Scientific Bulletin. Series F. Biotechnologies, Vol. XX, 2016
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

THE EVALUATION OF TRANSFER LEVEL FOR PESTICIDE RESIDUES, IN CASE OF PROCESSING BY SOLVENT EXTRACTION OF MEDICINAL PLANTS

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Abstract

There are multiple ways in which pesticide contamination can take place, depending on the method used for obtaining the raw material: by conventional agriculture or harvest from wild flora. Assessing the transfer level of residues in extracts, in controlled conditions of preparation and analysis, contributes to determining the risk level for the consumer, as well as making processing choices that lead to a low concentration of contaminants. We used an adapted QuEChERS extraction method for liquid samples to compare the level of pesticides in plant extracts obtained by ethylic alcohol and acetone extraction. This was done by extraction in the presence of MgSO4 and NaCl and cleanup by dispersion with PSA, active charcoal and Mg SO4. To achieve the appropriate level of sensitivity, the injection module of the equipment was used in Programmed Temperature Vaporization (PTV) mode, injecting 3 µl of sample. Calibration was performed by the standard addition technique, in which case the correlations were linear. Our results show that ethylic alcohol extraction, commonly used for obtaining tinctures, leads to different residue transfer levels, depending on their chemical structure and solubility (between 15 and 76,8 % recovery, for quintozene and terbuthylazine respectively). On the other hand, acetone extraction is selective for pesticides, and the crystallization of the resulting bioactive compounds leads to significantly reduced pesticide levels.

Key words*: extracts, medicinal plants, pesticides residues, QuEChERS.*

INTRODUCTION

The presence of pesticide residues in plant products is a prevailing problem, and their identification and quantification in the fresh or processed plant products is of great importance. MADR (Ministry of Agriculture and Rural Development), is concerned with tracking the levels of pesticide residues in plants but not so much in processed products (e.g. plant extracts). Their 2015 official report shows that 28.84% of vegetable samples had residue values lower than MRL (maximum residue limits), while 1.8% of samples exceeded MRL. The highest level of residues, in over 50% of the samples, was found in green salad, dill, lovage, scallion, parsley and celery. In fruits, the highest percentage of residues identifications (70%) was found in grapes, followed by strawberries (68%) and apples (67%). The most frequently identified pesticides were carbendazim and tebuconazol (MADR report). Organochlorurate pesticides residues are also frequently detected in fruits and vegetables, sometimes exceeding the accepted limit (Crentsil, 2011; Akan, 2014; Nsikak, 2011; Łozowicka, 2016, Dobrinas, 2011). Studies concerning the levels of pesticide residues during different processing steps show that residues can concentrate in the finite product (in the case of drying fruits or extracting fatty or volatile oils or other compounds) (Cortés, 2009), or can decrease (through washing, peeling, chopping, grinding, juicing or thermal preparation) (Elpiniki, 2011; Mekonen, 2015).

In medicinal plants, the presence of residues is the result of inadequate agricultural techniques, remanence of residues in the soil and/or contamination during the processing stages. Most studies were concerned with method validation and residue determination (LOQ = limit of quantitation < MRL), and in some instances the pesticide residues exceeded the approved levels (Hua, 2012; Sadowska, 2012; Amirahmadi, 2013; Brahushi, 2014; Agbeve1, 2014; Al-Othman, 2015). Consumers, oftentimes vulnerable segments of the population expect these products to be obtained in controlled conditions, following the GAP (Good Agricultural Practice) and GMP (Good Manufacturing Practice) rules. There is

therefore an intense concern with the evaluation of residue transfers from raw materials to products such as infusions (Rodrigues, 2005) tinctures (Kong, 2016) decocts, oils (Dugo, 2002; Garland, 2004) and concentrated fractions (Zuin, 2000).
In the European Pharmacopoeia, Ed. 8.0, for the pesticide residues parameter (2.8.13) there is a differentiation between residues in the raw plant and in processed products, and the accepted limit is adjusted with the following formulas:

If DER ≤ 10, then

$$MRLprep = MRL_{HD} \times DER$$

If DER>10, then

$$MRLprep = \frac{ADI \times M}{MDD_{HP} \times 100}$$

where: DER= drug/extraction ratio (ratio between the quantity of herbal drug used in manufacture and the quantity of herbal drug preparation obtained, $MRL_{prep.}$= officially accepted limit for pesticide residues in herbal drug preparation (mg/kg), MRL_{HD}= officially accepted limit for pesticide residues in raw material, ADI=acceptable daily intake (mg/kg), M=body mass (kg), MDD_{HP}=daily dose of herbal drug preparation (kg).
The present study is concerned with evaluating the transfer level of pesticide residues in extracts of plants initially fortified with pesticides. The QuEChERS technique was initially validated on vegetable and fruit matrices, and later tested and applied on various types of samples, such as cereals (Kolberg, 2010), fatty matrices (Wilkowska, 2011), water and soil (Brondi, 2011), medicinal plants (Sadowska-Rociek, 2013; Huebschmann, 2012; Amirahmadi, 2013), juicies and other liquid products (Kong, 2016; Cherta, 2013) or food supplements (Thomas, 2010; Dominguez, 2014). In the present study the QuEChERS method was adapted for liquid samples.

MATERIALS AND METHODS

Dried ivy leaf (*Hedera helix*) and dried sage leaf (*Salvia officinalis*) were fortified with pesticide solutions of known concentrations, prepared in the lab from individual and mixed standards bought from dr Ehrenstorfer. Their selection was made taking into account the real chance of contamination, both with pesticides used for plant protection and with banned pesticides that are still present in the environment due to their persistence. The ivy leaves were subjected to ethylic alcohol extraction 70% (ratio 1/30), concentrated 20:1 in the rotary evaporator. The final extract was conditioned in propylene glycol. The pesticide levels were assessed in the alcoholic phase and in the final propylene glycol conditioned phase. The sage leaves were extracted with acetone and the bioactive compound (BAC) was crystallized by solvent evaporation. The pesticide concentration was determined both in the acetone phase and in the crystallized substance. The analytical extraction of pesticides from plant extracts was performed with a modified QuEChERS protocol, for alcoholic, acetonic and propylene glycolic liquid samples and for solid BAC samples. The parameters for the analysis steps are in table 1.

Table 1. Extraction and cleanup methods

Type of sample	Extraction method	Cleanup method
EA 70%	4 ml extract, solvent evap. and, ACN solvent exchange 1: 1	PSA-25mg, CA-10 mg, MgSO4-250 mg, all for 1ml extract; filtration 0,22 µm PTFE
AC	4 ml extract without prep.	
PG	1 g sample, 10 ml ACN extraction, with NACl (0.5 g and Mg SO4 (2g)	PSA-25mg, CA-10 mg, MgSO4-150 mg, all for 1ml extract, filtration 0,22 µm PTFE
BAC	0.2 g, 5 ml ACN extraction	PSA-25 mg, Mg SO4-150 mg, all for 1ml extract

where: EA-ethylic alcohol, AC-acetone, PG-propylene glycol, BAC-bioactive compound. The analysis was performed using an Agilent GC-MS equipment (7890A-5975C)–SIM mode. The acquisition parameters were HP-5MS column, 60 m x 0.25 mm, 0.25 µm, MMI Inlet with PTV solvent vent: 60^{O} C (0.35 min) → 600 ^{O}C/ min to 325 ^{O}C (5 min) , then 20 ^{O}C/min to 220 ^{O}C; 3µL injection volume; Oven profile: 50^{o}C=ct, 1 min, 25^{o}C/ min→ 190^{o}C, 0 min, 3^{o}C/min→202^{o}C, 0min, 1,5^{o}C/min→240^{o}C, 0 min, 5^{o}C/min→250^{o}C, 0 min, 2^{o}C/min→ 266^{o}C, 8^{o}C/min→290^{o}C, 15.2

min, run time = 62.5 min; solvent delay: 9 min.

Quantitation was performing through standard method, by Chemstation software using individual parameters for peak integration. The method is validated for 100 compounds, and the representative compounds for this study are shown in table 2, with the specific quantitation ions in the SIM mode and the initial plant concentrations.

Table 2. Pesticides monitored in the study

Pesticide	Activity	Quant Ion	Initial concentration in plant (mg/kg)
Chlorprofam	plant growth regulator	213	0.47
Terbutilazin	herbicide	173	0.02
Quintozene	fungicide	237	0.21
Diazinon	fungicide	304	0.27
Prothiofos	insecticide	267	0.24
γ HCH	persistent OCl	217	0.20
Tolclofos methyl	fungicide	265	0.33
Malathion	insecticide	285	0.67
Chlorpirifos	insecticide	199	0.09
Fenthion	insecticide	278	0.23
Dieldrin	persistent OCl	263	0.20
Deltametrin	insecticid	181	2.27

RESULTS AND DISCUSSIONS

A partial chromatogram, with the monitored compounds is shown in Figure 1, Figure 2 and 3 show overlapping chromatograms of pesticides with standard addition and the calibration curve for diazinon.

The transfer ratio between the initial level of pesticides in the samples and the alcoholic extract is between 15 and 76.8 %, for quintozene and terbuthylazyne, respectively.

The transfer ratio between the initial pesticide level in samples and the PG extract is between 6.8 and 55.8 %, for quintozen and dieldrin, respectively.

The acetone extraction and further concentration in preparation for BAC is a selective method, both increasing the BAC purity and decreasing the level of pesticides in the final product by a significant percentage (transfer ratio: 0.05-0.7 % in the final product).

The residues concentrations found in the different preparation methods are shown in table 3.

Fig. 2. Overlaping 304 ion for diazinon

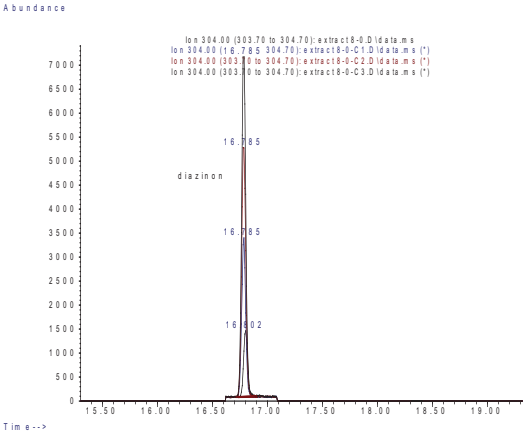

Fig. 3. Graphical representation of chromatographic responses using the standard addition method

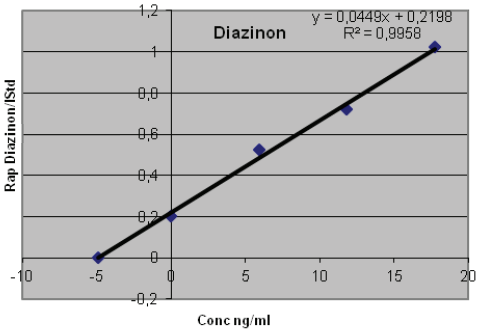

Fig.1 Section from the chromatogram with the monitored pesticides

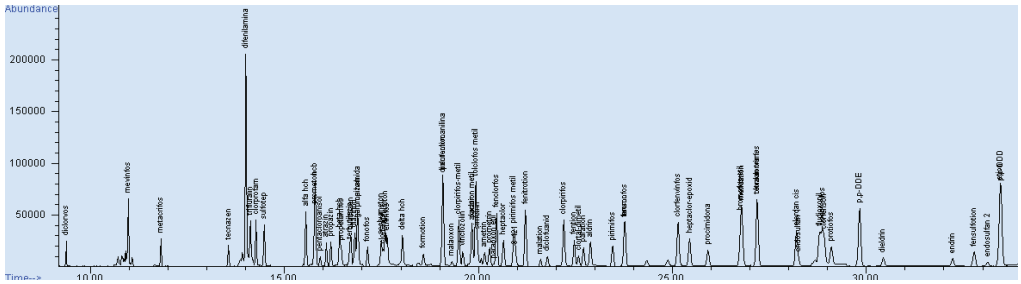

Table 3. The residues concentrations for different types of extraction

Pesticide	Recovery, % from initial quantity			
	AE extract	PG extract	acetonic extract	solid extract (BAC)
Chlorprofam	52	40	44	0.23
Terbutilazin	76.8	52	32	0.11
Quintozene	15	6.8	69	0.7
Diazinon	57.4	44	62	0.33
Prothiofos	28.6	15.5	69.5	0.44
γ HCH	75	50	72	0.5
Tolclofos methyl	51.6	20.73	80	0.3
Malathion	65	51	65	0.2
Chlorpirifos	50.2	30	99.5	0.55
Fenthion	27	17.7	41.5	0.05
Dieldrin	68.8	55.8	72	0.7
Deltametrin	45	25	80	0.1

CONCLUSIONS

The proposed analysis method is selective, and the peak separation in the SIM analysis conditions is appropriately used.

The quantification method through standard addition is adequate, as there is linearity between signals of aliquots with pesticides added in the final stage of analysis and the aliquot extracted without pesticide addition.

It is important to determine the ratio in which existing pesticides in the raw material transferred to extracts; extraction with alcohol allows retrieval of appreciable quantities of residues that remain at significant levels even if conditioning in propylene glycol is performed.

Acetone extraction followed by BAC crystallization was proved to be a very successful preparation method, as it leads to very low levels of residues in the solid extract for all pesticides (less than 1% of the initial concentration).

ACKNOWLEDGEMENTS

This project was partially funded under the Sectoral Operational Programme Promoting innovation in enterprises (POS-CCE), through project No 546/2013: "Innovative solutions to determine the extent of contamination by toxic residues - heavy metals and pesticides - in products of plant origin".

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