DEVELOPMENT AND VALIDATION OF NEW MULTIRESIDUE METHOD FOR THE DETERMINATION OF MULTICLASS PESTICIDE RESIDUE USING LC-MS/MS IN ONIONS

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Abstract

The LC-MS/MS was used in the ESI+ mode. The method was set for the detection of six multiclass pesticides in a single injection. The validation procedure for the method was in accordance with SANTE /11945/2015and it was carried out using blank onion samples spiked with a pesticide mix solution at four levels: 0.01, 0.02, 0.1 and 0.2 µg/mL, with carbofuran–D3 as the internal standard. The linearity of the method was investigated in the range from 0.01 to 0.20 mg/kg. The obtained R^2values for all investigated pesticides (formetanate hydrochloride, spirotetramat, spinosad, dimethomorph, metalaxyl-Mand mandipropamid)were higher than 0.99.The recoveries ranged from96.2 to 101.45 with the precision lower than 8.00%. The LODs were calculated using the Agilent MassHunter B.04.00 software, and the LOQs were experimentally set at 0.01 mg/kg.

Introduction

Onion (*Allium cepa*L.) is one of the most significant vegetables and belongs to the family *Alliaceae*[1]. Onion has weak competitive strength towards weeds which, in return, can lower the yield and the quality of the onion and in the extreme cases totally annihilate the crop. On the other hand, the onion has to be treated, against pathogens such as *Sclerotiumcepivorum, Botrytis allii*, *Botrytis cinerea*, *Botrytis squamosa*, *Peronospora destructor*, *Alternariaporri* and including viruses and bacteria. It also has to be protected against numerous pests such as species belonging to families *Scarabaeidae*, *Elateridae*, *Noctuideae*, *Triozabrassicae* and*Hylemia antique*, *Ceuthorrynchussaturalis*, *Frankliniellaoccidentalis* and *Thripstabaci*. Onion bulbs are quite often treated during the storage with the preparations containing maleic hydrazide which prevents germination[2].

Obtaining high and quality yields of agricultural plants is the ultimate goal of modern agricultural production. To achieve these results the use of pesticides is indispensable[3].In Serbia the formulations of four insecticides, 21 fungicides, 20 herbicides and two growth regulators were registered for use in onions[4].

The growing concern for human health related to pesticide residues in food, greatly changed the strategy of crop protection with a special emphesis on the quality and safety of food [5]. That is why the paper will deal with the validation of the multi-residue method for the determination of formetanate-hidrohlorid, spirotetramat, spinosad, dimethomorf, metalaxil-M and mandipropamidresidues in onions by liquid chromatography tandem mass spectrometry (LC-MS/MS) according to SANTE/11945/2015.

Materials and methods

Chemicals and apparatus. All reagents used were of analytical grade, >95% purity. Acetonitrile was purchased from Fisher Chemical (Leics, UK) and methanol (Ultra Gradient HPLC Grade) was purchased from J.T. Baker (Deventer, the Netherlands). Water was deionized (>18 M/cm) by the Elga Maxima system. QuEChERS Extraction Packets, EN Method (BondElute, P/N 5982-7550, Agilent Technologies) was used for extraction and Dispersive SPE, Fruits and Vegetables packets (BondElute, P/N 5982-5056, Agilent Technologies) for cleanup samples.The certified pesticide analytical standards of formetanate-hidrohlorid, spirotetramat, spinosad, dimethomorf, metalaxil-M and mandipropamid were purchased from Dr. Ehrenstorfer. Standard stock solutions were prepared in acetonitrile (1.0 mg/mL), while the working standard was in concentration of 10 µg/mL. This solution was used as spiking solution and also to prepare the standard solutions to obtain the calibration curves, by dilution with mixture of methanol and water (50/50,V/V; with 0.1% formic acid).

The LC-MS/MS analysis was performed on the Agilent 1200 HPLC system (Agilent Technologies, Waldronn, Germany) with an automatic degasser, a binary pump and an auto sampler connected to the Agilent 6410B Triple-Quad LC/MS system. The chromatographic separation was performed on the Zorbax XDB C18 analytical column of 50×4.6mm and 1.8 µm particle size (Agilent Technologies, the USA), which was maintained at 30 °C. The LC flow was maintained at 0.4 mL/min, the injection volume was 5 µL. The mobile phase gradient program started at 90% of B (water with 0.1% formic acid) held for 2 min, then decreases to 10% at 15 and 5% at 17 min, held for 3 min. The mobile returned to the initial composition at 5.0 min and equilibrated for another 5 min before the next injection. Electrospray ionisation was performed in the positive mode with the following parameters: resolution Q1 and Q3-wide (0.3 units) spray voltage-2000 V, gas temperature-325 °C, vaporizer-220 °C, gas flow (N_2)-5 L/min; nebuliser gas (N_2)-40 psi; the MassHunter software (version B.04. QQQ Agilent Technologies) controlled the LC-MS/MS system and processed the data. The data acquisition was in multiple reactions monitoring (MRM) mode. The ion transitions and mass parameters monitored for each analyte are listed in Table 1.

Validation

Linearity study, LOD and LOQ determinations: The evaluation of the analytical curves' linearity was done based on injections of the standard solutions prepared in organic solvent (mixture methanol and water) and also in blank union extract, atthe concentrations 10, 25, 50, 100 and 200 ng/mL, where this sequence was injected three times (n = 3). The corresponding range of pesticide concentrations in union extract were from 0.01 to 0.20 mg/kg. The limit of detection (LOD) was determined as the lowest concentration giving a response of three times the average baseline. The ratio signal/noise in the obtained chromatograms for the LOD was calculated by MassHunter Qualitative Software. The real LOQ was based on the accuracy and precision data, obtained via the recovery determinations and was defined as the lowest validated spike level meeting the requirements of a recovery within the range 70–120% and RSD≤20%.

Accuracy and precision (recovery experiments): The main goal of the recovery experiments is to determine the method accuracy, via comparison of the real concentration of each pesticide measured by performing the complete procedure with the known pesticide concentration initially added to the matrix. The method precision is expressed as the repeatability (RSD%) of the recovery determinations at the four differentspiking levels (10, 50, 100 and 200 mg/kg).The spiking procedure with 6 pesticides, added to blended and homogenized union, was done three times (n=3) at each spike level and also the blank union matrix analysis was performed two times. This blank extract was also used for preparationof standard solutions in matrix.

Extraction procedure

10g sample + 10mLMeCN + 100µL ISTD (10 µg/mL Carbofuran-D3)

↓ Shake vigorously for 1 min

Add 4g $MgSO_4$,1g NaCl, 1g Na_3Citrate dihydrate, 0.5g Na_2HCitrat sesquihydrate
Shake tube immediately for 1 min

↓ Centrifuge for 5 min at 3000 g

Transfer 5 ml of the extract into a PP tube contained $MgSO_4$,PSA
Shake for 30s

↓ Centrifuge for 5 min at 3000 g

Transfer 200µL into a vial, evaporate to dryness
Reconstitute in 200µL of mobile phaseLC-MS/MS →

Results

The validated method which uses the LC-MS/MS provides appropriate linearity, a very high sensitivity, good repeatability and can be applied with the high reliability to the analysis of pesticide residues in trace levels [7].Before the calibration andquantification of pesticides it was necessary to set an acquisition method. The determination of the acquisition method comprises: setting chromatographic conditions, determining the precursor and product ion so called monitoring mode of ion transfer (MRM or SRM), determining the fragmentation energy (Frag.) and the energy of collision cell (CE). For setting the MRM MassHunter Optimizer Softvere Version B03.01 (Agilent Technologies 2010) and Agilent G1733AA MassHunter Pesticide Dynamic MRM Database were used.

Table 1. Retention times, MRM and CE, Frag, R^2 and average recoveries of studied pesticides

Pesticide	**MRM (m/z)**	**Product ion (m/z)**	**Frag. (V)**	**CE (V)**	**Rt (min)**	**R^2**	**Avr. recovery ±RSD (%)**
formetanate	222.1	165.1 (93.1)	120	15 (36)	11.597	0.9997	98.5±4.25
metalaxil-M	280.2	220.1(192.1)	90	9 (13)	15.760	0.9978	96.2±7.19
dimethomorf	388.1	301.1(165.0)	120	30 (20)	17.024	0.9992	97.6±6.47
spirotetramat	374.0	302.0(330.0)	100	20 (20)	17.497	0.9999	99.8±5.36
mandipropamid	412.1	328.0(356.0)	100	5 (11)	18.677	1.0000	100.0±3.88
spinosad	732.5	142.0(98.0)	140	35 (55)	23.673	0.9999	101.4±2.32

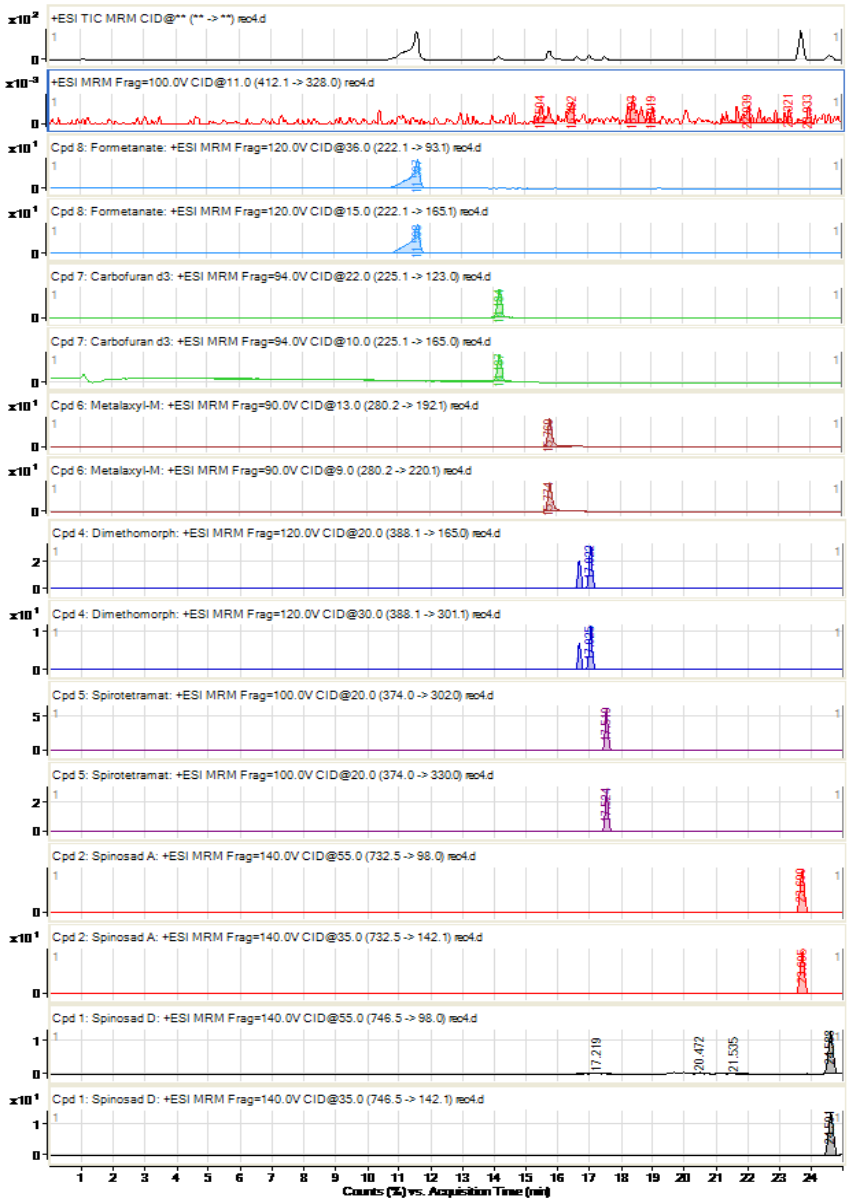

Figure 1. TIC chromatogram and MRM chromatograms of analysed pesticides

The methodlinearity was investigated in the range from 0.01 to 0.2 mg/kg. The obtained correlation coefficients (R^2) were higher than 0.99 for all investigated pesticides.The recoveries were in the 96.2-101.4% range and were characterized by precision lower than 8%.The LOQs of 0.01 mg/kg confirm that the method is appropriate for the determination of pesticide residuesaccording to the regulations of the Serbian and EU MRLs(Maximum Residue Levels).

Conclusions

A very fast, easy, cheap, robust and efficient multiresidue method, based on QuEChERS procedures and LC–MS/MS analysis, has been developed and validated for onion samples. The performance characteristics for the majority of the six studied pesticides were acceptable, according to the most recent EU guidelines for method validation. Good linearity of the calibration curves was obtained the range from 10 to 200 ng/mL, with $R^2 \geq 0.99$. Recoveries were in the range 96.2–101.4%, with RSD≤7.19%. The method has been proved to be successful as a real quantitative, multiresidue method for pesticide residues analysis in onion,

which is known to be a difficult matrix. It can be recommended for routine application in monitoring studies or surveys.

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