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Research Article

DETERMINATION OF FOUR NEONICOTINOID INSECTICIDE RESIDUES IN COTTON SEED OIL USING MATRIX SOLID-PHASE DISPERSION COUPLED TO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

Nageswara Rao. T

Andhra University, Visakhapatnam, Andhra Pradesh, India

ABSTRACT

A simple, sensitive and inexpensive method was developed using matrix solid-phase dispersion (MSPD), together with high performance liquid chromatographic method for determination of neonicotinoid insecticide residues (Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid) in cotton seed oil. The evaluated parameters included the type and amount of sorbent (silica gel, C18 and Florisil) and the nature of eluent (tetrahydrofuran, Millipore water and acetonitrile).The best results were obtained using 1.0 g of cotton seed oil sample, 1.0 g of C18 as sorbent and 20ml of tetrahydrofuran - Millipore water (2:8, (v/v)).The method was validated using in cotton seed oil samples spiked with insecticides at different concentration levels (0.03 and 0.3 µg/mL). Average recoveries (using each concentration six replicates) ranged 89-96%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.01-2.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.01 µg/mL and 0.03 µg/mL respectively.

Keywords: matrix solid-phase dispersion, neonicotinoid insecticides, HPLC-UV.

INTRODUCTION

The neonicotinoids, the newest major class of insecticides, have outstanding potency and systemic action for crop protection against piercing-sucking pests, and they are highly effective for flea control on cats and dogs. Their common names are acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam. They generally have low toxicity to mammals, birds and fish. The neonicotinoids are not protonated and have an electronegative nitro or cyano pharmacophore. Agonist recognition by the nicotinic receptor involves cation-Π interaction for nicotinoids in mammals and possibly a cationic subsite for interaction with the nitro or cyano substituent of neonicotinoids[1] in insects.

Various methods have been described for the determination of these Insecticides, using solid-phase extraction (SPE)[3-4], solid-phase micro extraction (SPME), supercritical fluid extraction (SFE) and matrix solid-phase dispersion (MSPD)[6], However, none of the published researches to date have reported the simultaneous analysis of chemical classes such as Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid in cotton seed oil.

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 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 insecticide recovery depends on the solubility of the insecticide in the eluting solvent, as well as the interactions between the matrix components, sorbent and eluent.

Due to the lack of literature reports concerning the use of MSPD as an extraction technique for insecticides belonging to different chemical classes from plants, this paper presents an MSPD method for determination of residue of insecticides in cotton seed oil. So, the present research considered four different chemical classes, namely Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid which analysis by high-performance liquid chromatography with ultraviolet detector (HPLC-UV).

1. EXPERIMENTAL

1.1. Standards, Reagents and samples

Certificated analytical standards of Imadacloprid (99.4%), Dinotefuran (98.9%), Thiamethoxam (99.1%) and Acetamiprid (99.6%) were obtained from international institute of biotechnology and toxicology (IIBAT). Common names and structures of the neonicotinoid insecticides evaluated here are shown in **Fig. 1**. Acetonitrile was purchased from Rankem, New Delhi, Analytical grade solvents, tetrahydrofuran was 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 cotton seed oil were purchased from local market. They were brought to the laboratory and stored in plastic bag at refrigerator condition until they were processed in the laboratory.

1.2. Standard stock solutions

The insecticide standard stock solutions were individually prepared in acetonitrile at a concentration level 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.

1.3. Sample preparation

Representative 1.0 g portions of cotton seed oil fortified with 100 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, before it was kept at refrigerator condition, until analysis.

1.4. Extraction procedure

1.0 g of cotton seed oil sample was weighed out and homogenized with 1.0 g of C18 – bonded silica for 5 min. The homogenized sample was transferred to an MSPD column consisting of a 20mL capacity polyethylene syringe containing 1.0 g flurosil and 1.0 g of anhydrous sodium sulfate. The elution was performed under vacuum with 20 mL of tetrahydrofuran - Millipore water (2:8, (v/v)). The eluent was collected into a round bottom flask and evaporated to near dryness. Finally make up with 5mL of acetonitrile and analysed by HPLC-UV system.

1.5. 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 x 4.6 mm and particle size 5 µm (Phenomenex Luna-C18) Column temperature was maintained at 40°C. The injected sample volume was 20µL. Mobile Phases A and B was Acetonitrile and 0.05M Potassium di hydrogen phosphate (25:75(v/v)). The flow- rate used was kept at 0.9 ml/min. A detector wavelength was 254nm. The

external standard method of Calibration was used for this analysis.

1.6. 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 0.03 and 0.3 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 insecticide giving a response of 10 times the baseline noise.

2. RESULTS AND DISCUSSION

2.1. Specificity

Specificity was confirmed by injecting the cotton seed oil control. There were no matrix peaks in the chromatograms to interfere with the analysis of insecticide residues shown in **Fig.2.** Furthermore, the retention times of Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid were constant at 3.7 ±0.2, 4.2 ± 0.2, 6.7 ± 0.2, 12.9 ± 0.2 min.

2.2. Linearity

Different known concentrations of insecticides (0.01, 0.05, 0.1, 0.5, 1.0, 2.0 µg/mL) were prepared in acetonitrile by diluting the stock solution. Each solution was prepared in triplicate. Injected the standard solutions and measured the peak area. 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 insecticides were used to calculate linear regression equations. These were Y= 87955.96X + 41.57, Y= 128224.3X + 12.36, Y=122346.74X+35.22 and Y=120021.15+23.11, with correlation coefficients of 0.9998, 0.9997, 0.9999 and 0.9997 for Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid respectively. A calibration curve showed in **Fig. 3.**

2.3. Accuracy and Precision

Recovery studies were carried out at 0.03 and 0.3 µg/mL fortification levels for Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid in cotton seed oil. The recovery data and relative standard deviation values obtained by this method are summarized in **Table 1.**

These numbers were calculated from four (6) replicate analyses of given sample (Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid) made by a single analyst on one day. The repeatability of method satisfactory (RSDs<3 %).

2.4. Detection and Quantification Limits

The limit of quantification was determined to be 0.03 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (89-96%, 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 a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

2.5. Storage Stability

A storage stability study was conducted at -20±1°C with cotton seed oil samples spiked with 0.1 µg/mL of Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid Samples were stored for a period of 30 days at this temperature. Analysed for the content of Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 3% for Imadacloprid, Dinotefuran, Thiamethoxam and Acetamiprid showing no significant loss of residues on storage. The results are

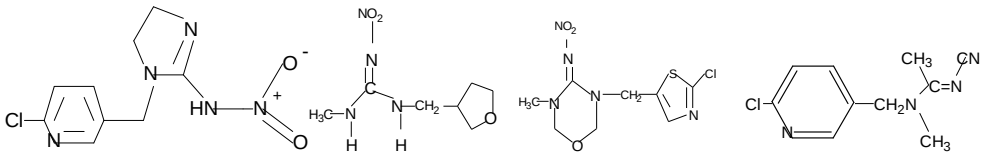
presented in **table 2.**

3. CONCLUSIONS

This paper describes for the first time a fast, simple sensitive analytical method based on MSPD-HPLC-UV was developed and validated for the simultaneous determination of four neonicotinoid insecticide residues in cotton seed oil.

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 cotton seed oil 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 0.05M Potassium di hydrogen phosphate yields good separation and resolution and the analysis time required for the chromatographic determination of the four Neonicotinoid insecticides is 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[7]. For all of the Neonicotinoid 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 insecticide residues on a large number of fruit samples.



madacloprid Dinotefuran Thiamethoxam Acetamiprid

Fig.1: Names and structures of four neonicotinoid insecticides evaluated

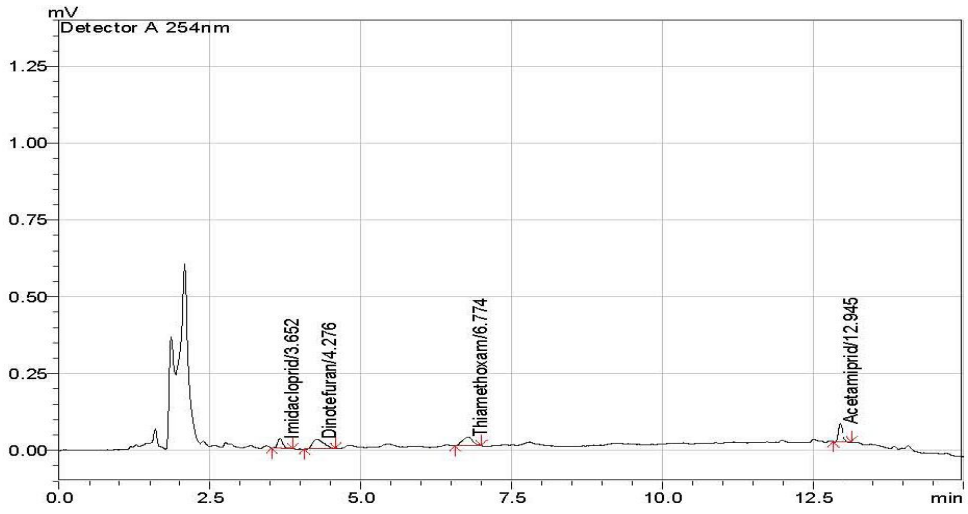


Fig. 2: Representative Chromatogram at fortification level of 0.03µg/mL

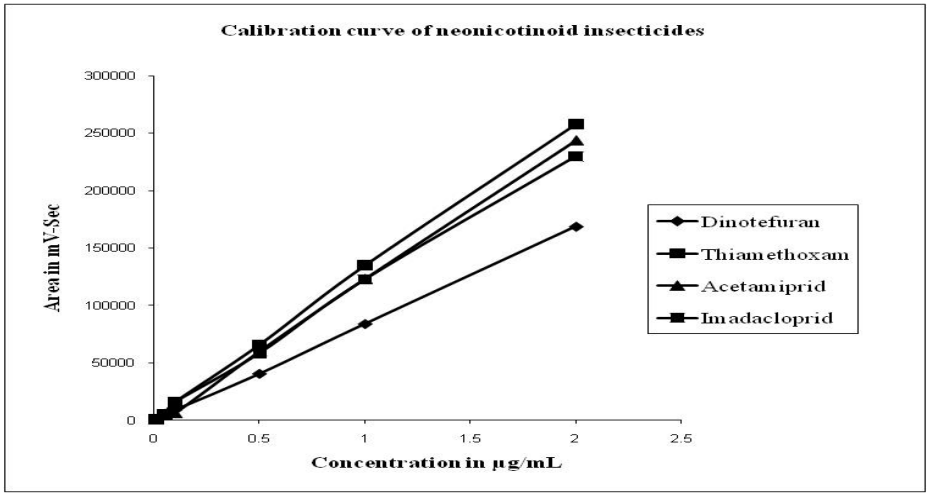


Fig. 3: Representative Calibration curve of neonicotinoid insecticides

Table1: Recoveries of the neonicotinoid insecticides from fortified cotton seed oil control sample (n=6)

Fortification Concentration in µg/mL	Replication	Recovery (%)			
		Imadacloprid	Dinotefuran	Thiamethoxam	Acetamiprid
0.03	R1	87	89	91	92
	R2	89	90	89	91
	R3	91	88	90	89
	R4	90	88	90	90
	R5	89	89	92	89
	R6	91	90	92	90
	Mean	**90**	**89**	**91**	**90**
	RSD	**1.69**	**1.00**	**1.34**	**1.30**
0.3	R1	94	91	94	92
	R2	94	92	92	90
	R3	95	90	95	93
	R4	95	92	96	94
	R5	93	93	93	94
	R6	92	92	94	92
	Mean	**94**	**92**	**94**	**93**
	RSD	**1.25**	**1.13**	**1.50**	**1.64**

Table2: Storage stability Details (n=6)

Fortified concentration in µg/mL	Storage Period in Days	Replication	Recovery in %			
			Imadacloprid	Dinotefuran	Thiamethoxam	Acetamiprid
0.1	0	R1	94	95	96	94
		R2	93	93	95	95
		R3	91	94	95	95
		R4	93	93	93	96
		R5	91	96	94	93
		R6	95	93	93	96
		Mean	**93**	**94**	**94**	**95**
		RSD	**1.73**	**1.35**	**1.28**	**1.23**
	30	R1	93	91	93	93
		R2	91	92	91	92
		R3	90	91	92	93
		R4	92	91	92	93
		R5	90	92	91	92
		R6	91	92	90	93
		Mean	**91**	**92**	**92**	**93**
		RSD	**1.28**	**0.60**	**1.15**	**0.56**

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5. REFERENCES

1. Motohiro Tomizawa and John E. Casida. Neonicotinoid insecticide toxicology. Annual review of Pharmacology and Toxicology. 2005; 45**:**247-268.
2. S.A.Barker. Applications of matrix solid-phase dispersion in food analysis. J.Chromatogr. 2000; 880: 63-68.
3. Chander Mohan, Yogesh Kumar, Jyotsana Madan and Navaneet Saxena. Multiresidue analysis of neonicotinoids by Solid-phase extraction technique using high-performance liquid chromatography. Environmental monitoring and assessment. 2010; 165: 573-576.
4. Seccia S, Fidente P., Montesano D, Morrica P. Determination of neonicotinod insecticides residues in bovine milk samples by solid-phase extraction clean-up and liquid chromatography with diode-array detection. J Chromatogr A. 2008; 1214(1-2): 115-120.
5. Serenella Seccia, Paola Fidente, Danilo Attard Barbini, Patrizia Morrica. Multiresidue determination of nicotinoid insecticide residues in drinking water by liquid chromatography with electrospray ionization mass spectrometry. Analytica Chimica Acta. 2005; 553: 21-26.
6. Paola Fidente, Serenella Seccia, Fabiana Vanni and Patrizia Morrica. Analysis of nicotinoid insecticide residues in honey by solid matrix partition clean-up and liquid chromatography-electrospray mass spectrometry. Journal of chromatography A. 2005; 1094: 175-178.
7. SANCO Guidelines. Method validation and quality control procedures for pesticide residues analysis in food and feed. Document NO. SANCO/10684/2009.