerance levels, obtained on three different days over two weeks, ranged within 2%. The analysis time, including sample preparation and determination, is only 15 min.

KEYWORDS: Thiabendazole; imazalil; *o*-phenylphenol; citrus fruit; tandem mass spectrometry

To prevent citrus fruit from deteriorating during storage and transportation, thiabendazole (TBZ), imazalil (IMA), and *o*-phenylphenol (OPP) have been widely used as fungicides (**Figure 1**). In Japan, tolerance levels of 10 mg/kg for TBZ and OPP and 5 mg/kg for IMA in citrus fruit have been established in the same manner as FAO/WHO (*1, 2*). One of the major obligations of public health agencies is to provide safe products for consumers through quantification of these fungicides in citrus fruit. If residues of these fungicides are detected over the tolerance levels from a citrus fruit sample, then all the citrus fruits which have been stored in the same container have to be recalled and discarded to protect consumers. Thus, it is very important for public health agencies not only to determine the residual fungicides around the tolerance levels in citrus fruit but also to obtain the results of determinations rapidly. However, these three fungicides are analyzed separately; IMA is usually analyzed by gas chromatography (GC) and TBZ and OPP are most often analyzed by high-performance liquid chromatography (LC), since they are very polar and nonvolatile and their determination by GC requires long and tedious derivatization procedures. Moreover, most of the analytical methods for these
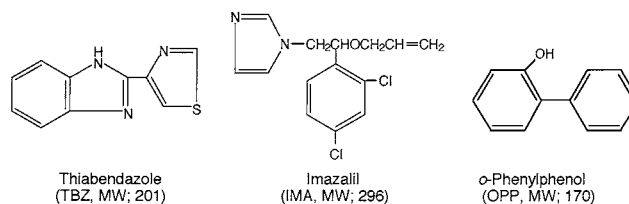

Figure 1. Chemical structures of thiabendazole, imazalil, and *o*-phenylphenol.

fungicides using GC or LC involve labor-intensive sample cleanup and strict settings of chromatographic conditions, because they are developed for accurate determination despite using low-selective and low-specific detectors (*3–8*). Recently, several methods using mass spectrometry connected with GC or LC have been reported (*9–16*). However, these are also not useful as rapid simultaneous determination methods, because all of these methods use chromatographic separation, such as GC or LC, which is applicable only to specific compounds with similar physicochemical properties and requires long separation times, even if an additional technique, such as temperature-programmed or gradient elution, is used. Consequently, there is a high need for a simple screening method which permits

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the rapid simultaneous determination of TBZ, IMA, and OPP around their tolerance levels.

The combination of tandem mass spectrometry (MS/MS) and direct sample injection to a MS/MS system, named flow-injection MS/MS, can be applied for food analysis as well as LC/MS or GC/MS. Because the first MS of MS/MS is used for separation of the target compounds from all ionized compounds according to the differences of mass and the second one is used for detection of the target compounds, the determination of the target compounds in food extracts does not require any separation system such as chromatography. This means that there is no need for long separation times, and it is easy to develop a rapid analysis of compounds having a wide range of physicochemical properties. We judged this method to be well suited for screening analysis; however, there is little precedent for its application to food analysis. Accordingly, we decided to develop a method for rapid simultaneous quantification of TBZ, IMA, and OPP in citrus fruit for screening analysis using flow-injection electrospray ionization tandem mass spectrometry (ESI MS/MS).

This paper describes the first use of flow-injection ESI MS/MS for food analysis.

MATERIALS AND METHODS

Chemicals and Reagents. Ethyl acetate, methanol, and anhydrous disodium sulfate (Na_2SO_4) were of pesticide analysis grade.

Thiabendazol, imazalil, and *o*-phenylphenol were purchased from Sigma (St. Louis, MO), from Riedel-de Haen (Hanover, Germany), and from Wako Pure Chemical Industries (Osaka, Japan), respectively. Thiabendazol-$^{13}C_6$ (chemical purity >99.9%) and imazalil-d_5 (chemical purity >99.9%) were obtained from Hayashi Pure Chemical Industries (Osaka, Japan). *p*-Phenylphenol-d_9 (chemical purity >98.0%) was purchased from C/D/N Isotopes (Quebec, Canada). These chemicals, labeled with stable isotopes, were used as internal standards. Each stock solution of fungicides and their internal standards was prepared by dissolving 50 mg in 50 mL of methanol. Subsequent dilutions were made with ethyl acetate. All the working standard were stored in 10-mL light-resistant vials at 5 °C and were stable for up to 1 week.

Apparatus. The flow-injection ESI MS/MS system consisted of an HP1100 series binary pump and an autosampler (Hewlett Packard, Palo Alto, CA) and a Quattro II triple-quadrupole tandem mass spectrometer (Micromass UK Ltd., Altrincham, UK) equipped with a Z-spray API source. PEEK tubing (100 cm × 0.5 mm i.d.) was used to connect the tandem mass spectrometer with the autosampler. For optimization of the Z-spray probe position, a model 100 syringe pump (KD Scientific Inc., Boston, MA) was connected to the tandem mass spectrometer using fused silica tubing.

Flow-Injection ESI MS/MS Conditions. Full-scan data of product ions were collected from 150 to 500 *m/z* at a rate of 0.17 scans/s to optimize MS/MS conditions, and multiple reaction monitoring (MRM) data were collected at 0.5 s of dwell time and 0.1 s of delay time for each analyte. Electrospray ionization mass spectra recorded for TBZ and IMA gave [M + H]$^+$ at *m/z* 202 and 297 in positive-ion mode, respectively, and the spectrum recorded for OPP gave [M − H]$^-$ at *m/z* 169 in negative-ion mode. When each of these molecular ion species was used as a precursor ion for full-scan ESI tandem mass spectra, TBZ and IMA gave product ion spectra with clear cleavage of the precursor ions; however, OPP gave the product ion spectrum without the cleavage. The following product ions that show the highest intensity were selected as the monitor ions for MRM in order to develop highly sensitive quantification: TBZ, *m/z* 131; IMA, *m/z* 159; and OPP, *m/z* 169. For the purpose of highly sensitive MRM determinations, each solution of three fungicides prepared in ethyl acetate (1 µg/mL) was flow-injected into the ESI MS/MS, and the intensity of each precursor ion and monitor ion was monitored under selected cone voltages (10−60 V) and collision energies (5−60 eV), respectively. These compound-specific cone voltages and collision energies are listed in **Table 1**.

Table 1. Compound-Specific ESI MS/MS Parameters for the Fungicides and the Corresponding Internal Standard

analyte	precursor ion (*m/z*)	monitor ion (*m/z*)	cone voltage (V)	collision energy (eV)	ion mode (ESI)
thiabendazole	202 ([M + H]$^+$)	131	40	40	+
thiabendazole-$^{13}C_6$	208 ([M + H]$^+$)	181	40	40	+
imazalil	297 ([M + H]$^+$)	159	35	25	+
imazalil-d_5	302 ([M + H]$^+$)	159	35	35	+
o-phenylphenol	169 ([M − H]$^-$)	169	35	10	−
p-phenylphenol-d_9	178 ([M − H]$^-$)	178	45	10	−

Collision-induced dissociation was performed using argon as the collision gas at a pressure of 1.9×10^{-3} mbar in the collision cell.

Because the ion source temperature and desolvation temperature could not be changed for each target compound, the optimal temperatures were selected using OPP solution, which showed the weakest intensity among the three fungicides. These temperatures were optimized at 100 and 300 °C, respectively.

The carrier liquid was a mixture of methanol−water (90:10, v/v), and the flow rate was 200 µL/min.

The position of the Z-spray probe was optimized using a 10 µg/mL solution of OPP in a mixture of methanol−water (90:10, v/v) at a flow rate of 200 µL/min by a syringe pump connected to the MS/MS system.

Sample Preparation. Citrus fruit was sliced and homogenized with a mixer. A 5-g aliquot of a representative sample was weighed into a 250-mL centrifuge tube and added 0.5 mL of a mixed internal standard ethyl acetate solution (50 µg/mL of imazalil-d_5 and 100 µg/mL each of thiabendazol-$^{13}C_6$ and *p*-phenylphenol-d_9). The mixture was blended with 20 g of anhydrous Na_2SO_4, 1.5 g of anhydrous disodium hydrophosphate (Na_2HPO_4), and 50 mL of ethyl acetate using a high-speed blender. After centrifugation (3100 rpm, 8 min), a 1-µL aliquot of the supernatant was injected into the flow-injection ESI MS/MS system.

Quantitation. Calibration curves were constructed by using peak area ratios of fungicides to each internal standard. Recoveries were calculated as the ratio of the peak area ratio of the analyte to the internal standard from the fortified samples to the corresponding peak area ratio of standard solutions.

RESULTS AND DISCUSSION

Optimization of Flow-Injection ESI MS/MS Conditions. The performance of the flow-injection MS/MS system was optimized by evaluating the effects of sample injection parameters (carrier liquid compositions, carrier flow rate, injection volume, and size of the connection tube of the MS/MS interface with the injection port) on mass spectral ion intensity. The optimization of these sample injection parameters is discussed below.

Carrier Liquid. The development of a highly sensitive determination using flow-injection ESI MS/MS requires that the carrier liquid can promote ionization of the precursor ion. Methanol, acetonitrile, water, and their mixed solutions are usually used as carrier liquids for ESI MS/MS. The promotion effects of ionization of these solvents increase in the following order: acetonitrile, methanol, water. On the other hand, it is very common that the higher the concentration of an organic solvent used for a carrier liquid is, the lower the surface tension of the sprayed drop becomes, the larger the surface area of the sprayed drop becomes, and the larger the amount of ion becomes that can be detected using electrospray ionization (*17*). Because methanol both promotes ionization and decreases the surface tension of the sprayed drop, we investigated whether methanol can be used as the carrier liquid for determination of TBZ, IMA, and OPP. When each of the three fungicides in ethyl acetate solution was analyzed by flow-injection ESI MS/MS, the monitor ions of TBZ and IMA on each MRM profile showed
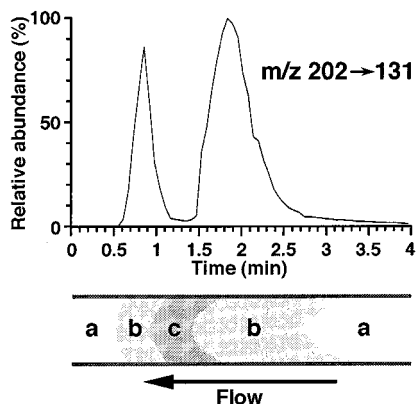
ESI MS/MS Analysis for Fungicides in Citrus Friut

J. Agric. Food Chem., Vol. 51, No. 4, 2003   **863**

Figure 2. MRM profile of thiabendazole under flow-injection ESI MS/MS conditions and corresponding schematic representation of tube inside. Operating conditions: tube size, 100 cm × 0.5 mm i.d.; injection volume, 100 μL; others, see Materials and Methods. (a) Carrier liquid, (b) sample solution and carrier liquid, (c) sample solution.

satisfactory intensity; however; the monitor ion of OPP did not show the same result as those of TBZ and IMA. This means that methanol does not sufficiently promote the ionization of OPP; therefore, we decided to add promoter chemicals to methanol. In electrospray ionization, formic acid and acetic acid in the positive mode and ammonia and triethylamine in the negative mode are commonly used as additives for promoting ionization. But we were not able to use these additives because both positive- and negative-ion modes were chosen for the simultaneous determination of TBZ, IMA, and OPP in this study. Although water increases the surface tension of the sprayed drop, it also can promote ionization more effectively than methanol. So we examined the effects of selected concentrations (10−40%) of water on the intensity of the precursor ions of each of the three fungicides. For TBZ and IMA, only a decrease of the ion intensity was observed in association with the increase in concentration of water in the carrier liquid, because water caused the surface tension of the sprayed drop to increase rather than to promote increased ionization for these two fungicides. In contrast, in the case of OPP, 10−30% of water increased the ion intensity, but an increase over 30% decreased the ion intensity. There was no difference in the intensities using between 10 and 30% of water; the observed intensites were 1.2 times higher than those obtained using only methanol. For the simultaneous determination of the three fungicides using flow-injection ESI MS/MS, it was essential that the intensities of all fungicides were shown sufficiently; hence, we decided to use a mixture of methanol−water (90:10, v/v) as the carrier liquid.

In flow-injection ESI MS/MS, the carrier liquid flow rate has a great influence on the measuring time. To develop the rapid determination method for TBZ, IMA, and OPP, we chose 200 μL/min as the carrier liquid flow rate, which was the maximum flow rate of our MS/MS system.

Sample Injection. When 100 μL of a mixed standard solution of TBZ, IMA, and OPP in ethyl acetate solution (1 μg/mL each) was injected into the flow-injection ESI MS/MS system, two peaks appeared on each MRM profile of the three fungicides; the results for TBZ are shown in **Figure 2**. This phenomenon means that the fungicides in the completely mixed sample solution with carrier liquid (**Figure 2b**) are able to be ionized and the fungicides in only a sample solution (ethyl acetate; **Figure 2c**) are not able or are difficult to be ionized. In a word, the efficient ionization of each of the three fungicides requires that the sample solution mixes with the carrier liquid completely.

Key parameters in the control of the mixing of these solutions are the sample volume, the mixing time, which is the same as the moving time of sample solution from injection port to MS/ MS interface, and the area of contacting surface between sample solution and carrier liquid. Since it was obvious that the small-volume sample solution was easily mixed with the carrier liquid, we decided that the sample volume was 1 μL, which was the minimum volume required for the autosampler used in our system. The mixing time is related to the tube length, and the area of contacting surface between sample solution and carrier liquid is related to the tube inside diameter. Therefore, the effects of tube length (50−to 200 cm) and tube i.d. (0.13−0.75 mm) were investigated by evaluating their effects on ion intensity and peak shape using a mixed standard solution. The short tube or the small i.d. resulted in a significantly short measuring time and high intensity of each monitor ion; however, peak tailing appeared on each MRM profile, and less quantitative accuracy was found because of the deficiencies of data points due to lack of peak width. On the other hand, although the long tube or the large i.d. resulted in a long measuring time and low intensity, it exhibited peak symmetry and kept enough data points for quantitative analysis. To ensure quantitative accuracy, the number of data points for one peak should be over 15. As described in the Materials and Methods, 0.5 s was needed to detect and record the intencity of one selected monitor ion, which is a selected product ion originated from a selected precursor ion, and 0.1 s was the time required between finishing monitoring one mass and starting monitoring the next mass. Because in flow-injection ESI MS/MS, six target compounds, namely TBZ, IMA, and OPP and their corresponding internal standards (described later), were injected simultaneously and measured separately, one data point for one compound was obtained by recording for 0.5 s at 3.1-s intervals; hence it took 3.6 s to obtain one data point for each of the six compounds. To ensure quantitative accuracy, peak width (from peak start to peak end) requires over 54 s. On the basis of the results of these preliminary experiments, we chose a tube 100 cm in length by 0.5 mm in i.d., which gave a peak width of about 78 s and produced satisfactory reproducibility and quantitative data as the screening method for fungicides in citrus fruits.

Selection of Internal Standards. To improve reproducibility and accuracy, it is essential to use an internal standard. In particular, using a stable isotopically labeled internal standard is reasonable for mass spectrometric analysis because there is no difference in the physicochemical behavior from a corresponding compound, except for molecular weight. It can correct for various influences of the sample matrix on suppressing or promoting ionization of the analyte. In this study, there is the possibility of a decrease of the relative contribution of the analyte to the total ion current, because the crude sample extract of citrus fruit without cleanup was directly injected to the MS/ MS interface for development of a rapid analysis (described later). Therefore, we decided to use the three commercially available fungicides labeled with stable isotopes. Thiabendazole-$^{13}C_6$ and imazalil-d_5 were available in powder form on the market, while *o*-phenylphenol-$^{13}C_6$ was in hexane solution. Because the concentration of *o*-phenylphenol-$^{13}C_6$ solution on the market was too weak for it to be used as an internal standard in this study, *p*-phenylphenol-d_9, which was available in powder form on the market, was selected as the internal standard for OPP.

ESI MS/MS conditions (ionization mode, precursor ion, monitor ion, cone voltage, and collision energy; listed in **Table**

1) for each of the three internal standards were optimized in the same manner as described in the Materials and Methods section.

Next, all of the precursor ions (molecular ion species) and isotopic ions of TBZ, IMA, and OPP and their internal standards were compared with each other, because the overlapping of the ions could lead to serious errors in MRM determination using flow-injection ESI MS/MS. Neither the precursor ions of TBZ and OPP nor those of the corresponding internal standards overlapped each other, including their isotopic ions. An isotopic ion of IMA, however, was detected at the same *m/z* as the precursor ion of the internal standard (imazalil-d_5, *m/z* 302, [M+H]$^+$). Therefore, we measured the full-scan ESI tandem mass spectra using the isotopic ion of IMA (*m/z* 302) as a precursor ion and confirmed that the product ion at *m/z* 159, which is the same *m/z* as the monitor ion of imazalil-d_5, was not detected. Accordingly, we judged that imazalil-d_5 was able to be used as the internal standard for the MRM determination of IMA.

Sample Preparation. For the purpose of highly sensitive and accurate determination in the conventional method, two or three extractions and several segmental cleanup steps are needed, but these operations obviously consume time. To develop a rapid screening method for TBZ, IMA, and OPP in citrus fruits, it is necessary that the sample preparation, including the extraction and the cleanup steps, is as simple as possible. Since a stable isotopically labeled internal standard shows the same physico-chemical behavior as a corresponding compound, adding the internal standard immediately after sample weighing enables corrections for sample loss to be made. This means that the time of extraction can be reduced while keeping good recovery using this technique. Moreover, using a stable isotopically labeled internal standard can also correct for various influences of the sample matrix on the ionization of the analyte, and so it became possible to omit the cleanup steps. Accordingly, we elected to use only one extraction after addition of the internal standards and no cleanup steps for the present sample preparation.

Because the crude sample extract of citrus fruit without cleanup, which contained a high concentration of coexisting substances, was directly injected to the MS/MS interface, some influence of the extraction solvent and its volume on the results of quantifications could be expected. According to the conventional method, the extraction solvent was examined using methanol, acetonitrile, acetone, and ethyl acetate. The influence of these solutions caused peak tailing on the MRM profile of each analyte, and their influences increased in the following order: ethyl acetate, acetone, acetonitrile, methanol. Although the stable isotopically internal standard could correct for the influence of the sample matrix on the ionization of the analyte, it could not correct too much for the influence of the high concentration of the sample matrix. Therefore, we extracted a 5-g lemon sample with 50 mL of ethyl acetate and added the three fungicides and their corresponding internal standards around the tolerance level to check the influence of the concentration of the sample matrix. Because satisfactory quantitative results were shown with no ion suppression and no peak tailing, we selected these extraction conditions. To verify the reproducibility and the accuracy of determination of the residual fungicides around the tolerance level, we decided the fortified values of the three internal standards were the same as the tolerance levels of the corresponding fungicides; hence, 0.5 mL of the mixture of the internal standards (50 μg/mL of
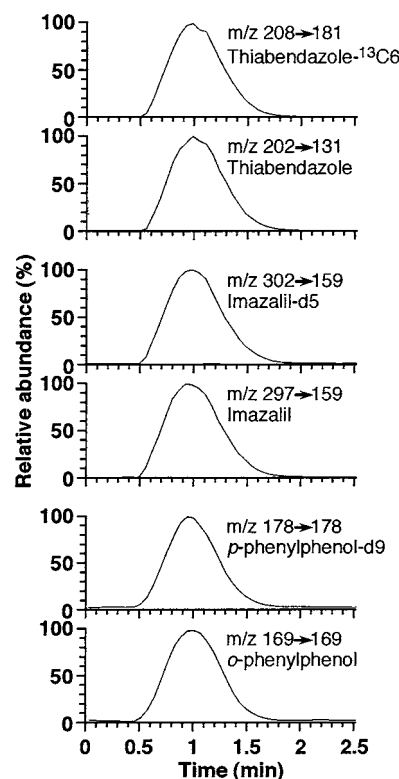

Figure 3. MRM profiles of the standard fungicides under flow-injection ESI MS/MS conditions. The concentrations are 1 μg/mL for TBZ and OPP and 0.5 μg/mL for IMA.

imazalil-d_5 and 100 μg/mL each of thiabendazole-$^{13}C_6$ and *p*-phenylphenol-d_9) was added to a 5-g sample.

Typical MRM profiles of the three standard fungicides and the corresponding internal standards are shown in **Figure 3**. These profiles were obtained under the present flow-injection ESI MS/MS conditions using a mixture of 1 μg/mL each of TBZ and OPP and their internal standards and 0.5 μg/mL of IMA and its internal standard. **Figure 3** indicates that satisfactory intensities of the three fungicides and their internal standards at each tolerance level were observed.

Quantitation. *Calibration Curves.* Calibration curves were linear over the ranges of 0.4−10 μg/mL (for TBZ and OPP) and 0.2−5 μg/mL (for IMA), with correlation coefficients over 0.999. These concentrations were equivarent to 0.4−10 times each tolerance level in citrus fruit under the present flow-injection ESI MS/MS conditions.

Recoveries. Citrus fruit samples were fortified with TBZ and OPP (10 and 5 mg/kg, respectively) and IMA (5 and 2.5 mg/kg) and analyzed according to the sample preparation procedures described in the Materials and Methods. Typical MRM profiles of the fortified lemon samples at half the tolerance levels (TBZ and OPP; 5 mg/kg; IMA, 2.5 mg/kg) are shown in **Figure 4**. The recoveries and corresponding coefficients of variation (CVs) are listed in **Table 2**. The average recoveries for TBZ ranged from 101 to 103%, with the CVs ranging from 0.7 to 1.7%. For IMA and OPP, the average recoveries ranged from 99 to 112% and from 62 to 101%, with the CVs ranging from 1.0 to 1.9% and from 0.7 to 8.4%, respectively. For TBZ and IMA, the recoveries and the CVs were not influenced by the kind of samples. In the case of OPP, however, the recoveries were lower in the following order: lemon, orange, grapefruit. Although it seemed that there was an effect of ion suppression by the sample matrix, which was too much to be corrected for by the internal
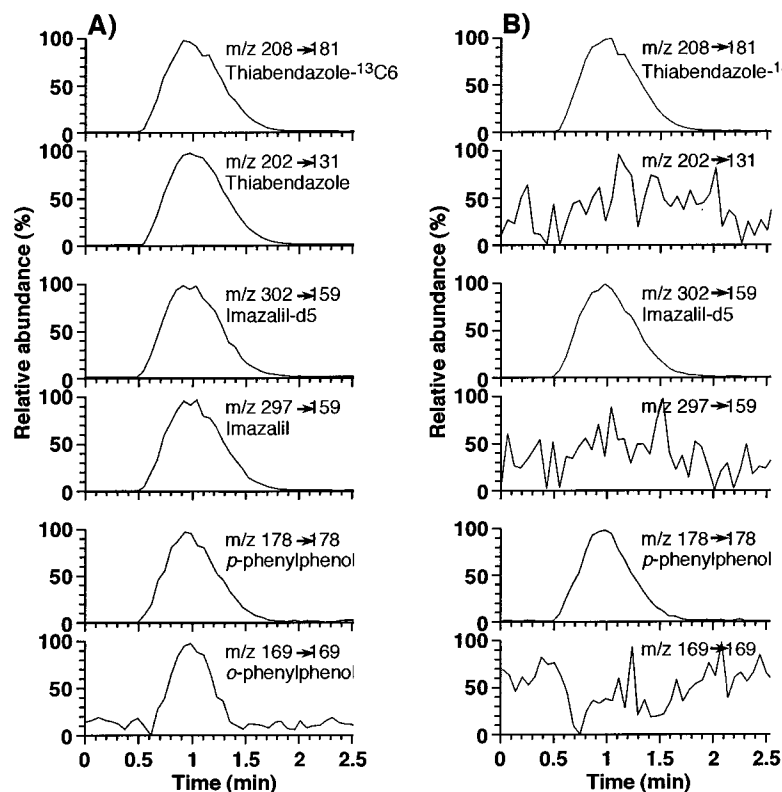
ESI MS/MS Analysis for Fungicides in Citrus Friut

J. Agric. Food Chem., Vol. 51, No. 4, 2003 **865**

Figure 4. Typical MRM profiles of lemon samples under flow-injection ESI MS/MS conditions. (A) Fortified at 5 mg/kg of TBZ and OPP and 2.5 mg/kg of IMA; (B) lemon (control).

Table 2. Recoveries of Thiabendazole, Imazalil, and *o*-Phenylphenol from Citrus Fruits

	thiabendazole			imazalil			*o*-phenylphenol		
sample	added (mg/kg)	recovery[a] (%)	CV[b] (%)	added (mg/kg)	recovery[a] (%)	CV[b] (%)	added (mg/kg)	recovery[a] (%)	CV[b] (%)
lemon	10	101.4	1.71	5	100.7	0.96	10	100.6	0.73
	5	103.0	1.65	2.5	99.1	1.28	5	95.8	8.36
orange	10	100.6	0.69	5	100.4	1.78	10	96.0	1.71
	5	100.8	0.73	2.5	112.4	1.24	5	70.3	3.39
grapefruit	10	100.5	1.22	5	100.4	1.45	10	77.2	4.20
	5	103.2	1.28	2.5	101.0	1.92	5	61.9	3.64

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

standard, we can still recommend the lowest recovery of OPP (grapefruit) as a satisfactory result for a screening method.

Quantification Limits. On the basis of the results of the above recovery tests and the MRM profiles of the fortified samples at half the tolerance levels, the limits of quantification have been estimated to be 1 mg/kg for TBZ and IMA and 5 mg/kg for OPP in citrus fruit (S/N ratio >10).

Method Reliability and Analyte Stability. The recovery tests of lemon samples fortified with three fungicides at the tolerance levels were carried out on three different days over two weeks in the same manner as descrived above. The CVs of the three average recoveries ranged within 2%.

For the purpose of the confirmation of analyte stability, we analyzed the same sample solutions stored overnight at 5 °C which were made from fortified lemon samples descrived above in the Recoveries section. In comparison with the results of quantifications, no significant differences were observed between two days. However, we have no concern about storing the sample solution awaiting analysis, because the analysis time of flow-injection MS/MS for one sample in this study is only 5 min, and the most important goal in the development of this method is rapid screening analysis.

Analysis of Commercial Samples. To investigate the availability of this method, citrus fruit samples on the market (5 lemons and 10 each of oranges and grapefruits) were analyzed according to the present method. TBZ and IMA were detected from 10 samples and 11 samples, respectively. The highest levels of fungicides found in citrus fruits in this study were 4.3 mg/kg for TBZ and 2.9 mg/kg for IMA. These levels are significantly lower than the tolerance levels established by FAO/ WHO and Japan for these compounds in citrus fruits. OPP was also detected from two samples; however, in these samples it appeared as trace peaks at levels below the limit of quantification. Analysis of three fungicides was finished simultaneously less than 15 min from cutting the citrus fruit sample, which is much shorter than the conventional analytical method.

Conclusions. A flow-injection method with electrospray ionization tandem mass spectrometry for the simultaneous quantitative determination of TBZ, IMA, and OPP in citrus fruit was developed with the following characteristics: (1) The high selectivity of tandem mass spectrometry makes it possible to establish a simple and rapid determination method without chromatographic separation. (2) The internal standards labeled with a stable isotope are effective in correcting for the influence

866 *J. Agric. Food Chem.,* Vol. 51, No. 4, 2003

Ito et al.

of directly injected sample extract without cleanup on the ionizations of analytes and the sample loss of only one extraction. (3) The reproducibility and the accuracy of quantification of fungicides in citrus fruit around the tolerance levels established by FAO/WHO and Japan were verified by adding the internal standard at the tolerance levels. (4) The analysis time required, that is, from cutting the citrus fruit to getting the determination report, is less than 15 min.

Because of these characteristics, we strongly recommend the simultaneous quantification method presented in this paper for the screening analysis of TBZ, IMA, and OPP in citrus fruit.

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