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Simultaneous Determination of Toxic Organophosphorus Insecticide Residues in Honey Followed by Matrix Solid-Phase Dispersion Coupled to High-Performance Liquid Chromatography with Ultraviolet Detection

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Abstract

Matrix solid-phase dispersion has been developed for the extraction and preconcentration of organophosphorus insecticide residues (Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos) in honey. After extraction residues were determined by high performance liquid chromatographic method. The evaluated parameters included the type and amount of sorbent (silica gel, C18 and Activated Florisil) and the nature of eluent (n-hexane and dichloromethane). The best results were obtained using 2.0 g of honey sample, 2.0 g of C18 as sorbent and 20 mL of n-hexane -dichloromethane (1:1, (v/v)).The method was validated using honey sample spiked with insecticides at different concentration levels (0.01 and 0.1 µg/mL). Average recoveries (using each concentration six replicates) ranged 87-96%, with relative standard deviations less than 3%, calibration solutions concentration in the range 0.01-2.0 mg/L and limit of detection (LOD) and limit of quantification (LOQ) were 0.003 mg/L and 0.01 mg/L respectively.

Keywords: Matrix solid-phase dispersion, Organophosphorus insecticides, HPLC-UV

Introduction

A molecule of an organ phosphorous substance contains two kinds of groups, the electronegative group 'x' forming a relatively unstable anhydride bond with phosphorous and an alkoxy group having a strong bond with phosphorous. The hydrolysis of organophosphorus compounds by phosphatases is the main metabolic way leading to a complete loss of toxicity in the organisms of humans, warm blooded animals, insects and plants.

The toxic action of organophosphorus compounds on insects is due to inhibition of the activity of acetylcholinesterase in the cholinergic synapses of the nervous system. (The space between nerve cells, called a synapase). When organophosphorus compounds enter the organism of an insect, the activity of the acetylcholinesterase sharply drops. Indications of poisoning appear very rapidly in hyperactivation, next paralysis and then leading to death. Most organophosphorus insecticides have a high initial toxicity, and pests perish during the first few hours after treatment.

Substances of this group are good for controlling larvae and adult individual of pests but their ovicidal action is weak, which is associated with low permeability of egg shells. Oil solutions of some organophosphorus compounds penetrate quite well into the eggs of insects and mites, causing their death.

The activity of an embryon's cells and its growth are not violated but acetylcholine accumulates in the embryon. The larvae die owing to the inability of moving when it leaves the egg. This is why plantings should be treated with oil formulations of such a kind not long before the larvae hatch from the eggs.

Upon perennial use of organophosphorus insecticides, specific resistance appears in insects or mites. Specific resistance to organophosphorus compounds appears more rapidly in the species of insects that produce several generations in a single season. But the most dangerous is the development of resistance in phytophagous mites. The economical harm form this phenomenon is very great especially in cotton-growing regions.

Organophosphorus compounds are mainly highly and moderately toxic to humans and warm-blooded animals. Upon entering into the body they affect the cholinergic synapses of the central nervous system and the peripheral nerve muscle connections [1].

Many compounds of this group have an acute and moderate chronic toxicity that manifests itself with the frequent introduction of toxic and sublethal doses. The high activity of phosphatases, carboxyesterases and amidases in the organisms of warm blooded animals result in the rapid decomposition of organophosphorus compounds into non toxic water soluble products which is excreted through urine. The dermal toxicity of organophosphorus insecticide is not high but toxicants like methyl-parathion are highly toxic when they get into the skin.

Several methods have been used for the determination of organophosphorus insecticide residues using solid-phase extraction (SPE), solid-phase micro extraction (SPME), supercritical fluid extraction (SFE), Single drop micro extraction (SDME) and matrix solid-phase dispersion (MSPD). However, none of the published researches to date have reported the simultaneous analysis of chemical classes such as Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos in honey.

The matrix solid-phase dispersion (MSPD) technique was developed by Barker in 1989 [2]. It has advantages over conventional techniques because it employs small amounts of sample and solvent, and the extraction procedure consists of only a few experimental steps. MSPD evolved from the solid-phase extraction (SPE) technique, modified for application to solid and semi-solid matrices. The MSPD

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procedure is based on the use of a sorbent, which acts as an abrasive in order to produce a modified "opening" of the solid matrix, facilitating the extraction process when using a suitable solvent for eluting the analytes. The use of MSPD for organophosphorus insecticides recovery depends on the solubility of the organophosphorus insecticides in the eluting solvent, as well as the interactions between the matrix components, sorbent and eluent [3]. Due to the lack of literature reports concerning the use of MSPD as an extraction technique for organophosphorus insecticides belonging to different chemical classes from plants, soil water and food products, this paper presents an MSPD method for determination of residue of organophosphorus insecticides in honey [4-7]. So, the present research considered five different chemical classes, namely Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos which analysis by high-performance liquid chromatography with ultraviolet detector (HPLC-UV).

Experimental

Standards, reagents and samples

Certificated analytical standards of Monocrotophos (99.4%), Triazophos (98.2%), Phosalone (99.2%) Profenofos (99.1%) and Chlorpyrifos (99.8%) were obtained from Sigma Aldrich. Common names and structures of the organophosphorus insecticides evaluated here are shown in Figure 1. Acetonitrile was purchased from Rankem, New Delhi, Analytical grade solvents, dichloromethane and n-hexane, were supplied from Merck Limited, Mumbai, C18-bonded silica (50 µm) from phenomenex (Torrance, CA, USA), Florisil (60-100 mesh) from Fluka Chemie GmbH CH-9471 Buchs, AR grade sodium sulphate from Merck Limited, Mumbai and honey was purchased from local market. They were brought to the laboratory and stored in plastic bag under refrigerator condition until they were processed in the laboratory.

Standard stock solutions

The organophosphorus insecticide standard stock solutions were individually prepared in acetonitrile at a concentration level of 100 µg/mL and stored in a freezer at -18°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using acetonitrile, immediately prior to sample preparation.

Sample preparation

Representative 2.0 g portions of honey fortified with 10 µL of working standard solution. The mixture was then gently blended in the mortar for 30 min, to assess the homogeneity of the sample. The sample was allowed to stand at room temperature for one hour, until analysis.

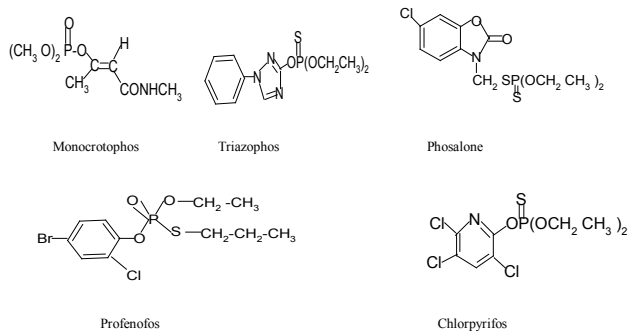

Figure1: Names and structures of five organophosphorous insecticide evaluated.

Extraction procedure

2.0 g of honey sample was weighed out and homogenized with 2.0 g of C18 –bonded silica for 5 min. The homogenized sample was transferred to an MSPD column consisting of a 20 mL capacity polyethylene syringe containing 2.0 g florisil and 2.0 g of anhydrous sodium sulfate. The elution was performed under vacuum with 20 mL of n-hexane-dichloromethane (1:1). The eluent was collected into a 50 mL glass tube and then evaporated under gentle stream of nitrogen, with the water bath temperature set at 40-45°C. Residue was dissolved with 5 mL of acetonitrile.

Chromatographic separation parameters

The HPLC-UV system used, consisted Shimadzu high performance liquid chromatography with LC-20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed Phase C18 analytical column of 250 mm×4.6 mm and particle size 5.0 µm (Phenomenex) Column temperature was maintained at 30°C. The injected sample volume was 20 µL. Mobile Phases A and B were acetonitrile and Milli-Q water (75:25(v/v)). The flow- rate used was kept at 1.2 mL/min. The detector wavelength was 230 nm. The external standard method was used for this analysis.

Method validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.01 and 0.1 mg/kg. Linearity was determined by different known concentrations (0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, µg/mL) was determined as the lowest concentration of a given herbicide giving a response of 10 times the baseline noise.

Results and Discussion

Specificity

Specificity was confirmed by injecting the honey control. There were no matrix peaks in the chromatograms to interfere with the analysis of herbicide residues shown in Figure 1 & Figure 2. Furthermore, the retention times of Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos were constant at 3.8 ± 0.2, 4.7 ± 0.2, 7.2 ± 0.2, 8.8 ± 0.2 and 12.6 ± 0.2 min.

Linearity

Different known concentrations of organophosphorus insecticides (0.01, 0.05, 0.1, 0.5, 1.0, 2.0 µg/mL) were prepared in acetonitrile by diluting the stock solution. The standard solutions were injected and recorded the peak areas. A calibration curve has been plotted of concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six solutions. The peak areas obtained from different concentrations of organophosphorus insecticides were used to calculate linear regression equations. These were Y= 132256.12X+84.23, Y=105266.62X+32.18, Y=115461.52X+12.93, Y=123968.33X+25.12 and 150918.15. +51.36

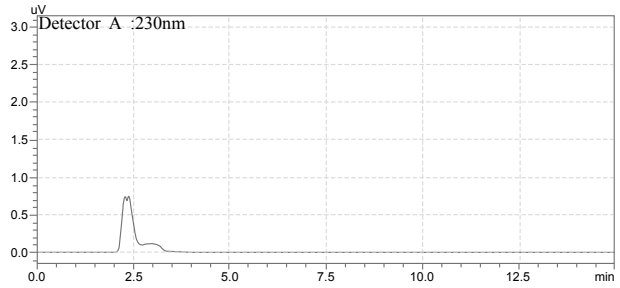

Figure 2: A representative chromatogram obtained from control honey sample.

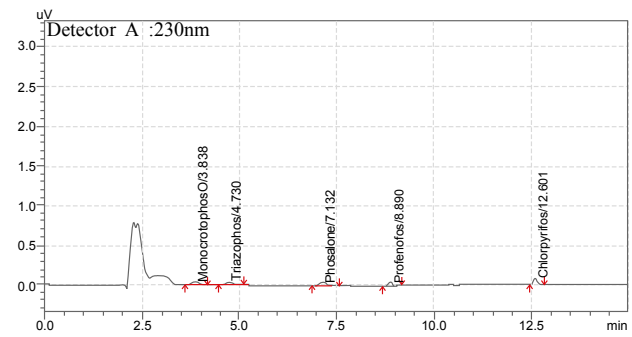

Figure 3: Representative chromatogram at fortification level of 0.01 µg/mL.

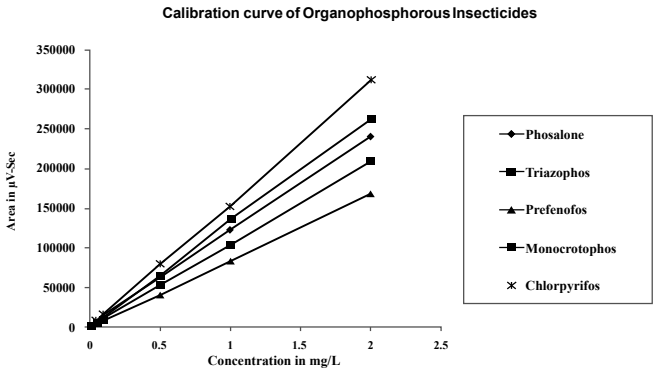

Figure 4: Representative Calibration curve of organophosphorous insecticides.

with correlation coefficients of 0.9998, 0.9999, 0.9996, 0.9999 and 0.9997 for Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos, respectively. A calibration curve was show in Figure 2.

Accuracy and precision

Recovery studies were carried out at 0.01 and 0.1 µg/mL fortification levels for Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos in honey. The recovery data and relative standard deviation values obtained by this method are summarized in Table 1.

These numbers were calculated from five (6) replicate analysis of given sample (Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos) made by a single analyst on one day. The repeatability of method was satisfactory (RSDs<3%) Figure 3.

Detection and quantification limits

The limit of quantification was determined to be 0.01 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (87-96%, RSD<3%) were achieved. This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.01 µg/mL at retention time of the peak of interest Figure 4.

Storage stability

A storage stability study was conducted at -20 ± 1°C with honey samples spiked with 0.5 µg/mL of Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos Samples were stored for a period of 30 days at this temperature. Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos contents were analysed before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only 2% for Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos showing no significant loss of residues on storage. The results are presented in Table 2.

Conclusions

This paper describes for the first time a fast, simple sensitive analytical method based on MSPD with HPLC-UV was developed and validated for the simultaneous determination of five organophosphorus insecticides residues in honey. The MSPD extraction procedure of the described method is very simple and requires no sample preparation or pre-treatment, providing adequate clean-up of the matrix. Whole honey extracts are very clean, with no interfering peaks at the retention time of the target compounds, indicating good selectivity of the proposed method.

Fortification Concentration in µg/mL	Replication	Recovery (%)				
		Monocrotophos	Triazophos	Phosalone	Profenofos	Chlorpyrifos
	R1	85	87	90	89	88
	R2	89	88	86	88	85
	R3	91	91	90	85	85
0.01	R4	87	86	87	90	86
	R5	87	85	86	86	89
	R6	88	87	86	88	91
	Mean	88	87	89	89	87
	RSD	2.32	2.37	2.22	2.07	2.77
	R1	95	91	96	93	90
	R2	98	92	92	92	93
	R3	96	91	94	92	91
0.1	R4	95	93	93	94	95
	R5	95	94	94	95	93
	R6	93	92	93	92	92
	Mean	95	92	94	93	92
	RSD	1.71	1.60	1.46	1.36	1.90

Table 1: Recoveries of the organophosphorous insecticides from fortified honey control sample (n=6).

The mobile phase acetonitrile and Milli-Q water yields good separation and resolution and the analysis time required for the chromatographic determination of the five organophosphorus insecticides are very short (around 15 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and according to the

Fortified concentration in µg/mL	Storage Period in Days	Replication	Recovery in %				
			Monocrotophos	Triazophos	Phosalone	Profenofos	Chlorpyrifos
		R1	96	94	92	91	92
		R2	93	92	94	93	93
		R3	96	93	95	91	95
	0	R4	95	94	94	95	95
		R5	94	92	96	92	93
		R6	94	92	91	92	91
0.5		Mean	95	93	94	92	93
		RSD	1.28	1.06	1.99	1.63	1.72
		R1	90	88	90	88	89
		R2	92	90	89	89	90
		R3	90	89	90	90	91
	30	R4	91	91	91	91	92
		R5	94	90	92	89	91
		R6	93	91	92	89	89
		Mean	92	90	91	91	90
		RSD	1.78	1.30	1.34	1.16	1.34

Table 2: Storage stability Details (n=6).

SANCO guidelines [8,9]. For all of the organophosphorus insecticides the sensitivity of the method was good enough to ensure reliable determination levels lower than the respective MRLs. Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of organophosphorus insecticide residues on a large number of honey samples.

Acknowledgement

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Keywords: Matrix solid-phase dispersion; Organophosphorus insecticides; HPLC-UV

Introduction

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Experimental

Standards, reagents and samples

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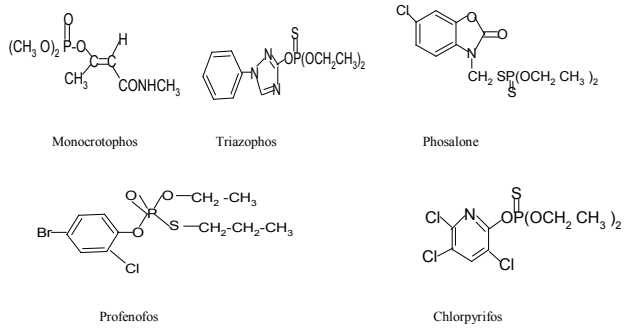

Figure 1: Names and structures of five organophosphorous insecticide evaluated.

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Chromatographic separation parameters

The HPLC-UV system used, consisted Shimadzu high performance liquid chromatography with LC-20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed Phase C18 analytical column of 250 mm×4.6 mm and particle size 5.0 µm (Phenomenex) Column temperature was maintained at 30°C. The injected sample volume was 20 µL. Mobile Phases A and B were acetonitrile and Milli-Q water (75:25(v/v)). The flow- rate used was kept at 1.2 mL/min. The detector wavelength was 230 nm. The external standard method was used for this analysis.

Method validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.01 and 0.1 mg/kg. Linearity was determined by different known concentrations (0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, µg/mL) was determined as the lowest concentration of a given herbicide giving a response of 10 times the baseline noise.

Results and Discussion

Specificity

Specificity was confirmed by injecting the honey control. There were no matrix peaks in the chromatograms to interfere with the analysis of herbicide residues shown in Figure 1 & Figure 2. Furthermore, the retention times of Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos were constant at 3.8 ± 0.2, 4.7 ± 0.2, 7.2 ± 0.2, 8.8 ± 0.2 and 12.6 ± 0.2 min.

Linearity

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Accuracy and precision

Recovery studies were carried out at 0.01 and 0.1 µg/mL fortification levels for Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos in honey. The recovery data and relative standard deviation values obtained by this method are summarized in Table 1.

These numbers were calculated from five (6) replicate analysis of given sample (Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos) made by a single analyst on one day. The repeatability of method was satisfactory (RSDs<3%) Figure 3.

Detection and quantification limits

The limit of quantification was determined to be 0.01 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (87-96%, RSD<3%) were achieved. This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.01 µg/mL at retention time of the peak of interest Figure 4.

Storage stability

A storage stability study was conducted at -20 ± 1°C with honey samples spiked with 0.5 µg/mL of Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos Samples were stored for a period of 30 days at this temperature. Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos contents were analysed before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only 2% for Monocrotophos, Triazophos, Phosalone, Profenofos and Chlorpyrifos showing no significant loss of residues on storage. The results are presented in Table 2.

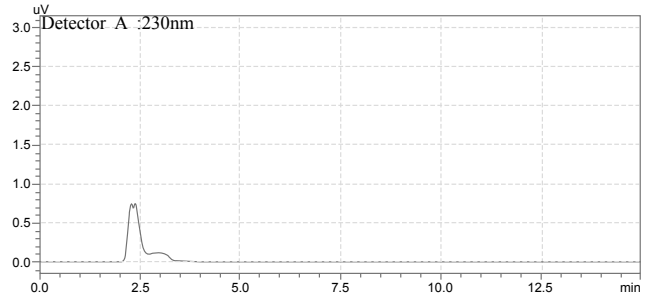

Figure 2: A representative chromatogram obtained from control honey sample.

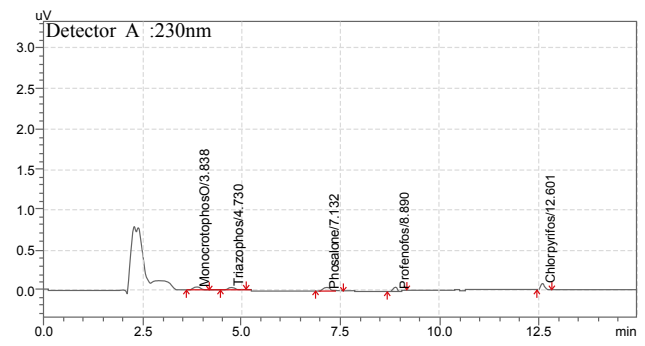

Figure 3: Representative chromatogram at fortification level of 0.01 µg/mL.

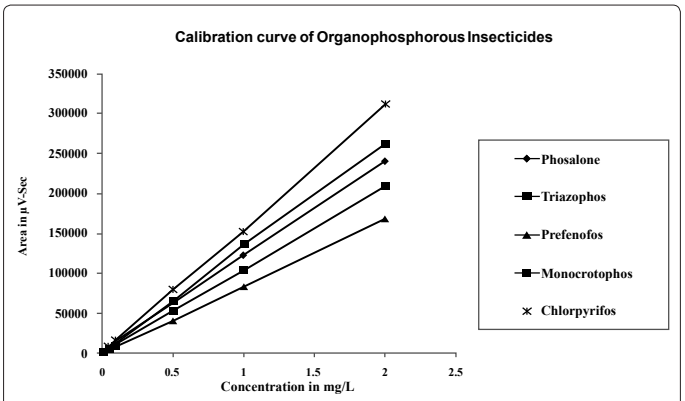

Figure 4: Representative Calibration curve of organophosphorous insecticides.

Fortification Concentration in µg/mL		Recovery (%)				
	Replication	Monocrotophos	Triazophos	Phosalone	Profenofos	Chlorpyrifos
	R1	85	87	90	89	88
	R2	89	88	86	88	85
	R3	91	91	90	85	85
0.01	R4	87	86	87	90	86
	R5	87	85	86	86	89
	R6	88	87	86	88	91
	Mean	88	87	89	89	87
	RSD	2.32	2.37	2.22	2.07	2.77
	R1	95	91	96	93	90
	R2	98	92	92	92	93
	R3	96	91	94	92	91
0.1	R4	95	93	93	94	95
	R5	95	94	94	95	93
	R6	93	92	93	92	92
	Mean	95	92	94	93	92
	RSD	1.71	1.60	1.46	1.36	1.90

Table 1: Recoveries of the organophosphorous insecticides from fortified honey control sample (n=6).

Fortified concentration in µg/mL	Storage Period in Days	Replication	Recovery in %				
			Monocrotophos	Triazophos	Phosalone	Profenofos	Chlorpyrifos
		R1	96	94	92	91	92
		R2	93	92	94	93	93
		R3	96	93	95	91	95
	0	R4	95	94	94	95	95
		R5	94	92	96	92	93
		R6	94	92	91	92	91
0.5		Mean	95	93	94	92	93
		RSD	1.28	1.06	1.99	1.63	1.72
		R1	90	88	90	88	89
		R2	92	90	89	89	90
		R3	90	89	90	90	91
	30	R4	91	91	91	91	92
		R5	94	90	92	89	91
		R6	93	91	92	89	89
		Mean	92	90	91	91	90
		RSD	1.78	1.30	1.34	1.16	1.34

Table 2: Storage stability Details (n=6).

Conclusions

This paper describes for the first time a fast, simple sensitive analytical method based on MSPD with HPLC-UV was developed and validated for the simultaneous determination of five organophosphorus insecticides residues in honey. The MSPD extraction procedure of the described method is very simple and requires no sample preparation or pre-treatment, providing adequate clean-up of the matrix. Whole honey extracts are very clean, with no interfering peaks at the retention time of the target compounds, indicating good selectivity of the proposed method.

The mobile phase acetonitrile and Milli-Q water yields good separation and resolution and the analysis time required for the chromatographic determination of the five organophosphorus insecticides are very short (around 15 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and according to the SANCO guidelines [8,9]. For all of the organophosphorus insecticides the sensitivity of the method was good enough to ensure reliable determination levels lower than the respective MRLs. Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of organophosphorus insecticide residues on a large number of honey samples.

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