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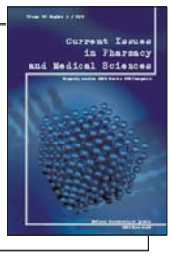

Determination of imazalil and thiabendazole by UPLC- Q-TOF-MS – an analysis of a grapefruit extract-containing dietary supplement

PAWEŁ SZPOT*, KRZYSZTOF BAŃKA, GRZEGORZ BUSZEWICZ, ROMAN MĄDRO

* Chair and Department of Forensic Medicine, Medical University of Lublin, Poland

ABSTRACT

The report presents a method for determining imazalil and thiabendazole in a liquid grapefruit extract-containing dietary supplement using the ultra performance liquid chromatography coupled to quadrupole-time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS) system. The employment of hybrid quadrupole coupled with time-of-flight analyzer characterized by a very high resolution allows for identifying the investigated compounds and for employing the developed technique in routine quality control of natural extracts-containing foodstuffs. The limit of detection for imazalil was 0.25 ng/ml, while for thiabendazole its value was 2 ng/ml. The recovery of the investigated fungicides was within the range of 84-109 %. In the studied dietary supplement, the investigators demonstrated trace amounts of imazalil and thiabendazole, what may be explained by biocide diffusion in to the flesh of fruits employed in manufacturing products of this type.

Keywords: fungicides, imazalil, thiabendazole, Q-TOF, dietary supplement

INTRODUCTION

Various types of biocides are widely used in agriculture and post-harvest protection of food. They are one of the most frequently detected contaminants in the environmental samples such as natural water and effluent of a wastewater-treatment plant [1,3].

Dietary supplements are foodstuffs intended to supplement normal diet. They are subject to the same control procedures as other products designed to be eaten. Manufacturers of food should adhere to standards of maximum levels of residual pesticides, especially when such fungicide-treated intermediate products as fruits or vegetables are employed in the manufacturing process. In post-harvest protection of citruses against fungi development, imazalil and thiabendazole (Fig. 1) are most commonly used [2,12,13]. Maximum Residue Levels (MRLs) of tested fungicides in citrus fruits are 5 mg/kg (Commission Directive 2005/74/EC and 2007/73/EC). According to our knowledge, there are no rules governing MRLs of imazalil and thiabendazole in dietary supplements.

Corresponding author
* Chair and Department of Forensic Medicine,
Medical University of Lublin,
8 Jaczewskiego Str., 20-090 Lublin, Poland
e-mail: pawel.szpot@wp.pl

The compounds have been already determined in fruits and vegetables [2,5,7,11-14], but according to data from the literature on the subject, no such determinations have been done in a grapefruit extract containing dietary supplement. Ortelli et al. showed that 13% of studied citrus fruits contained pesticides in concentrations exceeding the MRLs [5]. All of the methods referenced in this work are based on liquid chromatography tandem mass spectrometry. The best results were obtained when using liquid chromatography coupled to quadrupole-time-of-flight tandem mass spectrometry due to the high sensitivity and the possibility of metabolites identification and products of degradation of post-harvest fungicides [7,11]. In consequence of a steadily increasing use of components of plant origin in dietary and food supplements, there is a need for developing new methods that would allow for identifying not only active substances in a supplement, but also fungicide contamination of such products [9].

The objective of the study was to develop and validate a method for determining trace amounts of imazalil and thiabendazole employing ultra performance liquid chromatography coupled to quadrupole-time-of-flight tandem mass spectrometry and to assess whether there were fungicides employed as antimycotic fruit protection in

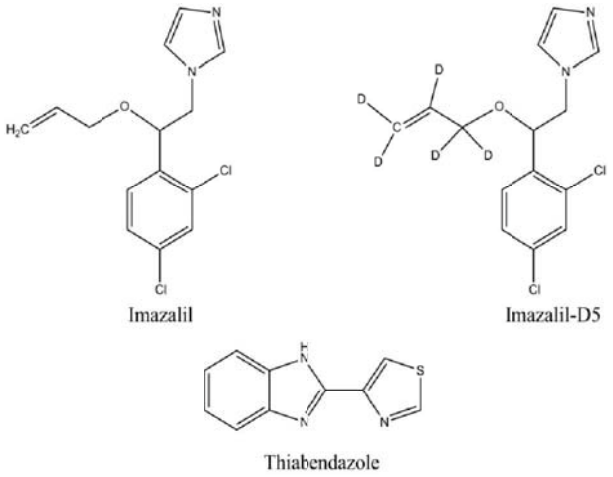

Fig. 1. Structure of imazalil, imazalil-D5 (ISTD) and thiabendazole

dietary supplement derived from seeds, pulp and white membranes of grapefruit.

MATERIAL AND METHODS

Reagents and standards. Water, acetonitrile, methanol, acetone (Chromasolv® LC-MS, Fluka, Germany), imazalil, thiabendazole, diisopropyl ether, ammonium chloride, formic acid (Fluka, Germany), imazalil-D5 employed as an internal standard (Dr. Ehrenstorfer GmbH, Augsburg, Germany). Standard solutions of imazalil and thiabendazole were prepared in methanol, while the solution of imazalil-D5 were prepared in acetone.

Equipment. Chromatographic analysis was performed using an ultra performance liquid chromatograph (UPLC 1290, Agilent Technologies, USA). The separation was done employing a Poroshell 120 EC-C18 column 3.0 x 100 mm; 2.7 µm (Agilent Technologies, USA) with a thermostat at 40°C. Experimentally selected chromatographic separation parameters are presented in Table 1 (Tab.1).

Detection of the investigated compounds was achieved using hybrid quadrupole coupled with time-of-flight analyzer (Q-TOF-MS 6540, Agilent Technologies, USA). The spectrometer was equipped with an ESI Jet Stream source; identification and determination of the investigated fungicides were carried out in the SCAN mode within the range of 100–1000 m/z. Operating spectrometer parameters are presented in Table 1 (Tab. 1).

Study material. The analysis was performed using a widely used commercially available liquid dietary supplement that contained grapefruit extract.

Sample preparation. An amount of 200 µl of the unfiltered studied supplement was transferred to 12 ml test tubes, subsequently adding 200 µl of buffer (ammonium chloride – pH 3) and internal standard (imazalil D5) in the volume allowing for achieving final sample concentration of 5 ng/ml. Liquid-liquid extraction with diisopropyl ether was carried out for 20 min. The samples were centrifuged at 5500 rpm and the organic phase was transferred to 2 ml Eppendorf tubes and evaporated to dryness under a stream of nitrogen (at 45°C). The extract was dissolved in 50 µl of 1:1 acetonitrile/water mixture and analyzed by UPLC-Q-TOF-MS.

Table 1. Chromatographic conditions and spectrometer operating parameters

Mobile phase composition	A – 0.1% formic acid in water B – 0.1% formic acid in acetonitrile			
Gradient	Time (min)	% B	Flow (ml/min)	
	0	5	0,4	
	5	70	0,4	
	7	100	0,4	
Injection volume	10 µl			
Spectrometer operating mode	4 GHz High Resolution			
Reference masses [m/z]	121.0509 922.0098			
Voltages [V]	VCap – 4000 Skimmer – 45			
Ionization	ESI Jet Stream (positive ions)			
Source	Gas temp. – 300°C Drying gas – 10 l/min. Nebulizer – 35 psig (N_2) Sheath gas temp. – 400°C Sheath gas flow – 12 l/min.			
Acquisition rate/time	Rate – 5 spectra/s Time – 200 ms/spectrum			
Mass range [m/z]	100 – 1000			
Studies substances	Thiabendazole		Imazalil	
Retention time [min]	2.90		4.81	
Data acquisition	Quantitative ion $[M+H]^+$	Qualitative ion $[M\text{-}HCN]^+$	Quantitative ion $[M+H:^{35}Cl]^+$	Qualitative ion $[M+H:^{37}Cl]^+$
m/z	202.0435	175.0323	297.0556	299.0527
Fragmentor voltage [V]	220		100	

DISCUSSION OF THE RESULTS

The investigated supplement with grapefruit extract was found to contain trace amounts of both fungicides employed in antimycotic protection of citrus fruits. The concentration of imazalil in the supplement was 4.26±0.12 ng/ml (n=3), while the value for thiabendazole amounted to 15.66±0.47 ng/ml (n=3). Thus, the results may support the notion that the investigated fungicides may diffuse through the peel into the fruit flesh. Kruve et al. [2] demonstrated that trace amounts of imazalil penetrated the fruit flesh and even thorough washing of fruits could not prevent the process.

The employed high resolution mode (4GHz) of the quadrupole time-of-flight mass spectrometer (Q-TOF) allowed for reducing a negative effect of biological matrix, which was also described by other authors [5,10,12].

The effective linear concentration range of the method is from 0.25 to 25 ng/ml for imazalil and 2 to 50ng/ml for thiabendazole. Correlation coefficients (R^2) were > 0.999. In this paper for the limit of detection (LOD) was used lowest calibration level (LCL). LOD can not be determined based on the signal-to-noise ratio, because of the lack of chemical noise in the chromatogram. This problem was already described [6,8]. The precision was expressed as relative standard deviation (%RSD, n=3). The recovery of fungicides was determined using the ratio of the ana-

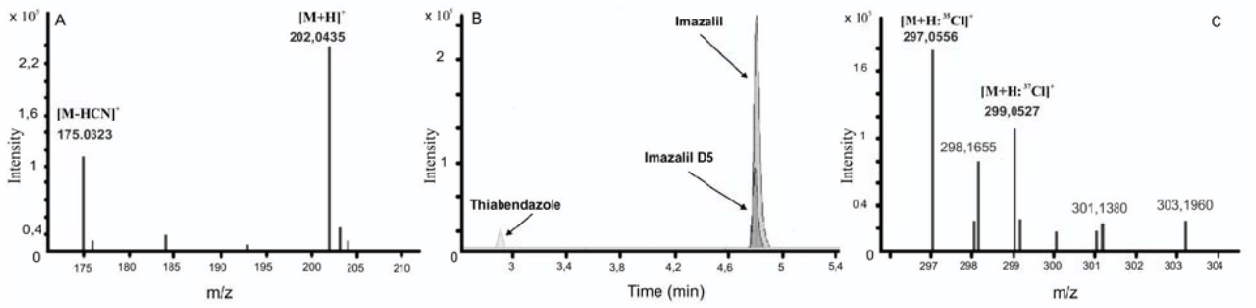

Fig. 2. Mass spectra of thiabendazole (A), imazalil (C) and chromatogram illustrating analysis of dietary supplement (B)

lytical signal from 3 extracts of 5 ng/ml solutions of imazalil and thiabendazole to the signal from the non-extracted methanol standards of equal concentrations. The results of method validation has proven that the employed technique of determining imazalil and thiabendazole is characterized by a high extraction efficiency and is comparable to recovery achieved while isolating the substances from citrus juice and fruits [4,14]. Moreover, the very high sensitivity of the developed method allows for identifying and determining imazalil and thiabendazole even at trace concentrations (Tab. 2).

Table 2. Validation parameters

	Imazalil	Thiabendazole
Recovery [%]	109	84
Correlation coefficient (linearity range)	0.9998 (0.25–25 ng/ml)	0.9993 (2–50 ng/ml)
Precision [% RSD]	0.79	5.29
Limit of detection	0.25 ng/ml	2 ng/ml
Limit of quantification (2xLOD)	0.5 ng/ml	4 ng/ml

Additionally, the employment of a modern UPLC-Q-TOF-MS system and an ESI Jet Stream-type ion source allows for observing isotope ions of the studied fungicides in the mass spectrum (Fig. 2); the said ions are helpful in compound identification and may also be successfully employed as confirmative ions [11].

CONCLUSIONS

Employing ultra performance liquid chromatography coupled to quadrupole-time-of-flight tandem mass spectrometry allows for determining imazalil and thiabendazole in dietary supplements with a high sensitivity, specificity and detection linearity; it also makes it possible to use the developed technique in routine quality inspection of foodstuffs containing natural extracts. The investigated dietary supplement contains trace amounts of imazalil and thiabendazole, what may be an effect of biocide diffusion into the flesh of fruits employed in manufacturing such products.

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