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Original scientific paper

Determination of linuron in chamomile by LC-MS/MS using the QuEChERS extraction method

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SUMMARY

Linuron is a selective herbicide used for the control of broadleaf weeds. Its mode of action is the inhibition of photosynthesis. The QuEChERS method for extraction of linuron residues from chamomile was used. The LC–MS/MS method was used for determination of linuron residues. Its linearity was studied in a range of 0.025-0.50 µg/ml using matrix-matched calibration, and the determination coefficient (R^2) was higher than 0.99. Blank chamomile samples were spiked with linuron solution at three concentration levels yielding recoveries of over 90%. The internal standard added in all samples was isoproturon–d6. There were no linuron residues in chamomile flowers, while the residues ranged from 0.010 to 0.040 mg/kg in the flower stalk samples.

Keywords: Linuron; Residues; QuEChERS; LC-MS/MS; Chamomile

INTRODUCTION

Chamomile (*Matricaria chamomilla* L.), the family Asteraceae, is very important and highly appreciated as a medicinal plant, foodstuff and raw material for the cosmetic industry. It is native to southern and eastern Europe, especially to Germany, Hungary, France, Russia and Serbia (Singh.etal., 2011; Hutchings, 1989; Bolofo & Johnson, 1988) but it is important mainly in the south and southeast of Brazil (Vieira etal., 2010) and other countries. A wide range of raw and refined products based on chamomile crops are available: chamomile flowers (*Matricariae flos*), chamomile fines, chamomile herb with flowers, chamomile herb, chamomile for extraction (industrial chamomile), chamomile root, chamomile oil (*Matricariae aetheroleum*), chamomile fluid extract and chamomile tincture. In Serbia, chamomile is mostly used and traded as a medicinal plant (Singh et al., 2011; Jovanović-Radovanov et al., 2012; Stevanović et al., 2007). There are only a few studies on possible herbicide uses for weed control in chamomile crops in Serbia. The efficiency of weed control and selectivity towards chamomile as the protected crop are very important aspects of herbicide application. Control of pesticide residues in agricultural products allows assessment of conformity of production with good agricultural practices applied in the conventional, integrated and organic production, and determination of origin and

cause of the residues found (Baša & Gregorčič 2006; Baša et al., 2009). But herbicide residual levels in medical plants are of immense importance. The application rates of herbicides in chamomile crops are lower in comparison to other crops treated by the same active substances. Momčilović et al. (1999) detected higher levels of mecoprop (1.9 mg/kg), linuron (0.54-0.63 mg/kg), fluazifop-P-butyl (0.78 mg/kg) and cycloxydim (1.8-3.16 mg/kg) than the maximum residue level (MRL) prescribed for chamomile flowers, which is 0.1 mg/kg (Regulation (EC) No 396/2005; Commission Regulation (EU) No. 212/2013).

Linuron (Figure 1) is a non-selective herbicide used for the control of grasses and broadleaf weeds. It works by inhibiting photosynthesis. Linuron is a white powder with a melting point at 3994 °C and water solubility of 75 ppm at 25 °C (Ebato & Yonebayashi2005).

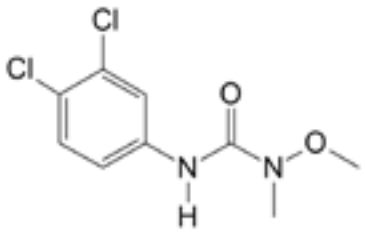

Figure 1. Structural formula of linuron

In recent years, the LC-MS has been widely used for the analysis of pesticide residues in fruits, vegetables and other food samples. More recently, the coupling of LC with tandem mass spectrometry detection (MS/MS) has gradually become significant for pesticide residue analyses (Soler & Pic, 2007; Vuković et al., 2012b). Thus the LC–MS/MS with electro-spray ionization (ESI) has been demonstrated as a suitable technique for pesticide residue analysis (Dreassi et al., 2010).

That is why the LC-MS/MS was used in this study for determination of linuron residues in chamomile flowers and stalks after QuEChERS extraction. The determined concentrations were compared with the MRL for linuron in chamomile (0.1 mg/kg) (Pravilnik, 2014).

MATERIALS AND METHODS

1. Chemicals and apparatus

All solvents used in the experiment were of chromatography grade and obtained from Merck (Darmstadt, Germany). The certified pesticide analytical standard of linuron (99.5 %) was purchased from Dr. Ehrenstorfer (Augsburg, Germany) and the internal standard isoproturon–d6 was purchased from Sigma Aldrich (99.8 %). An internal standard was added to make a concentration of 10 μg/ml in acetonitril. The stock standard solution was prepared by dissolving the analytical standard of linuron in acetonitrile (1mg/ml), while the working standard was obtained by diluting the stock standard with acetonitrile, resulting in the final mass concentration of 10 μg/ml. Magnesium sulphate, disodium hydrogencitrate sesquihydrate, trisodium citrate dihydrate, sodium chloride and formic acid (analytical reagent grade) were purchased from Fisher Scientific UK (Loughborough, UK). Bondesil primary secondary amine (PSA, 40 μm) was obtained from Agilent Technologies (Australia Pty Ltd). For LC analysis, an Agilent 1200 (Agilent Technologies, USA) HPLC system with a binary pump was used. It was equipped with a reversed-phase C18 analytical column of 50×4.6 mm and 1.8 μm particle size (Zorbax Eclipse XDB-C18, Agilent). The mobile phase was methanol (solvent A) and Milli-Q water (solvent B), both containing 0.1% formic acid in gradient mode, with the flow rate of 0.6 ml/min. The elution program was started with 50% B. It was linearly decreased to 30% B in 12 min and held constantly for 3 min. The stop time was 15 minutes with the post run of 3 minutes.

For the mass spectrometric analysis, an Agilent 6410 Triple-Quad LC/MS system was applied, and the Agilent MassHunter B.04.00 software was used for data acquisition and processing. The analysis was performed in positive ion modes. The ESI source values were as follows: drying gas (nitrogen) temperature 300 °C, vaporizer 200 °C, drying gas flow rate 5 l/min, nebulizer pressure 50 psi and capillary voltage 2500 V. The detection was performed using multiple reactions monitoring (MRM).

2. Validation parameters

2.1 Limit of detection, limit of quantification (LOD and LOQ) and linearity

The evaluation of the calibration curves' linearity was done based on the injections of standard solutions prepared in mobile phase and also in extracts of blank chamomile flowers and blank chamomile stalk samples, at the concentrations of 0.025, 0.05, 0.1, 0.25 and 0.5 μg/ml, adding the internal standard isoproturon–d6 (0.1 μg/ml). The calibration solutions

at each concentration level were injected three times. From the calibration curves' linearity data and the repeatability (RSD, %) at the lowest concentration levels, the instrument LOD and LOQ (LOD*i* and LOQ*i*, respectively) and also the method LOD and LOQ (LODm and LOQm, respectively) were estimated. The LOD*i* was calculated from the six replicate injections at the lowest detectable level, according to Equation 1

$$LODi\ (ng/ml) = 3 \times RSD \times concentration \quad (1)$$

(using the Agilent MassHunter Qualitative Analysis B 04.00). From these calculated values, the best estimated LOD*i* value was established. As a rule, this concentration should always be really injected and be detectable repeatedly all six times at that level. The (estimated) LOQ*i* was defined as Equation 2.

$$LOQi = 10 \times RSD \times concentration \quad (2)$$

And it becomes Equation 3.

$$LOQi = 3.3 \times LODi \quad (3)$$

The real LOQm 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 of 70-120% and a RSD ≤ 20 % (Pizzutti et al., 2007).

2.2 Recovery

The main goal of the recovery experiments was to determine the method accuracy by comparing the real pesticide concentration, measured by performing a complete procedure, with known pesticide concentration initially added to the matrix. Method precision was expressed as repeatability (RSD, %) of the recovery determinations at three different spiking levels (0.1, 0.25 and 0.5 mg/kg).

3. Sample preparation

Linuron was extracted from chamomile flowers and stalk samples using an extraction procedure based on the QuEChERS methodology (Anastassiades et al., 2003). For the chamomile extracts, the amount was reduced to 2 g of fine homogenized sample. The samples were then mixed with 10 ml of water before extraction. Then 100 μl of internal standard solution was added and extraction was done with 10 ml of MeCN. After extraction on a vortex mixer for 1 minute, 6.0 g of magnesium sulfate anhydrous, 1.5 g of sodium chloride, 1.5 g of trisodium citrate dihydrate and 0.75 g of disodium hydrogencitrate sesquihydrate were added and the mixture was shaken vigorously for 1 min and centrifuged for 5 minutes at 3000 rpm. After centrifugation, 1 ml of supernatant was transferred into a clean-up tube containing 900 mg of $MgSO_4$ and 150 mg of PSA. After centrifugation for 5 minutes at 4500 rpm, 0.5 ml of supernatant was evaporated to dryness and reconstituted in 0.5 ml of mobile phase.

RESULTS

A summary on the MRM transitions and MS operating parameters selected for the analysis of linuron and isoproturon–d6 in ESI, positive mode, is in Table 1.

4. Calibration, LOD and LOQ

The chamomile control (flower + stalk) based matrix used for calibration and for recovery studies was analyzed to verify the absence of linuron before performing the analysis. The calibration curves based on matrix-matched standards were obtained at concentration levels from 0.025 to 0.50 μg/ml at five levels (in triplicate). The matrix effect was observed comparing the slopes obtained for the calibration curves of matrix-matched standard, for each chamomile flower and stalk sample with the slope calibration curve in mobile phase. The increase response signal occurs for linuron in chamomile flowers (3.79 %) but the detector response was significantly enhanced by chamomile stalks where the matrix effect

Table 1. MRM transitions of linuron and isoproturon-d6.

Pesticide	Formula	M (g/mol)	Precursor ion	Product ion	Frag (V)	CE (V)
Linuron	$C_9H_{10}Cl_2N_2O_2$	249.1	249 249	182 160	70 70	18 18
Ispoproturon-d6	$C_{12}H_{12}D6N_2O$	212.3	213	78	135	17

was 19.38% (Vuković et al., 2012a; Council Directive 96/23/EC, 2002). Good linearity was achieved for linuron in chamomile flower and stalk samples with coefficients of determination (R^2) higher than 0.99 (Figure 2).

For linuron, there were no differences between the estimated instrument LOD and LOQ values calculated from the results obtained with standard solutions prepared in mobile phase and in chamomile flowers. But for chamomile stalks there were differences between the estimated instrument LOD and LOQ values which indicate that these parameters were influenced by the chamomile stalk matrix. In general, the LOD for linuron in chamomile flowers was 0.001 mg/kg, while it was 0.002 mg/kg in stalks. The LOQ value in chamomile flowers was 0.004 mg/kg, while it was 0.007 mg/kg in stalks.

5. Recovery

Recovery studies were performed along with the fortification experiments at three levels (0.10, 0.25 and 0.50 mg/kg) in three replicates with an addition of the internal standard isoproturon-d6. The pesticide-free samples were spiked before the QuEChERS method was applied and analyzed as previously described. The average recovery for chamomile flowers was 94.7 ± 7.18% and it was 95.4 ± 8.03% for stalks (Tables 2 and 3). Precision was assessed in terms of repeatability at 10 mg/kg.

A good repeatability (n = 6) with RSDs of 7.18% for chamomile flowers and 8.03% for chamomile stalks was obtained and it was calculated through recovery.

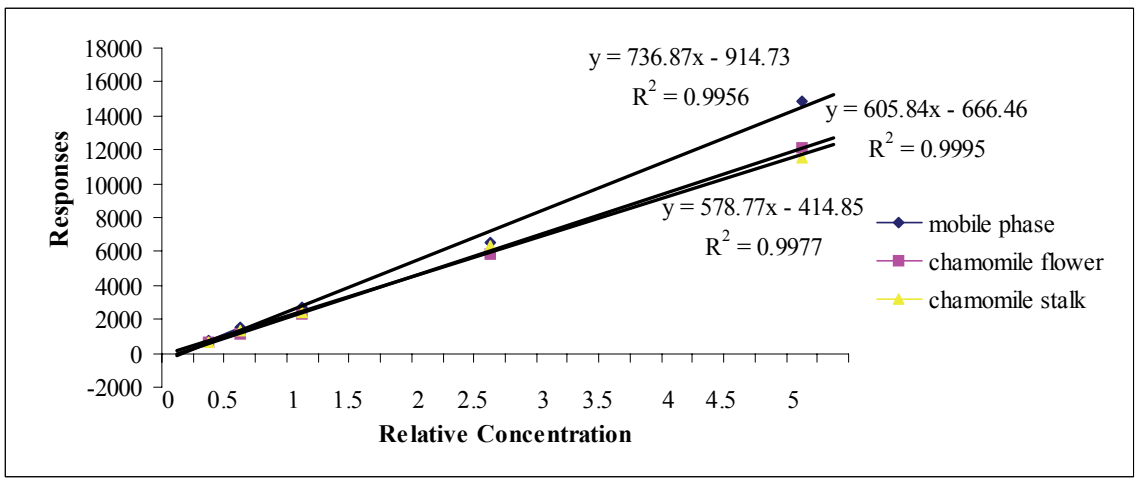

Figure 2. Calibration curve of linuron in mobile phase, chamomile flower and chamomile stalk

Table 2. Recoveries in chamomile flowers

Concentration (mg/kg)	Replicates			Average recovery (%)	RSD (%)
	1	2	3		
0.10	80.2	72.6	86.7	79.8	8.83
0.25	97.2	90.8	102.0	96.7	5.81
0.50	112.3	111.5	94.1	107.6	6.90

Table 3. Recoveries in chamomile stalks

Concentration (mg/kg)	Replicates			Average recovery (%)	RSD (%)
	1	2	3		
0.10	96.7	91.7	77.1	88.5	11.50
0.25	105.2	94.8	92.4	97.5	6.98
0.50	96.4	97.8	106.8	100.3	5.60

6. Sample analysis

The analysis comprised fifteen samples of chamomile flowers and stalks each. There were no linuron residues found in chamomile flowers, while residues were found in a range from 0.010 to 0.040 mg/kg in the flower stalk samples (Figure 3 and Table 4).

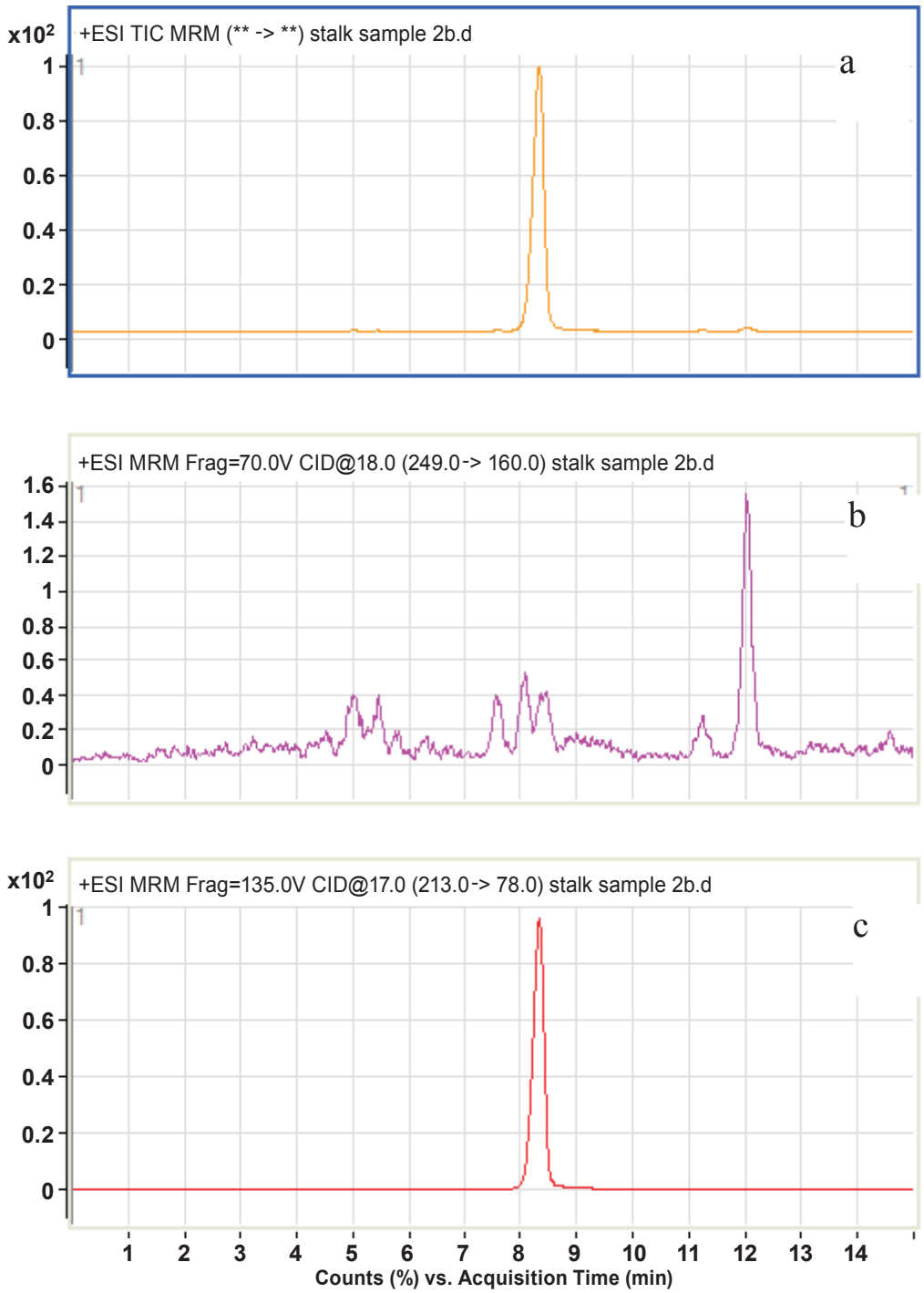

Figure 3. LC-MS/MS chromatograms of chamomile stalk (a – TIC chromatogram, b – MRM chromatogram of linuron, c – MRM chromatogram of isporoturon-d6)

Table 4. Detected linuron concentrations in samples

Sample	Detected concentration (mg/kg)	
	Chamomile flower	Chamomile stalk
1	< LOQ	0.014
2	< LOQ	0.012
3	< LOQ	0.040
4	< LOQ	0.010
5	< LOQ	0.028
6	< LOQ	0.019
7	< LOQ	0.037
8	< LOQ	0.018
9	< LOQ	0.031
10	< LOQ	0.010
11	< LOQ	0.015
12	< LOQ	0.017
13	< LOQ	0.023
14	< LOQ	0.010
15	< LOQ	0.040

DISCUSSION

An efficient, sensitive and specific method was developed for determining linuron residues in chamomile flowers and stalks by LC-MS/MS. The calibration curves were determined using matrix-matched standards and exhibited an excellent linearity of 0.025-0.50 μg/ml for both. The linearity of R^2 was over 0.99. The matrix influence was significant in chamomile stalks (19.38%). The LOD for linuron in chamomile flowers was 0.001 mg/kg, while it was 0.002 mg/kg in stalks. The LOQ value in chamomile flowers was 0.004 mg/kg, and 0.007 mg/kg in stalks. The average recovery was 94.7 ± 7.18% for chamomile flowers, and 95.4 ± 8.03 % for stalks. This validated method was successfully applied for the analysis of pesticide residues in chamomile flowers and stalks. No linuron residues were detected in the chamomile flower samples, i.e. the detection was below LOQ (0.002 mg/kg), while the detected pesticide residues in the stalk samples were below MRL and ranged from 0.10 to 0.40 mg/kg.

Lozano et al. (2012) determined the residues of 86 pesticides (insecticides, fungicides and herbicides) in teas and chamomile. Analysing four chamomile samples, pesticide residues were detected in all of them but the concentration of one or more pesticides exceeded their MRLs in three samples.

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Određivanje linurona u kamilici LC-MS/MS tehnikom i QuEChERS metodom ekstrakcije

REZIME

Linuron je selektivni herbicid, koji se koristi za suzbijanje širokolisnih korova. Njegov mehanizam delovanja je inhibicija fotosinteze (fotosistema II). Za ekstrakciju ostataka linurona iz uzoraka kamilice korišćena je QuEChERS metoda. Određivanje nivoa ostataka linurona vršeno je tečnom hromatografijom sa masenim spektroskopijom. Linearnost metode je ispitivana u opsegu koncentracija od 0.025 – 0.50 µg/ml, korišćenjem metode kalibracije u matriksu, pri čemu je koeficijent određivanja (R^2) bio veći od 0.99. Tačnost metode je ispitivana obogaćivanjem kontrolnih uzoraka kamilice na tri koncentraciona nivoa. Prinos ekstrakcije je bio preko 90 %. Interni standard korišćen za analizu je bio izoproturon-D6. U cvetu kamilice nisu nađeni ostaci linurona, dok su u uzorcima drške bili u opsegu od 0.010-0.040 mg/kg.

Ključne reči: Linuron; Ostaci; QuEChERS; LC-MS/MS; Kamilica