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Determination of Multiclass Pesticides in Dry Herbs Using GC-ECD

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ABSTRACT

Development and validation of a multi-residues analytical method for ten pesticides in aniseeds according to European guidelines was achieved. Baseline separation was achieved for most of the tested pesticides. GC/ECD equipped with 2 different polarity columns was used in this study for quantification and confirmation of the results. Method showed good linearity down to 0.025 μgml^{-1} and up to 0.25 μgml^{-1} levels. 2 g sample size offered extra ease of the method performance and minimized interferences and matrix load to the chromatograph injector and columns. The chosen pesticides possess wide range of physico-chemical properties in order to demonstrate the validity of the method to be applied for a wider range of pesticides. Matrix matched external standard method was employed for quantification using pesticide free blank extract. Accuracy (recovery) data ranged from 82.29 % (chlorothalonil) to 116.3 % (cypermethrin). Also, the method showed acceptable repeatability not higher than 13.94 % (deltamethrin). The overall mean recovery (102.54%) and its RSD (8.41%) show the adequacy of the whole method with expanded uncertainty of 16.82 %. The presented method showed acceptable accuracy results that fall in the required range between 80 % and 120 % and precision (RSD) not higher than 20 %. The obtained results meet the EU standard requirements and legislations to consider a method accurate and reproducible.

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INTRODUCTION

As population of the world grows, it becomes important to produce enough food to satisfy the needs of its inhabitants. Agrochemicals are used for crop protection, pest control and quality preservation (Soler *et al.*, 2005). The growth in pesticides consumption, poor agricultural practices and illegal use create significant risks to human health. Even following good agricultural practices, pesticide residues in food are unavoidable. Analytical chemistry plays important role in decreasing the potential hazards of pesticides to humans, by developing sensitive methods and monitoring pesticide residues in foodstuff. The desire to increase sample throughput while meeting the mandated detection limits is the main challenge facing the food safety testing labs today (Lehotay, 2007). Developing broad spectrum, low cost and quick sample preparation techniques especially for complex matrices can provide a solution. The common established extraction techniques are based on complex solvent extraction methods (Stan, 2000). These procedures have some cons such as time, labour consuming and require high amount of solvents (Fernandez *et al.*, 2000). That's why conventional extraction techniques are replaced with faster and easier protocols and the research in this field is up-warding day after day.

Herbs and medicinal plants are liable to contain pesticides residues that accumulated through different stages of cultivation and during the post harvest storage (Hajou *et al.*, 2004). Herbs are commonly used for several purposes worldwide in culinary, herbal tea preparations and in folk medicinal uses. Dry herbs form a challenging matrix in pesticide residue analysis as they contain high concentrations of interfering compounds such as lipids, chlorophyll, and sugars.

The aim of this work is to develop and validate a rapid, specific and sensitive multiresidue-analytical method for the routine analysis of ten widely used pesticides in aniseeds at concentration levels lower than their respective MRLs (EU pesticides database).

MATERIALS AND METHODS

1- Chemicals and Reagents:

Pesticides standards were purchased from Sigma–Aldrich, Germany. Anhydrous magnesium sulfate was purchased from Merck, Germany. Primary Secondary Amine (PSA) was purchased from Supelco, UK. Glacial

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acetic acid was purchased from Merck. All organic solvents used in the study were pesticide grade or HPLC grade and purchased from Merck, Germany.

2- Standard Preparation:

Individual stock solutions were prepared in toluene at 1.0 mgml^{-1} . A standard mixture solution, with all pesticides was prepared at $1.0 \text{ } \mu\text{gml}^{-1}$ in acetonitrile containing 0.1 % acetic acid. All standard solutions were stored at -20°C . Matrix-matched multi-standard solutions were prepared at 0.05 ppm using blank extracts of aniseeds.

3- Extraction Procedure:

Aniseed samples were extracted using modified QuEChERS method. 100 g sample was comminuted in an electrical grinder to give fine powder. 2 g of the ground material was taken into a 50 Teflon centrifuge tube and 10 ml deionized water was added and left to soak for 10 min. 10 ml acetonitrile (1% acetic acid) was added and the tube was shaken vigorously for 1 min. 4 g magnesium sulfate anhydrous (MgSO_4) and 1 g sodium chloride (NaCl) were added and the tube was shaken immediately for 1 min. The tube was centrifuged for 5 min at 3000 rpm. 1 ml aliquot of the upper layer was transferred into a Dispersive Solid Phase Extraction (DSPE) centrifuge tube and was shaken for 1 min. The DSPE tube was centrifuged for 1 min at 3000 rpm and 0.7 ml was filtered through 0.45 micron filter into a PTFE capped autosampler vial.

4- Instrumentation:

An Agilent 7890A gas chromatograph was used to carry out the present study. The system is equipped with two fused silica capillary columns, HP-5 and HP-35, of similar parameters, *i.e.*, length 30 m, 0.25 mm i.d., film thickness 0.25 μm . Each column is connected to a micro Electron Capture Detector (μECD). Oven temperature programme consisted of 1 min hold at 100°C , rise at $25^\circ\text{C}/\text{min}$ to 170°C , hold for 1 min, rise at $3^\circ\text{C}/\text{min}$ to 230°C , then hold for 1 min, rise at $8^\circ\text{C}/\text{min}$ to 300°C , then isothermal for 2min. Injector temperature 300°C /min. Carrier gas nitrogen, 2 mL/min. Purge gas, nitrogen 50 mL/min at 0.75 min. Detector μECD ^{63}Ni , temperature 320°C . Injection volume was 1 μL (splitless).

5- Method Validation:

The validation of the analytical method was carried out according to the European guidelines (European Commission Document SANCO/10684/2009).

5-1- Linearity and LOQ:

The evaluation of the calibration curve's linearity was carried out based on injections of the matrix matched standard solutions of each matrix at the concentrations 0.025, 0.05, 0.1 and 0.25 μgml^{-1} . Relative standard deviations (RSDs) and determination coefficients (r^2) were estimated for each pesticide. The Limits of Quantification (LOQs) were calculated according to Fajgelj and Ambrus (2000). The LOQ was established as the lowest concentration assayed, which gave satisfactory recovery (80–120%) and precision ($\leq 20\%$ RSD). For this purpose, six independent replicates of blank aniseeds samples were spiked with pesticides at a level of 0.05 mg/kg and analyzed against matrix matched reference.

5-2- Accuracy and precision:

The accuracy of the method was estimated by means of recovery experiments while repeatability of the method was evaluated through the Relative Standard Deviation (RSD). Accuracy and precision of the method were determined via performing recovery studies at the level of 0.05 mgkg^{-1} . Pesticide free samples of aniseeds were used as blank control matrix in recovery experiments and for the preparation of matrix-matched multi-level calibration solutions. The blank samples were previously analyzed to assure they are free of the tested pesticides. 2 g sample (six replicates) was fortified with the multi-standard solution in acetonitrile to give the required concentration and left to stand for 30 min prior extraction to allow pesticides absorption onto the dampened matrix. Samples were extracted using the mentioned procedure. Matrix matched external standard method was employed for the residues quantification.

5-3- Matrix matched standard:

2 g blank sample of ground aniseeds was extracted and clean-up was performed according to the mentioned procedure. Multi-standard solution was added prior the GC determination step to obtain the required concentration.

RESULTS AND DISCUSSION

1- Pesticides:

The selected pesticides show wide range of physico-chemical properties. Ten GC/ECD amenable pesticides were investigated in this study (Table 1). The physical-chemical properties of the analyzed pesticides varied

between the semi polar (K_{ow} 2.92, chlorothalonil) to low polarity (K_{ow} 6.6, cypermethrin). The molecular weights ranged from 265.9 (chlorothalonil) to 505.2 (deltamethrin) (Tomlin, 2004-2005). However, the selected pesticides are used for insect pests control except chlorothalonil used as a fungicide. The selected pesticides belong to different chemical groups i.e. pyrethroid, chloronitrile and arylpyrrole.

Table 1: Analyzed pesticides: polarity expressed as K_{ow} , molecular weight, action and chemical group.

Pesticide	K_{ow}	M.W	Action	Chemical group
Deltamethrin	4.6	505.2	insecticide	Pyrethroid
Cyfluthrin	5.9	434.3	insecticide	Pyrethroid
Fenvalerate	5.01	419.9	insecticide	Pyrethroid
Tefluthrin	6.4	418.7	insecticide	Pyrethroid
Permethrin	6.1	391.3	insecticide	Pyrethroid
Chlorothalonil	2.92	265.9	fungicide	Chloronitrile
Chlorfenapyr	4.83	407.6	insecticide	Arylpyrrole
Esfenvalerate	6.22	419.9	insecticide	Pyrethroid
Flucythrinate	4.7	451.5	insecticide	Pyrethroid
Cypermethrin	6.6	416.3	insecticide	Pyrethroid

2- Chromatographic Separation, Detector Response Range and Confirmation:

Baseline separation was achieved for most of the tested pesticides under the above mentioned chromatographic conditions. μ ECD dynamic linear range was up to 0.5 ppm for all the tested pesticides. European Commission guidelines (SANCO document no.10684/2009) mentioned that selective detectors employed with GC such as, ECD, when used with simultaneous separation in 2 different column types, offer acceptable level of confirmation and such verification method should be acknowledged when reporting the results.

3- Method Development:

Herbs in general and especially if dry form a challenging matrix for pesticide residue analysts as they contain high concentrations of interfering compounds. Up to our knowledge, normally 10 g sample is taken for the determination of the pesticide residues (Abou-Arab *et al.*, 1999) and (Wang *et al.*, 2011). The extract is concentrated prior determination step which by then contains high concentrations of matrix. Thus causes a matrix high load to the chromatograph and give more signals affecting the specificity of the method. In the present study, 2 g sample was taken in order to minimise the effect of the interfering components, taking in consideration that the ground sample is sufficiently homogenized.

4- Method Validation:

Aniseeds matrix was selected as a representative material for the development of a multiresidue analysis method. The method is still valid for a variety of dry herb matrices.

4-1- Linearity range and determination coefficient (r^2):

The evaluation of the analytical curves linearity was achieved based on injections of the standard solutions prepared in blank extract of aniseeds at the concentrations 0.025, 0.05, 0.1 and 0.25 μgml^{-1} . Determination coefficient (r^2) ranged from 0.96 to 0.98.

4-2- Matrix matched calibration standard:

Matrix-matched standards were prepared at 0.05 mg/kg (LOQ of the method) using blank extract of aniseeds. The specificity (interference) of the method was tested by the analysis of blank samples. Chromatographic analysis of the blank samples showed no peaks corresponding to the tested pesticides. Thus, indicated that the used blank samples were free of the tested pesticides also were free of interfering matrix compounds that would give false positive results.

4-3- Accuracy, precision and method LOQ:

Data in table 2 show good rate of recoveries of the tested pesticides. It ranged from 82.29 % (chlorothalonil) and 116.3 % (cypermethrin). Also, the method showed accepted repeatability that ranged from 3.53 % (permethrin) to 13.94 % (deltamethrin). However, results obtained by Wang *et al.*, 2011, Hajou *et al.*, 2004 and Tekel and Hatik, 1996, varied from 80-120 % for recovery rates and up to 20 % for repeatability which support the results obtained in the present study.

Table 2: Accuracy and precision data of the analyzed pesticides in aniseeds.

Pesticide	0.05 mg/kg		LOQ (mg/kg)	MRL (mg/kg)
	Recovery (%)	RSD		
Deltamethrin	107.34	13.94 13.94809204 8	0.05	0.05
Cyfluthrin	93.03	12.7	0.05	0.1
Fenvalerate	110.18	13.7	0.05	0.05
Tefluthrin	109.35	5.45	0.05	0.05
Permethrin	99.29	3.53	0.05	0.1
Chlorothalonil	82.29	6.01	0.05	0.1
Chlorfenapyr	97.86	6.00	0.05	0.1
Esfenvalerate	106.02	5.53	0.05	0.05
Flucythrinate	103.74	3.81	0.05	0.1
Cypermethrin	116.30	5.55	0.05	0.1
Mean	102.54	8.41		
Uncertainty	16.82			

The overall mean recovery (102.54%) and its RSD (8.41%) show the adequacy of the whole method. Considering a coverage factor ($k = 2$), (Kmellar *et al.*, 2008) an expanded uncertainty was estimated as being 16.82. This expanded uncertainty value is in agreement with the estimation made from the European Proficiency Test schemes, where an expanded uncertainty budget of $\pm 50\%$ is applicable as the default value, (European Commission Document SANCO/10684/2009). The obtained results support the method to meet the European mandates for method validation, which require accuracy range between 80 and 120 % and precession RSD not higher than 20 %, (European Commission Document SANCO/10684/2009).

Conclusion:

Development and validation of a multi-residues analytical method for ten pesticides in aniseeds according to European guidelines was achieved. Baseline separation was achieved for most of the tested pesticides. GC/ECD equipped with 2 different polarity columns was used in this study for quantification and confirmation of the results. 2 g sample size offered extra ease of the method performance and minimized interferences and matrix load to the chromatograph injector and columns. The chosen pesticides possess wide range of physico-chemical properties in order to demonstrate the validity of the method to be applied for a wider range of pesticides. The presented method showed acceptable accuracy results that fall in the required range between 80 % and 120 % and precision (RSD) not higher than 20 %. The obtained results meet the EU standard requirements and legislations to consider a method accurate and reproducible.

REFERENCES

- Abou-Arab, A.K., K.M. Soliman, M.E. El Tantawy, I.R. Badaea and K. Naguib, 1999. Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *Food Chemistry*, 67: 357-363.
- EU-Pesticides Database. Active Substances (Directive 91/414/EEC); EU-Pesticide MRLs (Regulation (EC) No 893/2010). 2010.
- European Commission Document SANCO/10684/2009. Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed. pp 3-40.
- Fajgelj, A. and A. Ambrus, 2000. Principles and practices of method validation, The Royal Society of Chemistry, UK.
- Fernandez, M., Y. Pico and J. Manes, 2000. Determination of carbamate residues in fruits and vegetables by matrix solid-phase dispersion and liquid chromatography–mass spectrometry. *J. Chromatography A*, 871: 43-56.
- Hajou, R.M., F.U. Afifi and A.H. Battah, 2004. Comparative determination of multi-pesticide residues in *Pimpinella anisum* using two different AOAC methods. *Food Chemistry*, 88: 469-478.
- Luke, M.A., J.E. Froberg and H.T. Masumoto, 1975. Extraction and cleanup of organochlorine, organophosphorus, organonitrogen and hydrocarbon pesticides in produce for the determination by gas-liquid chromatography. *J. AOAC Int.*, 58: 1020-1026.
- Kmellar, B., P. Fodora, L. Parejab, C. Ferrerc, M.A. Martinez-Urozc, A. Valverdec and A.R. Fernandez-Albac, 2008. Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography–tandem mass spectrometry. *J. Chromatography A*, 1215: 37-50.
- Lehotay, S.J., 2007. Determination of pesticide residues in foods by Acetonitrile extraction and partitioning with magnesium sulphate: collaborative study. *J. AOAC int.*, 90: 485-520.
- Soler, C., J. Manes and Y. Pico, 2005. Routine application using single quadrupole liquid chromatography–mass spectrometry to pesticides analysis in citrus fruits. *J. Chromatography A*, 1088, 224-233.

Stan, H.J., 2000. Pesticide residue analysis in foodstuffs applying capillary gas chromatography with mass spectrometric detection State-of-the-art use of modified DFG-multimethod S19 and automated data evaluation. *J. Chromatography A*, 892: 347-377.

Tekel, J. and S. Hatik, 1996. Pesticide residue analyses in plant material by chromatographic methods: clean-up procedures and selective detectors. *J. chromatography A*, 754: 379-410.

Tomlin, C.D.S. Ed., 2004-2005. The British Crop Protection Council. UK. The e-Pesticide Manual, Version 3.1, 13th ed.

Wang, Y., Y.J. Hong, S.C. Mab, J. Lua and R.C. Lina, 2011. Determination of 195 pesticide residues in Chinese herbs by gas chromatography–mass spectrometry using analyte protectants. *J. Chromatography A*, 1218: 334-342.