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Investigation of pesticide residues in apple fruits from conventional and integrated pest management

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Abstract

This paper describes method validation for determination of more than 40 pesticides in apples using GC technique. Target compounds belong to the groups of organochlorine, organophosphorus, carbamates, pyrethroids, triazoles, dicarboximides, strobilurins etc. The sample preparation consists of acetone extraction and subsequent cleanup/concentration by SPE with polymer-based sorbent. Single quadrupole GC-MS operating in SIM mode and electron impact ionization was used for identification and quantification of the pesticides. The average recoveries obtained for analytes ranged between 70 and 110 % at three fortification levels - 0.01, 0.1 and 0.2 mg kg⁻¹. The relative standard deviation was lower than 20 % for all tested compounds. The calculated limits of detection and limits of quantification were below 0.01 mg kg⁻¹ which were sufficiently low compared to the maximum residue levels (MRLs) established by European legislation. The proposed method was applied for determination of pesticide residue in four selected apple varieties after harvesting. Whole and processed fruits (peel and peeled fruits) were analyzed from different schemes of treatment: two conventional, one based on integrated pest management (IPM) and two variants based on organic production (controls). Higher level of pesticide residues were found in apple fruits under conventional conditions. Fenitrothion and chlorpyrifos residues were detected frequently in apple peel at concentrations up to 0.45 and 0.77 mg kg⁻¹, respectively. The levels found in the whole fruits of the same samples were much lower than in peels and below the respective MRLs (0.5 mg kg⁻¹ for both pesticides). In the apples from IPM were found only measurable residues of triadimenol, concentrated in the peels up to 0.05 mg kg⁻¹.

Key words: GC/MS Chromatography; Pesticide residues; apple fruit

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Introduction

Apple production in Bulgaria is one of the most vulnerable crops to pest damage due to serious problems caused by a number of pests namely apple scab (*Venturia inaequalis*), powdery mildew (*Phodoshpera leucotricha*), fire blight (*Erwinia amylovora*), codling moth (*Cydia pomonella* Linnaeus), leopard moth borer (*Zeuzera pyrina*) and aphids (Bobev et al. 1999; Kutinkova et al. 2006; Velcheva et al. 2006). In most cases the use of plant protection products (PPPs) is the only possibility for reducing these losses. Usually, the number of pesticide applications in conventionally treated orchards is about 10-15 times per year (Andreev et al. 2004). Nearly all of these treatments contain broad spectrum insecticides and fungicides.

In Bulgaria in 2007, 35 fungicides, 51 insecticides and 22 herbicides, containing a total of 62 active substances are in the list of authorized PPPs for placing on the market and use for apple production (National Service for Plant Protection. 2007). The large number of pesticide applications associated with apple production and the diversity of PPPs increases the potential risk for human exposure and harmful impact to the environment. To ensure food safety for consumers, the European Commission has established maximum residue limits (MRLs) for pesticides in foodstuffs, which are also regulated in Bulgarian legislation (European Parliament, 2005; Ministry of Health, Ordinance No 31. 2003). Adequate, reliable and rapid analytical methods for determination of pesticide residues are needed to provide control of the safety of production and PPPs usage.

Common procedure for analysis of pesticide residues consists of extraction from food commodity, cleaning of co-extracted materials and determination by instrumental techniques that separate analytes as gas chromatography (GC) or liquid chromatography (LC) (Alder et al., 2006). Multiresidue methods (MRMs) became a great opportunity for simultaneous determination of large number of pesticides and therefore are preferable because of their speed, low cost and high sample throughput. The main difficulty in development of MRMs is setting up optimum conditions in order to obtain satisfactory metrological parameters for analysis of pesticides with various physicochemical properties (Bolanos et al. 2007). Experienced residue analysts are aware that no validation report prepared in another

laboratory can be accepted without verification that the method does indeed work in the analyst's own laboratory (FDA Pesticide Analytical manual. 1999).

In order to minimize the negative effects that conventional apple production pose to the environment and human health, some alternative cultivation systems were developed (Tsakiris et al. 2004). Integrated pest management (IPM) is a sensitive approach that relies on current, comprehensive information on the life cycles of pests and their interaction with the environment. This information is used for drawing up of predictive models which allow growers to apply chemicals when is absolutely necessary. During last decades application of different IPM programs has started in many Bulgarian orchards (Andreev et al. 2003). The obtained results have showed that in orchards where the IPM approach was used the number of pesticides applications can be reduced considerably.

The aims of the present study were:

- to develop and validate GC-MS method for simultaneous determination of 42 pesticides frequently used in the apple growing process
- to estimate residual amounts of PPPs applied in different schemes of treatment: conventional agriculture and integrated pest management (IPM)
- to study the effectiveness of IPM approach for minimization of pesticide residues in apple production
- to investigate the pesticide residues distribution in apple fruit through analysis of residual pesticides in whole and processed fruits (peeled apples and peels).

Materials and methods

Samples

Totally fourteen representative samples of four different varieties of apples (Florina, Granny Smith, Erwin Baur and Prima) were collected in amount about 10 units (2.5 kg) during the harvest time and the sampling was performed according to Commission Directive 2002/63/EC (Comission Directive 2002/63/EC). The trees were treated by different schemes of application of PPPs: two conventional, one based on integrated pest management (IPM) and two variants based on organic production (controls). In order to obtain data about the pesticide distribution in the fruits from each treatment scheme, the laboratory sample from each variant was divided into three parts – a half of the sample was peeled and produce peeled fruits and peels, and remained half of whole fruits was processed unpeeled. All three derived

samples for each variant were chopped with kitchen robot and after homogenization, an analytical sample for each of them was stored at -18°C until their analysis.

Reagents

Analytical standards of the pesticides and ethion as internal standard (IS) with certified purity were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Riedel-de Haen (Seelze, Germany). Acetone, ethyl acetate and methanol (gas chromatography grade) were purchased from Merck KGaA (Darmstadt, Germany). The list of analytes with selected physicochemical properties (selected ions monitored, water partition coefficients and water solubility) are given in Table I. Stock solutions of each pesticide were prepared by dissolving in ethyl acetate or acetone at concentration ranged between $960\text{--}1420\mu\text{g ml}^{-1}$ and was stored in a freezer at -18°C . Mixed standard solution of all target pesticides was prepared in acetone at concentration $10\mu\text{g ml}^{-1}$ and was used for the preparation of working standards and fortification. Matrix-matched standard solutions were prepared by adding appropriate amounts of standard solutions to pesticide-free apple extract before the solid phase extraction step. Acetone solution of ethion with concentration $100\mu\text{g ml}^{-1}$ was used as IS in order to compensate sample and injection volume changes. An equal volume of IS was added to the final extracts immediately prior to GC analysis. Polymer-based sorbent LiChrolut® EN with surface area $1200\text{ m}^2\text{ g}$ and particle size $40\text{--}120\mu\text{m}$, and weight 500 mg was commercially prepacked in 6 ml cartridges and was purchased from Merck KGaA (Darmstadt, Germany).

Apparatus

A Thermo Finnigan Trace GC Ultra (Thermo Finnigan, Milan, Italy) coupled to Trace DSQ MSD (Thermo Finnigan, Austin, Texas, USA), equipped with split/splitless injector and Varian FactorFour capillary column VF 5ms, $30\text{ m} \times 0.25\text{ mm ID} \times 0.25\mu\text{m}$ film thickness was applied. A Thermo Finnigan AI 3000 autoinjector (Thermo Finnigan, Milan, Italy) was used to perform automatically $1\mu\text{l}$ injections. Xcalibur 1.3 data system software was used for data acquisition and processing. The carrier gas He was of purity 99.9995% (SIAD, Bulgaria). Vortex mixer V-1 plus (Boeco, Germany) and centrifuge Rotofix 32A (Hettich GmbH&Co.KG, Germany) were used in the sample preparation process. Vacuum manifold Vac-Elut (Varian Inc., The Netherlands) coupled to vacuum pump 2012C (Welch Rietschle Thomas, USA) was used for SPE. Ultrapure water was prepared with Water pro PS (Labcono, USA).

Sample preparation

The following procedure was previously described by Stajnbaher (Stajnbaher et al. 2003) but some changes were made in order to adjust and optimize the methodology for present study. The used sample preparation procedure for the three types of sample (peels, peeled apples and whole fruits) was as follows: 10 g of analytical samples were weighed into 50 ml centrifuge polypropylene tubes with screw caps, 20 ml of acetone were added, cap was tightened and the mixture was vigorously shaken by Vortex for 5 min. Then centrifugation was performed at 3000 rpm for 5 min and the supernatant was transferred in a glass cylinder in order to measure the volume. The exact half of the raw extract was diluted with water to 70 ml and was allowed to pass through the LiCrolut EN cartridge with maximum flow rate 8 ml min⁻¹. Before loading the cartridge was conditioned with 6 ml ethyl acetate, followed by 6 ml methanol and 8 ml ultra pure water. After loading the cartridge was washed with 10 ml water and the sorbent was dry under vacuum (about -20 mmHg) for approximately 30 min. Then the retained pesticide residues were eluted with 2 ml ethyl acetate followed by 7 ml mixture ethyl acetate:acetone 9:1, after that the eluates were evaporated under gentle nitrogen stream to final volume of 5 ml (1 g matrix in each ml extract). Finally 50 µl of IS were added and 1µl of the extract was injected in the GC-MS system.

GC-MS conditions

Splitless injection of 1 µl was carried out at 230°C and splitless time 1 min. The temperature program applied was as follows: 70°C hold 1 min, then increased at rate 30°C min⁻¹ to 190°C, hold 1 min again increased to 235°C with rate 5°C min⁻¹ and finally increased at rate 15°C min⁻¹ to 280°C, hold 8 min. The carrier gas flow rate was 1.0 ml min⁻¹. The mass spectrometer was operated in electron impact ionization mode (ionization energy 70 eV) at transfer line and ion source temperatures set on 250°C and 220°C, respectively. Selected ion monitoring (SIM) mode by detecting minimum 3 ions for each analyte was used (Table I). Depending on the number of monitored ions in one segment dwell time was set from 60 to 10 ms which resulted to scan times between 0.24 and 0.34 s. Performance criteria for estimation of analytical method were based on current European recommendations (European Commission, DG SANCO, Document N° SANCO/2007/3131; European Commission, Commission decision of 12 August 2002).

Results and discussion

Analytical parameters were calculated for the multi-residue method proposed (Table II). The accuracy of the method was expressed as recovery by fortified blank samples at three concentration levels - 0.01, 0.1 and 0.2 mg kg⁻¹ with three replicates for each level and three injections for each replicate (totally 9 injections per level). Recoveries obtained were in the range 70.2-109 % which complies with the requirements of European Commission concerning pesticide residues analysis in food (European Commission, DG SANCO, Document N° SANCO/2007/3131). Precision was calculated in terms of relative standard deviation (RSD) and was ranged between 2.8-20.4 %. Matrix matched standards at 5 concentrations 0.01, 0.05, 0.10, 0.25 and 1.0 mg kg⁻¹ and internal standard approach were used for calibration. The corresponding pesticides were added to the blank apple extract before SPE step, instead to the final extract as the custom proposes due to low elution degree of some pesticides (as pyrethroids) from the SPE cartridge. Prior addition and passing the matrix matched standards through the cartridge standardize SPE conditions both for the samples and the matrix matched standards and eliminate the differences.

The calibration curves for all investigated pesticides were linear with coefficients of determination higher than 0.98 in the range 0.01-1.0 mg kg⁻¹ with exception for endosulfan II for which the concentration range was 0.05-1.0 mg kg⁻¹. Limits of quantification (LOQs) calculated as the lowest concentration of compound that give a response ten times higher than background noise, ranged from 0.002 to 0.01 mg kg⁻¹ (with exception for endosulfan II – 0.011 mg kg⁻¹) and were lower than established MRLs. The obtained results showed that the proposed analytical method can be used for effective control of pesticide residues in apples.

The analysis of processed apple samples (whole fruits, peeled apples and peels) has showed presence of some pesticide residues (Table III) mostly in the peels and smaller amounts in the whole fruits of conventionally treated apples. Residues which exceed the set MRL were detected in only one sample – 0.060 mg kg⁻¹ fenitrothion in the whole fruits of variety Florina. The level of fenitrothion in the peels of the same sample was 7.5 times higher - 0.45 mg kg⁻¹. Additional calculations were made in order to find whether the level in the whole fruits exceed the toxicological limit for fenitrothion - acute reference dose (ARfD) of 0.013 mg kg⁻¹ bw d⁻¹ (Document 3010). The calculations made according to EU guidance (European Commission, DG SANCO, Document N° SANCO/3346/2001 rev 7) showed that the

predicted short term intake (PSTI) represent 6.1 % of the ARfD which means that the found violation does not present any risk for consumers as far as concern the acute toxicity. The highest amount of residues was found for chlorpyrifos in the peels of variety Florina – 0.77 mg kg⁻¹ (Figure 1). In the whole fruits of the same sample the residue level of chlorpyrifos was 0.11 mg kg⁻¹ which is nearly 5 times lower than the established MRL of 0.5 mg kg⁻¹. Measurable residues of tebuconazole, difenoconazole, myclobutanyl and hexaconazole were found only in apple peels. In whole fruits sub samples the residues found were below the lowest calibration level (LCL).

In the apples grown by the IPM approach only in a few samples residues of triadimenol and fenitrothion were found in the peels at concentrations ranged between 0.019 and 0.049 mg kg⁻¹. In the whole fruits triadimenol and pirimicarb were detected but below the LCL. In the peeled fruits neither conventionally nor IPM treated was found any pesticide residues.

Conclusions

The proposed analytical method possesses satisfactory metrological and economic characteristics and can be used for effective control of the most frequently applied PPPs. The analyses of real samples indicated that the IPM approach allows much safer production of apples compared to conventional application of PPPs. Obtained data related to the distribution of residues in apple fruit showed that the presence of pesticide residues can be minimized almost completely by peeling of the apple fruits.

Acknowledgements

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Caption of figures

Figure 1. Chromatograms of chlorpyrifos in a) matrix-matched standard solution – concentration 1.0 mg kg⁻¹ and b) peels of apple sample Florina variety contained 0.77 mg kg⁻¹ of the analyte.

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Table III. Levels (in mg kg⁻¹) of pesticide residues found in peels and whole fruits of the analyzed apple samples.

	Apple varieties	Prima		Erwin Baur		Florina		Granny Smith	
	MRL mg kg ⁻¹	Peels	Whole fruits	Peels	Whole fruits	Peels	Whole fruits	Peels	Whole fruits
Conventional treatment									
Chlorpyrifos	0.5	0.15	0.023	0.26	0.035	0.074÷0.77	0.010÷0.11	0.62	0.079
Tebuconazole	1*	<0.01**	-***	-	-	0.038	<0.01	0.013	<LCL
Hexaconazole	0.1	0.028	0.010	<0.01	-	<0.01	-	-	-
Myclobutanil	0.5	-	-	-	-	0.026	<0.01	0.018	<0.01
Difenoconazole	0.5*	-	-	-	-	0.040	<0.01	0.014	<0.01
Fenitrothion	0.01	-	-	-	-	0.45	0.06	-	-
Integrated Pest Management									
Pirimicarb	2*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	-
Triadimenol	0.2	0.045	<0.01	0.049	<0.01	0.019	<0.01	-	-
Fenitrothion	0.01	-	-	-	-	0.030	<0.01	-	-

* - Temporary MRLs
** - Detected but below the lowest calibration level (LCL)
*** - Not detected

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