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Chapter 5

Analysis of Pesticide Residues in Chili (*Capsicum annuum* L.) using Ultra Performance Liquid Chromatography with UV Detection

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Additional information is available at the end of the chapter

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Abstract

The aim of this study was to analyze the pesticide residues in chili samples, collected from farmer's field. Ultra performance liquid chromatography (UPLC) with BEH C_{18} column was used for this analysis work. A cheap and fast method for the simultaneous quantification of 12 residue of pesticides in chili has been developed. Samples were prepared according to Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) method and quantification was performed by using tunable ultra violet (TUV) detector. The method was applied for the analysis of the chili samples and results showed that most of the samples have detectable pesticide residues. The residues of acetamiprid and thiodicarb were detected only in three samples, whereas flubendiamide and mancozeb were detected in six samples and arbosulfan and Spinosad were detected in two and five samples, respectively. Out of the 30 chili samples, only 11 samples were found to be contaminated with pesticide residues with more than maximum residue limits (MRLs).

Keywords: pesticide residues, ultra performance liquid chromatography (UPLC), QuEChERS, chili

1. Introduction

Chili [*Capsicum annuum* L.] is one of the major spice crop in India. Indian chilies have gained global demand due to high color value and low pungency [1]. The total world production of red chili is estimated to be around 21 lakh tons, 45% of which is produced in India [2]. The

world spice production statistics records a bulk of 86% by volume, making the country the largest producer of spices, in addition to it being the largest consumer and exporter of spices in the global context [3, 4]. Chili has high medicinal value due to the abundance of availability of carotenoids, capsaicinoids [5], oleoresins, and mineral content [6]. Most of the studies have demonstrated that consumption of chili rich diets, increases in energy expenditure and oxidation of fat, and also it helps in the curing of many diseases [7].

Intensive agriculture practice receives most of the pesticides during different stages of cultivation. Pesticides increase crop productivity, reduce cost of production, improve quality, and thus help to increase in the farmers' income. The role and contribution of pesticides will be much more in the coming years, especially in the developing country like India. The demand for food continues to grow steadily due to growth of population. Although modern polar pesticides like organophosphorus and carbamates that replaced classical organochlorine pesticides are less persistent. There are more than 800 pesticide molecules used to control pests and also weeds [8, 9]. It is not possible to control the residues of pesticides in food commodities; hence, these compounds will accumulate in the human body after consumption through diets [10]. Hence, to overcome the effects of pesticides on different groups, the uniform maximum residue limits (MRL's) was established as 0.01 mg/kg for any pesticides [10].

In order to determine such a low level of detection of various analytes in the sample, a sophisticated instrument like gas chromatography (GC) or liquid chromatography (LC) have to be used for accurate separation and determination. With the advancement in the detectors in gas chromatography techniques namely electron capture detector (ECD), thermal conductivity detector (TCD), nitrogen phosphorus detector (NPD), and mass spectrometry detector (MSD), hence it is widely used in all analysis. Recently, polar and thermolabile pesticide analysis, liquid chromatography is used as alternative technique, where as these pesticides are not determinable by gas chromatography [11, 12]. For the analysis of wide range of polar pesticide residues in food commodities high-performance liquid chromatography mass spectrometry (HPLC–MS/MS) has become the important technique by choice [13].

Most of the published methods either expensive or involves laborious procedure for cleanup step during the extraction procedure, hence there is a chance of losing some quantity of analyte molecule. Similarly, some problems arise in the solvent exchange step, before applying the extract to the LC column, makes preparation of sample procedure less effective. Many challenges exists both in use of sophisticated equipments and sample handling procedure during pesticide residue analysis. In order to avoid such a complication in sample preparation, it is necessary to adopt Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) method. The ultra performance liquid chromatography (UPLC) is having more advantages than routine high-performance liquid chromatography (HPLC) system in terms of lesser retention time, resolution, and more sensitivity [14]. The UPLC separation was faster (six times) than regular HPLC system with monolithic column [15, 16]. And also, it consumes 80% of less mobile phase than normal HPLC system. The aim of the present study is to analyse the 12 pesticide residues with UPLC system using QuEChERS extraction method and critically determine the replacement of HPLC method with new UPLC method.

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2. Experimental

2.1. Chemicals and materials

The certified reference materials (CRM's) of acetamiprid (purity 99%), benomyl (99%), flubendiamide (98.5%), indoxacarb (98.5%), carbosulfan (99%), imidacloprid (98%), methomyl (99%), thiodicarb (96%), spinosad (99%), oxydemeton-methyl (99%), difenoconazole (98.5%), and mancozeb (98.5%) for this study were obtained from Dr. Ehrenstorfer GmbH, Augsburg, Germany. HPLC grade solvents (acetonitrile, methanol, acetic acid, and formic acid) were obtained from Merck India Ltd. (Mumbai, India). Mobile phase water was prepared using millipore water purification system. Anhydrous sodium acetate and magnesium sulfate were procured from Sigma-Aldrich (Germany). And primary secondary amine (40 μm, Bondesil PSA) was purchased from Agilent Technologies (Bangalore, India).

2.2. Selection of pesticides

As many as 12 pesticides (**Table 1**) were used in this study, which are liquid chromatography amenable. And these pesticides are monitered in chili for the export to European Union. The pesticides chosen were those most often sprayed in chili cultivation.

2.3. Collection and storage of chili samples

Thirty chili samples (**Tables 2** and **3**) were collected randomly from different farmers' field of Haveri district, Karnataka, India. Two kilograms of each sample was taken, sealed in polythene bags, and stored at −4°C in deep freezer for further processing.

Pesticides	Retention time (RT)	Correlation coefficient (R^2)	Limit of detection (LOD) (mg/kg)	Limit of quantification (LOQ) (mg/kg)
Acetamiprid	2.544	0.9969	0.0010	0.0030
Benomyl	3.420	0.9971	0.0005	0.0015
Flubendiamide	3.802	0.9988	0.0005	0.0015
Indoxacarb	4.502	1.0000	0.5000	0.1500
Carbosulfan	5.975	1.0000	0.0005	0.0015
Imidacloprid	6.200	0.9986	0.0005	0.0015
Methomyl	6.431	0.9999	0. 0005	0.0015
Thiodicarb	6.556	0.9998	0.0005	0.0015
Spinosad	8.738	0.9999	0.0005	0.0015
Oxydemeton-methyl	8.997	0.9970	0.0005	0.0015
Difenoconazole	10.013	1.0000	0.0005	0.0015
Mancozeb	10.561	0.9999	0.0005	0.0015

Table 1. Retention time (RT), correlation coefficient (R2), limit of detection (LOD), and limit of quantification (LOQ) of 12 reference standards.

Name of pesticides	No. of chili samples (Residues in ppm)															
	MRLs prescribed by EU in ppm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acetamiprid	0.30	**0.03**	ND	ND	**0.03**	ND	ND	ND	ND	ND	**0.04**	ND	ND	ND	ND	ND
Benomyl	0.10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flubendiamide	020	**0.20**	ND	ND	0.28	ND	ND	ND	ND	**0.35**	ND	ND	ND	ND	ND	**0.20**
Indoxacarb	0.30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carbosulfan	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**0.06**	ND
Imidacloprid	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methomyl	0.02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Thiodicarb	0.02	ND	ND	ND	**0.02**	ND	ND	ND	ND	ND	ND	ND	ND	ND	**0.02**	ND
Spinosad	2.00	**4.0**	ND	ND	**2.5**	ND	ND	ND	ND	**2.00**	ND	ND	ND	ND	ND	ND
Oxydemeton-methyl	0.01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Difenoconazole	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mancozeb	5.00	**5.0**	ND	ND	**5.1**	ND	ND	ND	ND	**5.0**	ND	ND	ND	ND	ND	**5.6**

Name of pesticides	No. of chili samples (Residues in ppm)															
	MRLs prescribed by EU in ppm	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Acetamiprid	0.30	0.30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benomyl	0.10	0.10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flubendiamide	0.20	0.20	ND	ND	ND	ND	ND	ND	ND	**0.22**	ND	ND	ND	ND	ND	**0.20**
Indoxacarb	0.30	0.30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Name of pesticides	No. of chili samples (Residues in ppm)															
	MRLs prescribed by EU in ppm	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Imidacloprid	1.00	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methomyl	0.02	0.02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Thiodicarb	0.02	0.02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**0.03**	ND	ND	ND
Spinosad	2.00	2.00	ND	ND	ND	**2.0**	ND	ND	ND	ND	ND	ND	ND	ND	ND	**2.2**
Oxydemeton-methyl	0.01	0.01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Difenoconazole	0.05	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mancozeb	5.00	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not detected.

Table 2. Monitoring of pesticide residues in chili samples collected from farmers field of Haveri district, Karnataka using UPLC.

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Sl. no	Name of pesticide	Number of positive samples	Incidence of residence (%)
1	Acetamiprid	3	10.00
2	Flubendiamide	6	20.00
3	Carbosulfan	2	6.66
4	Thiodicarb	3	10.00
5	Spinosad	5	16.66
6	Mancozeb	6	20.00

Table 3. Incidence of pesticide residues in 30 chili samples collected from farmer's field of Haveri district, Karnataka.

2.4. Preparation of reference standards

The individual stock solutions were prepared by exactly weighing 10 (±0.01) mg of certified reference standards in volumetric flask, dissolved in 10 ml methanol (1000 ppm), and were stored in a refrigerator −10 (±2)°C. Intermediate standards were prepared by diluting the stock solutions of 10 ppm and mix these with appropriate quantities for standard mixture preparation with acetonitrile. And these were stored at −10 (±2)°C and was used for 3 months. A working standard was prepared for diluting these intermediate stock solutions. Calibration plot was constructed using these standards.

2.5. Calibration

Five different standards of different concentrations like 500 ppt, 1 ppb, 10 ppb, 1 ppm, and 10 ppm were prepared using a serial dilution technique from 10 ppm concentration with acetonitrile as a solvent. For the same concentration levels, matrix matched standards were prepared in chili using the procedure mentioned in Section 2.6. Before doing this exercise, control chili samples were screened for the confirmation of absence of pesticide residues of the interest.

2.6. Sample preparation

Modified QuEChERS method was adopted for the preparation of the chili samples. The method involves crushing of 2 kg chili samples under ambient laboratory conditions. The 200 g of chili sample was further homogenized for 2 min and then 10 g of this sample were transferred in 50 ml polypropylene tubes and extracted with 10 ml acetonitrile (1% acetic acid)) in presence of 6 g anhydrous magnesium sulfate and 1.5 g sodium acetate. Then homogenization of the mixture was done at 15,000 rpm for about 2 min and centrifuged for 5 min at 6000 rpm. Dispersive solid phase extraction (d-SPE) was employed for the supernatant (1 ml) cleaning using 50 mg primary secondary amine (PSA) and 150 mg $MgSO_4$, which completely removes carbohydrates and fatty acids [17]. The supernatant was centrifuged at 3000 rpm for 5 min and the filtered through polyvinylidene difluoride (PVDF) membrane filter and transferred to auto sampler vial.

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2.7. UPLC analysis

UPLC analysis was carried out using an ACQUITY UPLC™ system (Waters, USA), and separation was performed using Acquity UPLC BEH C18 (100 mm × 2.1 mm) with 1.7 μm particle size. The mobile phases used were (A) acetonitrile and (B) 0.1% formic acid. The gradient was linear from 0 to 30% A for 11 min and from 30 to 100% A for 1 min, followed by washing with B and re-equilibration of the column for 2 min were maintained for re-equilibration of the column to original state. The optimized parameters used were 0.2 mL/min flow rate, 45°C column temperature, and 25°C sample temperature and volume of injection was 1 μL throughout the analysis. Absorbances were recorded on-line at 280 nm using TUV detector.

3. Results and discussion

3.1. Optimization of chromatographic separation conditions

Mobile phase namely acetonitrile was used for the optimization of the system for the separation of reference standards using UPLC BEH C18 column. Generally, with change in the concentration of formic acid, the retention time of the individual standard varies. With the optimized gradient steps, we got good separation of the 12 standards with 0.1% formic acid (**Figure 1**). The optimum parameters used for this experiments were as follows: the mobile phase gradient was linear from 0 to 30% A for 11 min and from 30 to 100% A for

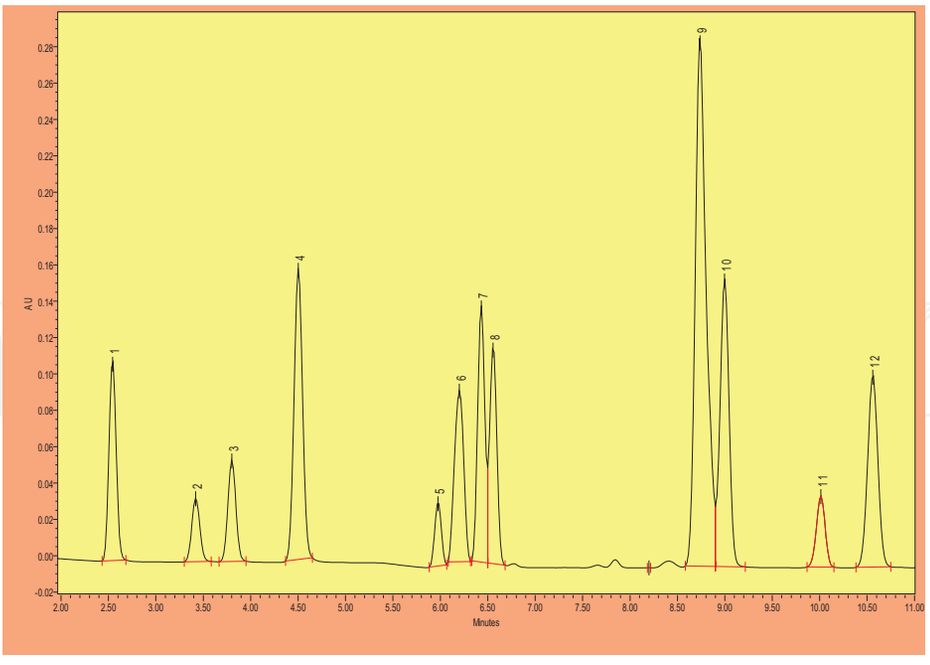

Figure 1. UPLC–UV chromatogram of a mixture of the following 12 pesticide reference standards, detected at 280 nm: acetamiprid (1), benomyl (2), flubendiamide (3), indoxacarb (4), carbosulfan (5), imidacloprid (6), methomyl (7), thiodicarb (8), spinosad (9), oxydemeton-methyl (10), difenoconazole (11), and mancozeb (12).

1 min, 0.2 mL/min flow rate, column and sample temperature were 45 and 25°C, respectively, injection volume was 1 μL and detection was done at 280 nm.

3.2. QuEChERS sample preparation method

As described, QuEChERS methodology [18, 19] have been adopted for the determination of 12 pesticide residues in chili. QuEChERS methodology have been devised in the year 2003 for the multiresidue analysis of pesticides in different matrices [20], and now it is universally accepted method [17]. In this procedure, extraction was performed with acetonitrile solvent initially and then partitioning step was carried out using salt mixture. A small amount of extract was further cleaned by using dispersive solid-phase extraction (d-SPE) method. Finally, extract was used for the determination of pesticide residues using UPLC. The advantages of this method include the large number of samples, and very low quantity of solvent and limited space are required [18, 21]. The acetonitrile has several advantages namely upon addition into salt, it will separate easily, good compatibility with d-SPE. The use of primary secondary amine removes acidic components, sugars and pigment molecules [18]. Another advantage is the removal of the waxes, lipids, and sugars during the freezing process. The pH of the extract will increases when it comes in contact with PSA [22]. This can be used as the stability of base-sensitive pesticides.

3.3. Method validation

Developed method has been validated after the optimization of the UPLC separation parameters. Limit of detections (LODs) were calculated using the signal to noise ratio by injecting 1 μL of dilute solutions.

3.3.1. Linearity

The calibration plot was constructed using the different concentrations namely 500 ppt, 1 ppb, 10 ppb, 1 ppm, and 10 ppm (**Figure. 2**) for checking the linearity of the method. Upto 10 ppm concentration, the response was linear for all the compounds, with correlation coefficient (R^2) values ranging from 0.9969 to 1.0000 (**Table 1**).

3.3.2. Accuracy and precision

Satisfactory results were found with recoveries between 85 and 100%. The relative standard deviation (RSD) was below 20%. The repeatability of the chromatographic method was determined by analyzing the chili samples spiked at different concentrations. The samples were injected 10 times with autosampler.

3.3.3. Limit of detection (LOD) and limit of quantification (LOQ)

For the blank sample of the chili, the limit of detection (LOD) of the compound can be measured using signal to noise ratio of 3 with obtained background noise. Then, for the limit of quantification (LOQ) of the method, S/N ratio was considered which was generally >10

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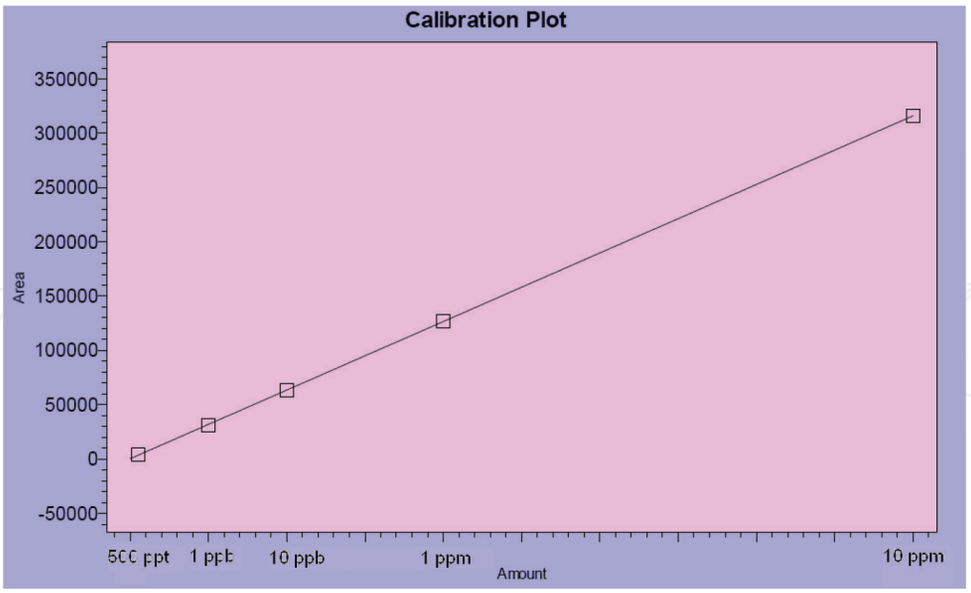

Figure 2. UPLC calibration plot of pesticide reference standards (500 ppt–10 ppm).

(**Table 1**). Effect of the matrix in the developed method was analyzed by comparing the standards in solvent with matrix-matched standards for five replicates. From the results obtained, it was evident that, no interfering peaks appeared and retention time (RT) of the tested analytes at spiked samples fully matched with those of standard samples. Each analyte molecule was eluted as separate symmetric peak.

3.3.4. Analysis of pesticide residues in chili samples

The validated method was employed for analysis of 30 samples collected from the different farmer's field of Haveri district, Karnataka, India. The optimized method was used for analysis of samples in triplicates. Results showed that most of the chili samples contained detectable pesticide residues (**Tables 2** and **3**). The residues of acetamiprid and thiodicarb were detected in three samples, whereas flubendiamide and mancozeb were detected in six samples, respectively, and carbosulfan and spinosad were detected in two and five number of samples, respectively (**Table 3**). The rest of the pesticides, that is, benomyl, indoxacarb, imidacloprid, methomyl, oxydemeton-methyl, and difenoconazole were not found in any of the samples. Out of the 30 chili samples, 19 samples did not contain any pesticide residues and 11 samples were found to be contaminated with residues with above MRLs.

4. Conclusion

Method has been developed with UPLC for the rapid detection and quantification of different pesticide residues in chili samples. The reliability of the method was checked by method validation in terms of linearity, precision, and accuracy in a range of 500 ppt–10 ppm, correlation coefficient (R^2) values were 0.9969. Average recoveries were more than 85–100%

for the wide range of pesticide analysis in chili samples. QuEChERS methodology has proved rapid and highly effective method. This validated method was successfully used for analysis of real chili samples. The results also emphasize the need for regular monitoring of a more number of samples for pesticide residues, especially chili sample which has to be exported. Finally, it is concluded that the developed method is suitable for routine use in laboratories with access to UPLC system and should be used for the rapid screening of chili samples.

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References

[1] Mathur R, Dangi RS, Dass SC, Malhotra RC. The hottest chilli variety in India. Current Science. 2000;**79**(3):287-288

[2] CRN India. Available via http://www.crnindia.com.2010. [Accessed: 26-10-2010]

[3] FAOSTAT. FAO Statistical Database. http://www.fao.org.2008. [Accessed: 10-11-2010]

[4] Peter KV, Nybe EV, Mini Raj N. Available Technologies to Raise Yield. Surv. Ind. Agric. The Hindu Year Book. 2006. Chennai:82-86

[5] Lee IO, Lee KH, Pyo JH, Kim JH, Choi YJ, Lee YC. Anti-inflammatory effect of capsaicin in Helicobacter pylori-infected gastric epithelial cells. Helicobacter. 2007;**12**(5):510-517

[6] Deepa GT, Chetti MB, Khetagoudar MC, Adavirao GM. Influence of vacuum packaging on seed quality and mineral contents in chilli (*Capsicum annuum* L.). Journal of Food Science and Technology. 2013;**50**(1):153-158

[7] Ahuja KD, Robertson IK, Geraghty DP, Ball MJ. Effects of chili consumption on post-prandial glucose, insulin, and energy metabolism. The American Journal of Clinical Nutrition. 2006;**84**(1):63-69

[8] Tomlin CDS (Ed.) The Pesticide Manual. 12th Ed. Surrey, UK: British Crop Protection Council; 2000

[9] Fernández, M, Picó Y, Mañs J. Analytical methods for pesticide residue determination in bee products. Journal of Food Protection. 2002;**65**(9):1502-1511

[10] Commission Directive 1999/39/EC of 6 May 1999. Amending Directive 96/5/EC on Processed Cereal-based Foods and Baby Foods for Infants and Young Children. Official Journal L124/8

[11] Picó Y, Font G, Moltó JC, Manes J. Pesticide residue determination in fruit and vegetables by liquid chromatography–Mass spectrometry. Journal of Chromatography A, 2000;**882**(1):153-173

[12] Torres CM, Picó Y, Manes J. Determination of pesticide residues in fruit and vegetables. Journal of Chromatography A. 1996;**754**(1):301-331

[13] Hajšlová J, Zrostlıkova J. Matrix effects in (ultra) trace analysis of pesticide residues in food and biotic matrices. Journal of Chromatography A. 2003:**1000**(1):181-197

[14] Yu K, Little D, Plumb R, Smith B. High-throughput quantification for a drug mixture in rat plasma–A comparison of Ultra Performance™ liquid chromatography/tandem mass spectrometry with high-performance liquid chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrometry. 2006;**20**(4):544-552

[15] Ayaz FA, Hayirlioglu-Ayaz S, Gruz J, Novak O, Strnad M. Separation, characterization, and quantitation of phenolic acids in a little-known blueberry (*Vaccinium arctostaphylos* L.) fruit by HPLC-MS. Journal of Agricultural and Food Chemistry. 2005;**53**(21):8116-8122

[16] Castellari M, Sartini E, Fabiani A, Arfelli G, Amati A. Analysis of wine phenolics by high-performance liquid chromatography using a monolithic type column. Journal of Chromatography A. 2002;**973**(1):221-227

[17] Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International. 2003;**86**(2):412-431

[18] Anastassiades M, Tasdelen B, Scherbaum E, Stajnbaher D Recent Developments in QuEChERS Methodology for Pesticide Multiresidue Analysis. Weinheim: Wiley-VCH; 2007

[19] European Committee for Standardization/Technical Committee 275 (Standards under development) Foods of Plant Origin. Determination of Pesticide Residues Using GC–MS and/or LC–MS (/MS) Following Acetonitrile Extraction/Partitioning and Cleanup by Dispersive SPE–QuEChERS Method. Brussels: European Committee for Standardization; 2007

[20] Lehotay SJ, Maštovská K, Yun SJ. Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. Journal of AOAC International. 2005;**88**(2):630-638

[21] Lehotay SJ, Kok AD, Hiemstra M, Bodegraven PV. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. Journal of AOAC International. 2005;**88**(2):595-614

[22] Lehotay SJ, Maštovská K, Lightfield AR. Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. Journal of AOAC International. 2005;**88**(2):615-629