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Short communication

Rapid determination of five post-harvest fungicides and metabolite in citrus fruits by liquid chromatography/time-of-flight mass spectrometry with atmospheric pressure photoionization

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ABSTRACT

Liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS) with atmospheric pressure photoionization (APPI) has been used for the determination of five post-harvest fungicides and metabolite, *o*-phenylphenol (OPP), diphenyl (DP), thiabendazole (TBZ), imazalil (IMZ), and its major metabolite R14821 (IMZ-M) in citrus fruits. Samples were extracted with diethyl ether and the extracts with minimal sample preparation were analyzed by LC/TOF-MS with APPI by measuring accurate mass. DP, IMZ, and IMZ-M were ionized in positive ion mode, and OPP and TBZ were in negative ion mode. The recoveries fortified to citrus fruits at 1 μg/g ranged from 70.5% to 101.4%. The detection limits (S/N = 3) were 0.002 μg/g (OPP, TBZ, and IMZ-M), 0.001 μg/g (IMZ), and 0.03 μg/g (DP).

1. Introduction

o-Phenylphenol (OPP), diphenyl (DP), thiabendazole (TBZ), and imazalil (IMZ) (Fig. 1) are commonly used for imported citrus fruits as a post-harvest treatment to inhibit the growth of mould during storage or long distance transport. The residues of them are frequently detected in imported citrus fruits. Detection rate of these fungicides in imported citrus fruits is over 70% in our survey (Akiyama, Yoshioka, & Ichihashi, 2005). Japanese maximum residue limits (MRLs) for these fungicides are based on CODEX standards. But for some fungicides, the MRLs of each country are different from CODEX standards. For instance, the MRL for OPP on citrus fruits in European Union (EU) is 12 μg/g (CODEX standard: 10 μg/g), the MRL for TBZ in EU is 5 μg/g (CODEX standard: 10 μg/g), and the MRL for IMZ in the United States is 10 μg/g (CODEX standard: 5 μg/g) (Ritenour, 2008). Thus, monitoring fungicide residue in citrus fruits has become important to ensure food safety.

A large number of methods have been reported for the determination of post-harvest fungicides: high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (Ito, Ikai, Oka, Hayakawa, & Kagami, 1998), gas chromatography (GC) (Matsumoto, 2001), HPLC and GC (Yamazaki & Ninomiya, 1999), liquid chromatography/mass spectrometry (LC/MS) (Fernández, Rodríguez, Picó, & Mañes, 2001), flow-injection tandem mass spectrometry (Ito et al., 2003), HPLC with fluorescence detection (Saad et al., 2004), and liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS) with electrospray ionization (ESI) (Thurman et al., 2005). However, there have been no published reports describing a simultaneous analytical method of these four fungicides.

Among these fungicides, DP is not ionized by ESI and atmospheric pressure chemical ionization (APCI) because of its low polarity. Atmospheric pressure photoionization (APPI) is suitable for the ionization of low or non-polar compounds such as pyrenes (Straube, Dekant, & Völkel, 2004), lipids (Muñoz-Garcia, Ro, Brown, & Williams, 2006), and pharmaceutical drugs (Cai, Hanold, & Syage, 2007).

In the previous published paper (Yoshioka, Akiyama, & Teranishi, 2004), we have reported the determination of these fungicides including DP and the major metabolite of IMZ, R14821 [1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)-1-ethanol] (IMZ-M) in citrus fruits by single quadrupole LC/MS with APPI. LC/TOF-MS gives accurate masses of ions, which provide molecular weight and elemental composition with high sensitivity. A number of analytical procedures by LC/TOF-MS have been reported in the literature: Pharmaceutical drugs (Zhang, Fountain, Bi, & Rossi, 2000; Zhang, Heinig, & Henion, 2000), lipids (Adachi et al., 2004), mycotoxins (Tanaka, Takino, Sugita-Konishi, & Tanaka, 2006), pesticides (Ferrer & Thurman, 2007; Sasaki, Yonekubo,

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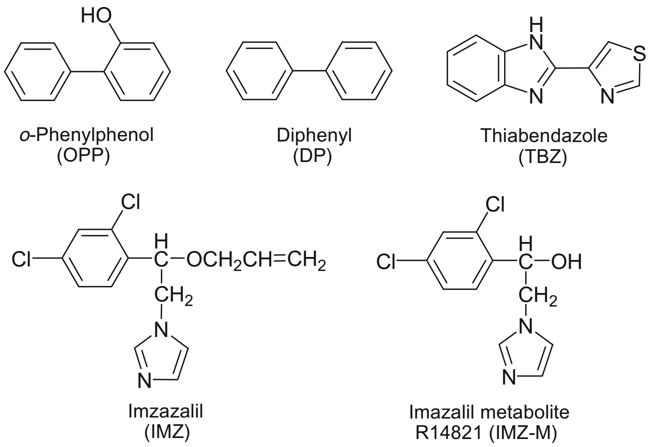

Fig. 1. Chemical structures of the fungicides.

& Hayakawa, 2006), antibiotics (Wang & Leung, 2007), and steroids (Blokland et al., 2008).

Recently, APPI-TOF-MS (flow-injection) methods (Banoub, Benjelloun-Mlayah, Ziarelli, Joly, & Delmas, 2007; Gómez-Ariza, Arias-Borrego, & García-Barrera, 2006; Song, Wellman, Yao, & Adcock, 2007) were reported, however, there have been very few reports of LC/TOF-MS combined with APPI. Ikekita et al. determined glucocorticosteroids in biological samples by APPI-LC/TOF-MS (Ikekita, Okano, & Ueki, 2005).

In this work, we applied LC/TOF-MS combined with APPI to fungicide analysis. This paper describes a rapid and simultaneous determination of OPP, DP, TBZ, IMZ, and IMZ-M in citrus fruits by LC–APPI–TOF-MS.

2. Materials and methods

2.1. Chemicals and reagents

OPP, DP, TBZ were purchased from Wako Pure Chemical (Osaka, Japan). IMZ and IMZ-M, 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)-1-ethanol were obtained from Kanto Chemical (Tokyo, Japan). All solvents used were of HPLC grade, and sodium chloride and sodium hydroxide were of reagent grade (Wako Pure Chemical). Water was purified by a Milli-Q Water Purification System (Millipore, Billerica, MA, USA). Stock solutions were prepared as 250 μg/mL solutions in methanol and stored in a refrigerator at 4 °C.

2.2. Liquid chromatography

HPLC analysis was carried out with an Agilent (Santa Clara, CA, USA) 1200 HPLC system equipped with an autosampler and a binary pump. HPLC separation was performed at 40 °C on an Inertsil ODS-3 (150 mm × 3 mm I.D., 5 μm, GL Sciences, Tokyo, Japan) analytical column. The solvent used in the mobile phase was methanol and water at the flow rate of 0.5 mL/min. In gradient-elution analysis, the first mobile phase was 60% methanol, increased linearly to 100% in 10 min, and held at 100% for 2 min. A return to the initial conditions was carried out in 5 min. The total cycle time was 17 min.

2.3. Mass spectrometry

Accurate mass measurements were performed with an Agilent 6210 time-of-flight MS equipped with an APPI interface. The drying gas and vaporizer temperature were set at 300 °C and 350 °C,

Table 1
Optimized MS parameters.

Analyte	Time (min)									
	3.0–4.2		4.2–6.0		6.0–7.5		7.5–9.5		9.5–12.0	
	TBZ		IMZ-M		OPP		IMZ		DP	
	Target	Qualifier	Target	Qualifier	Target	Qualifier	Target	Qualifier	Target	Qualifier
Ion mode	Negative		Positive		Negative		Positive		Positive	
Drying gas (L/min)	6.0		3.5		6.0		3.5		10.0	
Capillary voltage (V)	1500		1500		1500		1500		1800	
Elemental composition	$C_{10}H_6N_3S$	$C_9H_5N_2S$	$C_{11}H_{11}N_2OCl_2$	$C_8H_7OCl_2$	$C_{12}H_9O$	$C_{11}H_9$	$C_{14}H_{15}N_2OCl_2$	$C_7H_5Cl_2$	$C_{12}H_{10}$	$C_{12}H_9$
Calculated mass	200.0282	173.0173	257.0248	188.9873	169.0653	141.0704	297.0561	158.9768	154.0782	153.0704
Extracted ion range for quantitation	200.02–200.04	173.01–173.03	257.015–257.035	188.98–189.00	169.055–169.075	141.06–141.08	297.045–297.065	158.97–158.99	154.07–154.09	153.06–153.08
Fragmentor voltage (V)	160	240	190	240	140	250	180	270	180	250