



Determination of trace amounts of imazalil and thiabendazole in water-pipe “shisha” tobacco by UPLC-Q-TOF-MS

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ABSTRACT

The paper presents a method for determination of trace amounts of imazalil and thiabendazole in water-pipe tobacco. Fungicides were isolated with diisopropyl ether and analyzed by ultra-performance liquid chromatograph coupled with quadrupole – time of flight mass spectrometer (UPLC-Q-TOF-MS). The method for determination of imazalil and thiabendazole is highly sensitive. The lowest calibrated levels (LCL) were 0.2 ng/g and 10 ng/g respectively. Calibration curve was characterized by a very good linearity in the range for 0.2 – 100 ng/g for imazalil and 10 – 100 ng/g for thiabendazole (determination coefficient was 0.999 in both cases). The precisions (R.S.D.%) ranged from 1.56 % (imazalil) to 1.53 % (thiabendazole). Mean recovery was 129.44 % for imazalil and 84.73 % for thiabendazole. Usage of hybrid quadrupole coupled with time-of-flight analyzer characterized by a very high resolution allows to identify the investigated compounds and to apply the developed technique in routine quality control of fungicides in plant matrix.

Keywords: tobacco, water-pipe, imazalil, thiabendazole, UPLC-Q-TOF-MS

INTRODUCTION

Imazalil and thiabendazole (Fig. 1.) are post-harvest fungicides used in protection against the growth of fungi [18,19]. Trace amounts of their quantities were detected in dietary supplement [16]. They are commonly used in protection of the environment. These fungicides have been determined in such samples as natural water and effluent of a wastewater-treatment plant [6,7]. Imazalil and thiabendazole have also been determined in fruits and vegetables [12,17-20] but not in tobacco samples. Biocides, present in the tobacco were analyzed by various methods such as capillary gas chromatography [1], gas chromatography–tandem mass spectrometry (GC-MS) [5], gas chromatography–triple quadrupole mass spectrometry (GC-QQQ-MS) [8], two-dimensional gas chromatography (GC × GC) with fast acquisition time-of-flight (TOF) mass spectrometry (MS) [3], liquid chromatography–tandem mass spectrometry (LC-MS) [10] but not in liquid chromatography–tandem quadrupole–time-of-flight mass spectrometry (LC-Q-TOF-MS) to our knowledge. The CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) has published a list of

Guidance Residue Levels (GRLs) for 118 pesticides for tobacco and tobacco products [4]. Imazalil and thiabendazole are not present on the list. Fungicides are used in production of tobacco preservatives, therefore it is important to develop a method of determination the level of residues of these substances in products derived from this plant. In recent years, it has been observed that interest in water-pipe “shisha” smoking is growing [9]. Studies of water-pipe tobacco are based mainly on the analysis of smoke. [14,15]. Tobacco used in water-pipes is a particularly difficult to determine matrix, because it includes apart from plant material also large amounts of glycerol and flavorings. Therefore, it is important to develop sensitive method for determining fungicides which will be resistant to the negative matrix effect.

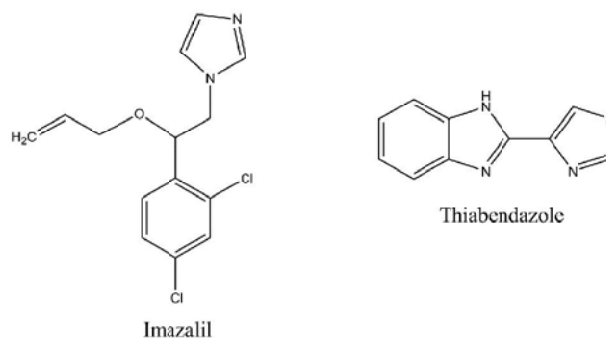


Fig. 1. Chemical structures of imazalil and thiabendazole

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This paper describes a method for determining trace amounts of imazalil and thiabendazole employing ultra-performance liquid chromatography coupled to quadrupole-time-of-flight tandem mass spectrometry in water-pipe tobacco.

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), glycerol (POCH, Poland), imazalil-D5 employed as an internal standard (Dr. Ehrenstorfer GmbH, Augsburg, Germany).

Equipment. Extracts were filtered by 0.45 µm PTFE Mini-UniPrep™ filter (Agilent Technologies, UK). 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. A mixture of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was used as a mobile phase. The gradient elution was carried out at constant flow 0.4 ml/min from 30%A (70%B) 0-5 min., 0%A (100%B) 5-7 min. and then 100%A (0%B) 7-8.5 min. The injection volume was 10 µl. Detection of fungicides was achieved using hybrid quadrupole coupled with time-of-flight analyzer (Q-TOF-MS 6540, Agilent Technologies, USA) with electrospray (ESI Jet Stream) source. Qualitative and quantitative analysis were carried out in the Extended Dynamic Range (2 GHz) MS mode within the range of 100 – 1000 m/z. Other operating spectrometer parameters are presented in our another work [16].

Blank material. Blank material was tobacco from cigarette suspended in glycerol at concentration of 100 mg/ml.

Stock solutions, calibration standards. Standard solutions of imazalil and thiabendazole were prepared in methanol, while the solution of imazalil-D5 was prepared in acetone. All standard solutions were stored at –20°C. Standard curves were prepared in blank material to yield final concentrations of 0.2, 0.5, 1, 2.5, 5, 10, 20, 50, 100 ng/g for imazalil and 10, 20, 50, 100 ng/g for thiabendazole.

Sample preparation: 2g of sample were transferred to 12 ml tubes adding internal standard (imazalil-D5) in the volume allowing to achieve final sample concentration of 10 ng/g, subsequently adding 500 µl of buffer (ammonium chloride – pH 3). Liquid-liquid extraction with diisopropyl ether was carried out for 20 min. Samples were centrifuged at 5500 rpm for 10 min. 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 100 µl of 1:1 acetonitrile/water (v/v)

mixture and centrifuged at 15000 rpm at 5°C for 10 min, filtered and analyzed by UPLC-Q-TOF-MS.

DISCUSSION OF THE RESULTS

The quantitative ions used for imazalil and thiabendazole were respectively: 297.0556 and 202.0435. The qualitative ion for imazalil was its isotope ³⁷Cl: 299,0527 whereas for thiabendazole ion 175.0323 was used. Retention times for studied fungicides are 4.59 min. and 2.74 min. respectively. We have successfully used isotope ions as qualitative ions in our other studies [16]. The method for determination of imazalil and thiabendazole has been validated. The lowest calibrated levels (LCL) were 0.2 ng/g and 10 ng/g respectively. LOD (the limit of detection – S/N 3/1) and LOQ (the limit of quantitation – S/N 10/1) cannot be determined, because we cannot observe the chemical noise in the chromatogram. This is due to high accuracy of determining the mass of the test compounds. These observations was described [13,16]. Calibration curve was characterized by a very good linearity in the range of 0.2–100 ng/g for imazalil and 10 – 100 ng/g for thiabendazole (determination coefficient was 0.999 in both cases). Precision as relative standard deviation (R.S.D.%) ranged from 1.56 % (imazalil 20 ng/g) to 1.53% (thiabendazole 50 ng/g). The recovery (in percent) was determined by comparing calculated concentration of analytes in the blank material matrix vs. standards in methanol at the same concentration. Mean recovery was 129.44 % for imazalil and 84.73 % for thiabendazole. Recovery of over 100% for certain pesticides is also possible which is confirmed by the literature data [8,11]. Matrix effects were calculated using equation described by Chambers et al. [2]:

$$\% \text{ Matrix effects} = \left(\frac{\text{Response extracted sample}}{\text{Response standard}} - 1 \right) \cdot 100$$

A negative result indicates suppression while positive result indicates enhancement of the analyte signal. Matrix effects for imazalil and thiabendazole amounted to –34.78% and –59.02 %, respectively. This means that the matrix has a negative impact on the quality of analytes signal.

Additionally, the method described in this paper was used in practice. For this purpose, we examined five samples in triplicate of water pipe tobacco available on the market. The results are shown in Table 1. In two samples, we investigated imazalil at the concentration below LCL. However, we have not found thiabendazole in any sample. The mean area peak of imazalil in the sample number one and two is twice as high as area peak of LCL (0.2 ng/g). Chromatogram of sample number two shows Fig. 2. These results may suggest that our blank matrix has a more negative effect than water-pipe tobacco on the recovery of the analyte from tobacco or that the matrix suppresses analyte ions, which come to the spectrometer. In addition,

it may suggest that different manufacturers use various amounts of glycerol for making their products. Fig. 3 shows that when imazalil elutes, the intensity of the matrix is very high. During the elution time of thiabendazole the intensity of matrix is low.

Table 1. The concentration of imazalil and thiabendazole in water-pipe tobacco samples

Sample	Flavor	Country	Imazalil [ng/g]	Thiabendazole [ng/g]
1	-	-	0.097	-
2	Watermelon	Egypt	0.090	-
3	Mint	India	-	-
4	Grape	Poland	-	-
5	Citrus fruits	Egypt	-	-

CONCLUSION

The sensitivity and specificity of the method for determination of imazalil and thiabendazole in water-pipe tobacco was high. Usage of hybrid quadruple coupled with time-of-flight analyzer characterized by a very high resolution allows to identify the investigated compounds and to apply the developed technique in routine quality control of fungicides in plant matrix.

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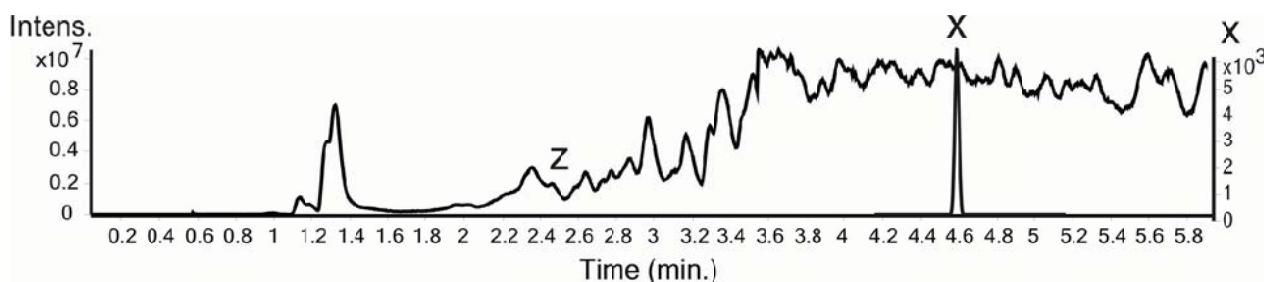


Fig. 2. Extract ion chromatogram (EIC) of imazalil (x) in sample 2. Total ion chromatogram (z)

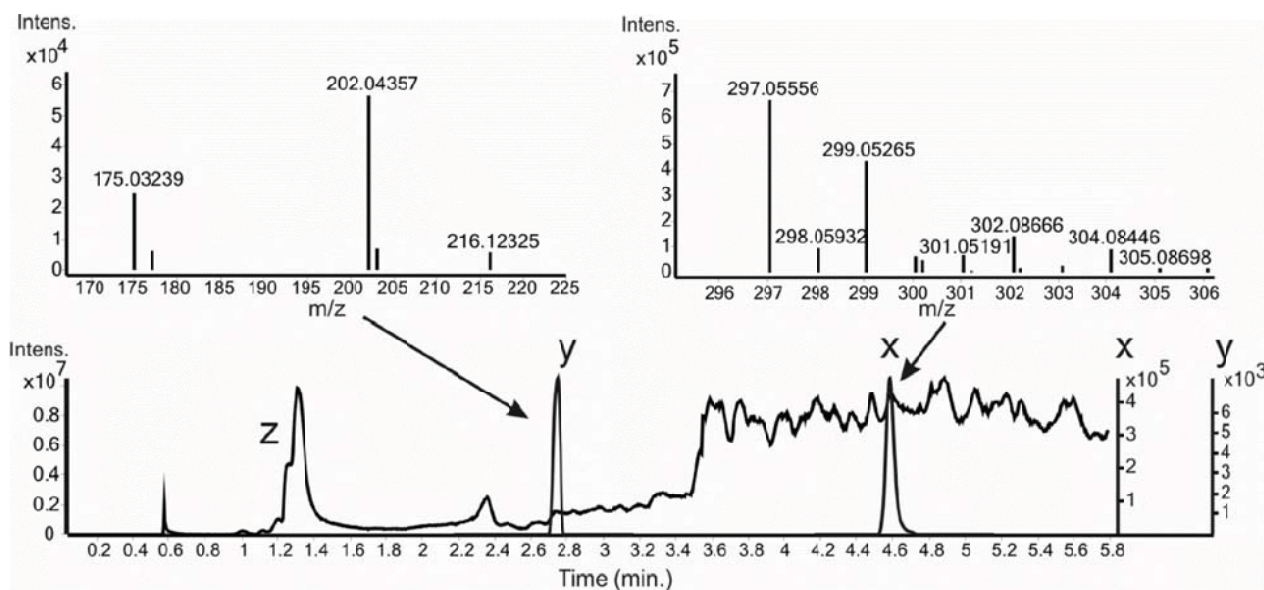


Fig. 3. Extract ion chromatogram (EIC) and Q-TOF-MS spectrum of imazalil (x) and thiabendazole (y). Total ion chromatogram (z)

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