LINURON RESIDUE FINDINGS IN CHAMOMILE FLOWERS

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Abstract

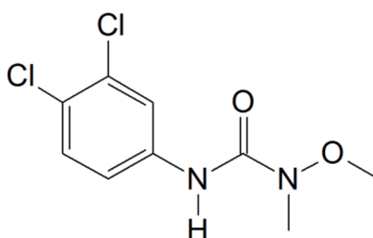
Text The aim of this study was to highlight the influence of herbicide usage in agriculture on linuron residues in chamomile flower. It was collected six chamomile flower samples. The validated LC-MS/MS method according to SANTE/11813/2017 was applied in the quantitative analyses. Two samples were with no linuron detection, while all the other detected concentrations exceeded maximum residue limit of 0.1 mg/kg. The detected concentrations were in the range 0.89 to 1.90 mg/kg.

Introduction

Chamomile is a member of the Asteraceae/Compositae family, and is represented by two common varieties German chamomile (*Chamomilla recutita*) and Roman chamomile (*Chamaemelum nobile*) [1]. Nowadays, it is a highly favored and much used medicinal plant in folk and traditional medicine. Its multitherapeutic, cosmetic, and nutritional values have been established through years of traditional and scientific use and research [2]. Considered to be one of the most ancient and versatile medicinal herbs known to mankind, dried chamomile flowers have numerous, widespread health implications thanks to their high level of disease-fighting antioxidants like terpenoids and flavonoids. Throughout the world the flowers are used in the form of a simple tea (tisane) as a gentle medicine for colicky babies and for adults with mild upset stomach or symptoms of mild stress. Extracts of the flowers and also the essential oil distilled from them provide other formulations that extend the range of medicinal benefits through antioxidant, anti-inflammatory, antifungal, and antibacterial activities. Several studies have indicated that chamomile has potential anticancer activity. Other uses are in cosmetics and as a flavoring agent in foods, beverages, bakery products, ice cream, and tobacco [3].

The common practice in chamomile production involves weed control using herbicides. Weeds are the serious hygiene concern, since contaminants in the final product (flowers, oil or extract) will detract from the specified quality. Considering there are no authorized plant protections product for use in medicinal crops, there are no recommendations on concentration required for efficient weed control and crop safety. Linuron (phenylurea) (Figure 1) is used as a selective herbicide to control both annual and broadleaf weeds, perennial broadleaf weeds and grassy weeds. It can be applied as a pre-emergence treatment and a post-emergence treatment. Linuron hinders electron transport in photosystem II in the photochemical step in photosynthesis [4].

Figure 1. Structural formula of linuron



The LC-MS has been widely used for the analysis of pesticide residues in fruits, vegetable and other food samples. The coupling of LC with tandem mass spectrometry detection (MS/MS) has become significant for pesticide residue analysis [5]. Experience shows the chemicals to be useful, but none is registered for use on chamomile in Serbia [6]. That is why in this study the liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the determination of linuron residues in chamomile flowers, after the QuEChERS extraction. The determined concentrations were compared with the maximum residue levels (MRLs) for linuron in chamomile, which is 0.1 mg/kg [7].

Experimental

Chemicals and apparatus. All solvents used were of chromatography grade and were obtained from J.T. Baker (Netherlands). The certified pesticide analytical standard of linuron (99.5 %) was purchased from Dr. Ehrenstorfer (Augsburg, Germany) and the internal standard carbofuran–D3 was purchased from Sigma Aldrich (CAS Number 1007459-98-4). The linuron stock standard solution was in the concentration of 1 mg/mL, while the working standard has the final mass concentration of 1 µg/mL in acetonitrile. Magnesium sulphate, disodium hydrogencitrate sesquihydrate, trisodium citrate dihydrate, sodium chloride and formic acid, primary secondary amine and graphitized black carbon (GBC) were purchased from Fisher Scientific UK (Loughborough, UK). For LC analysis, an Agilent 1200 (Agilent Technologies, USA) HPLC system with a binary pump was used. This 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 0.4 mL/min. The elution program was started with 90% B and held 2 min It was linearly decreased to 20% B in 15 min, 10% B in 20 min, 5% B in 25 min and it held constantly for 3 min. The stop time was 28 min with the post run of 5 min. The injection volume was 5 µL. For the mass spectrometric analysis, an Agilent 6410 Triple-Quad LC/MS system was applied. Agilent MassHunter B.06.00 software was used for the data acquisition and processing. The analysis was performed in the positive ion modes. The ESI source values were as follows: drying gas (nitrogen) temperature 350 °C, drying gas flow rate 10 L/min, nebulizer pressure 40 psi and capillary voltage 3500 V. The detection was performed using the multiple reactions monitoring mode (MRM).

Validation parameters. The analytical method based on a simple QuEChERS solvent-based extraction was validated according to SANTE N° 11813/2017[8].
The LOD was estimated from the chromatogram of the lowest level of calibration using the Agilent MassHunter software (Agilent Technologies, B.06.00) for those concentrations that provide a signal to noise ratio of 3:1. The LOQ was defined as the lowest validated spike level

which meets the requirements of a recovery within the range of 70–120% and a RSD≤20%. The LOQ was determined at 0.01 mg/kg in consideration of MRL (0.1 mg/kg). Recovery studies were done at two spiking levels (0.025 and 0.25 mg/kg). The method precision is expressed as the repeatability (RSD%) based on recovery obtained values.

Sample preparation. The linuron was extracted from chamomile flower samples using an extraction procedure based on the QuEChERS methodology. For the chamomile extracton the 2 g of fine homogenised sample was mixed with 8 mL of water. It was followed by adding 100 µL of IS solution (10 µg/ml) and by the extraction with 10 mL of MeCN. After extracting on vortex mixer for 1 min, 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 after that centrifuged for 5 min at 4000 rpm. After the centrifugation 6 mL of supernatant was transferred into a clean-up tube containing 900 mg of $MgSO_4$, 150 mg of PSA and 150 mg of GBC. After the centrifugation for 5 min at 4000 rpm, 5 µL of supernatant was injected into LC-MS/MS.

Results and discussion

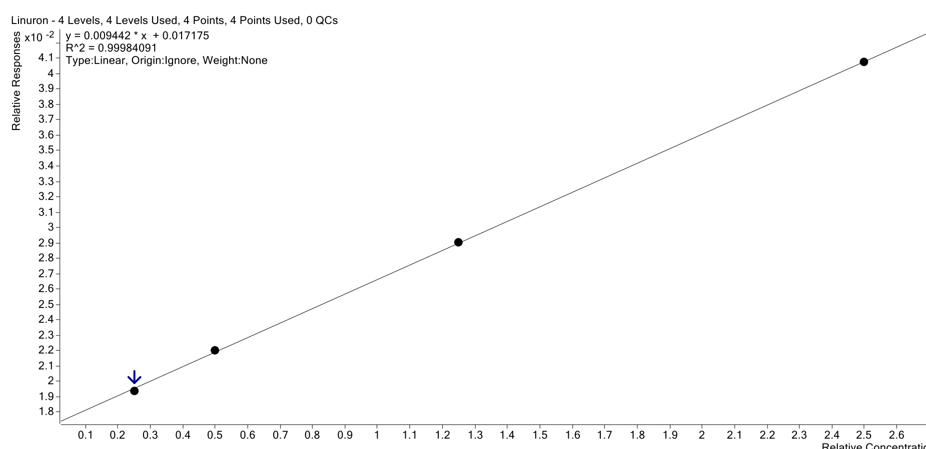
The summary of MRM transitions and LC-MS/MS operating parameters selected for the analysis of linuron and carbofuran–D3, as internal standard, in ESI positive mode are given in Table 1.

Table 1. MRM transitions of linuron and carbofuran-D3

Pesticide	Formula	Rt (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Frag (V)	CE (V)
Linuron	$C_9H_{10}Cl_2N_2O_2$	16.01	249 249	*Q* 182.3 *q* 160.1	100 100	8 20
Carbofuran-D3	$C_{12}H_{12}D_3NO_3$	13.28	225.1 225.1	*Q* 165 *q* 123.1	94 94	10 22

The previously developed LC-MS/MS method underwent a preliminary validation. The procedure exhibited an excellent linearity (R^2=0.9998) in the 5 – 50 ng/mL (corresponding 0.025-0.25 mg/kg) range with satisfactory precision expressed as relative standard deviation, RSD= 10.2% (*Figure 2*).

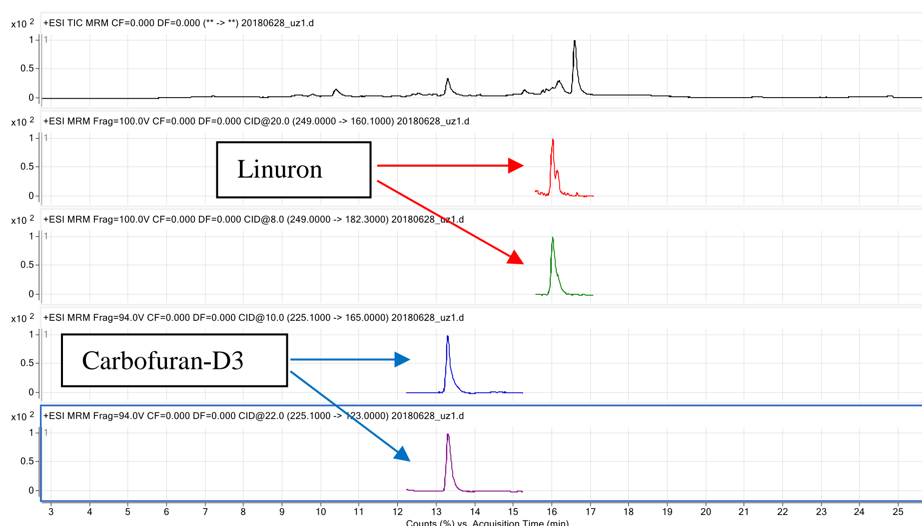
Figure 2. Linuron calibration curve



The accuracy and precision were determined via recovery experiments, spiking blank sample of chamomile at 0.025 and 0.25 mg/kg, at five replicates per level. The obtained recoveries

varied from 93.9% (RSDr=9.5%, level 0.25 mg/kg) to 99.9% (RSDr=3.8%, level 0.25 mg/kg) LOQ is experimentally set and confirmed at 0.025 mg/kg. These values are suitable for monitoring pesticides in plant material according to the EU Reg. 396/2005 [9] and National Regulation (Off. gazette RS 22/2018).
The analyses of six chamomile flower samples indicate that two samples were with no linurom detection, while the four samples were with the linuron detection above the MRL. The founding's were 0.89, 0.96, 1.09 to 1.90 mg/kg.

Figure 3. TIC chromatogram with MRM chromatograms of chamomile sample (linuron C=1.90 mg/kg)



Conclusion

An efficient, sensitive and specific method has been developed for the determination of linuron in chamomile flowers and stalks with LC-MS/MS, validated in accordance with SANTE/11813/2017 Document. This validated method was successfully applied for the analysis of linuron residues in six samples of chamomile flowers. No linuron residues were detected in 33.3% of invstigated samples, while all the other detections were above MRL of 0.1 mg/kg. This indicate that linuron was applied during chamomile production, and inplies that the constat monitoring of medical plants must be done.

Acknowledgements

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