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RESEARCH ARTICLE

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A NOVEL METHOD FOR DETERMINATION OF CYCLANILIDE AND ITS METABOLITE RESIDUES IN COTTON SEED OIL

ABSTRACT

A simple, sensitive and inexpensive method was developed using solid-phase extraction, together with high performance liquid chromatographic method with uv detection for determination of cyclanilide and its metabolite (2, 4 dichloroaniline) residues. The evaluated parameters include the extracts by gel permeation chromatography (clean-up module GPC) on bio beads S-X3 polystyrene gel using a mixture of ethyl acetate/ cyclohexane (1:1, v/v) as eluent (5.0 mL/min) and an automated gel permeation chromatography. The method was validated using cotton oil samples spiked with cyclanilide and its metabolite (2,4 dichloroaniline) at different concentration levels (0.05 and 0.5 µg/mL). Average recoveries (using each concentration six replicates) ranged 89-95%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.05-5.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.02 µg/mL and 0.05 µg/mL respectively.

Key words: solid-phase extraction, cyclanilide, metabolite, gel permeation chromatography, LOQ and LOD.

INTRODUCTION

Cyclanilide[1] is plant growth regulator that is registered for use in cotton at different stages of growth. It can be used to suppress vegetative growth when used in combination with mepiquat chloride or it can enhance defoliation and boll opening when used in combination with ethephon. Cyclanilide appears to synergize or enhance the activity of the primary growth regulator, because mepiquat chloride or ethephon alone will dominance and enhance lateral branching in apple nursery stock and in kidney beans.

Various methods have been described for the determination of these residues, using solid-phase micro extraction (SPME) [5, 8], Supercritical fluid extraction (SFE) [3] and liquid – liquid extraction[4]. However, none of the published researches to date have reported the simultaneous analysis of cyclanilide and its metabolite (2, 4 dichloroaniline) in cotton seed oil.

EXPERIMENTAL

Standards, Reagents and samples

The analytical standards of Cyclanilide (99.5%) and 2, 4 dichloroaniline (99.2%), were obtained from Sigma Aldrich. Acetonitrile was purchased from Rankem, New Delhi, Analytical grade solvents, ethyl acetate, cyclohexane and methanol were supplied from Merck Limited and cotton seed oil was purchased from local market.

Standard stock solutions

The Cyclanilide and 2, 4 dichloroaniline 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.

Sample preparation

Representative 10.0 g portions of cotton seed oil fortified with 10 µL of working standard solution. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

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Extraction procedure

The representative 10g of cotton oil sample was weighed into a separate 250 ml extraction flask. Added 2.0 g of calflo E and 1.0 g of Celite 545(both activated overnight at 135°C) and added 2.25 ml of acetone, 21.75 ml of acetonitrile and 1.0 mL of concentrated hydrochloric acid. Mixed and homogenized with ultrasonic bath for 2 minutes. Transferred 20 mL of the specimen extract into a 50 mL PE-Vial and centrifuged for 2 minutes at 3000 rpm.

Clean-up procedure

An aliquot of 10 mL of the centrifuged raw extract was gently evaporated after addition of 0.5 mL of iso-octane as keeper to a remainder using a rotary evaporator. The residue was dissolved with ethyl acetate/cyclohexane (1:1, v/v) to a volume of 10 mL (V2). Clean-up of 10.0 mL (V3) of the extracts by gel permeation chromatography (clean-up module GPC) on bio beads S-X3 polystyrene gel using a mixture of ethyl acetate/ cyclohexane (1:1, v/v) as eluent (10.0 mL/min) and an automated gel permeation chromatography. The GPC extract was gently evaporated to dryness using a rotary evaporator and a gentle steam of nitrogen. The residue was re-dissoved in 10 mL of methanol / water (1:1, v/v). The final extracts were analysed by HPLC.

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 0.1% formic acid (75:25 (v/v)). The flow- rate used was kept at 1.0 mL/min. A detector wavelength was 250 nm. The external standard method of Calibration was used for this analysis.

Method validation

Method validation[6] 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 0.05 and 0.5 mg/kg. Linearity was determined by different known concentrations (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 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 fungicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

Specificity

Specificity was confirmed by injecting the oil control. There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues shown in (**Figure 1 and 2).** Furthermore, the retention times of Cyclanilide and 2, 4 dichloroaniline were constant at 5.4 ± 0.2and 4.6 ± 0.2, min.

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Linearity

Different known concentrations of standards (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 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 standards were used to calculate linear regression equations. These were Y=131171.3X + 34.50 and Y=120045.15 + 41.13, with correlation coefficients of 0.9999 and 0.9998 for Cyclanilide and 2, 4 dichloroaniline respectively. A calibration curve showed in (**Figure 3).**

Accuracy and Precision

Recovery studies were carried out at 0.05 and 0.5 µg/mL fortification levels for Cyclanilide and 2, 4 dichloroaniline in 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 (Cyclanilide and 2, 4 dichloroaniline) made by a single analyst on one day. The repeatability of method satisfactory (RSDs<2 %).

Detection and Quantification Limits

The limit of quantification was determined to be 0.05 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (89-95%, RSD<2%) 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 0.05 µg/mL at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

Storage Stability

A storage stability[2] study was conducted at -20 ± 1°C with oil samples spiked with 0.1 µg/mL of Cyclanilide and 2, 4 dichloroaniline Samples were stored for a period of 30 days at this temperature. Analysed for the content of Cyclanilide and 2, 4 dichloroaniline before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 2% for Cyclanilide and 2, 4 dichloroaniline showing no significant loss of residues on storage. The results are presented in **table 2.**

CONCLUSIONS

This paper describes a fast, simple sensitive analytical method based on SPE-HPLC-UV simultaneous determination of Cyclanilide and 2, 4 dichloroaniline residues in cotton seed oil. The SPE extraction procedure is very simple and inexpensive method for determination of Cyclanilide and 2, 4 dichloroaniline residues in cotton oil. The mobile phase Acetonitrile and 0.1% formic acid showed good separation and resolution and the analysis time required for the chromatographic determination of the Cyclanilide and 2, 4 dichloroaniline 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. Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of Cyclanilide and 2, 4 dichloroaniline residues on a large number of seed, oil, fruit, water and soil samples.

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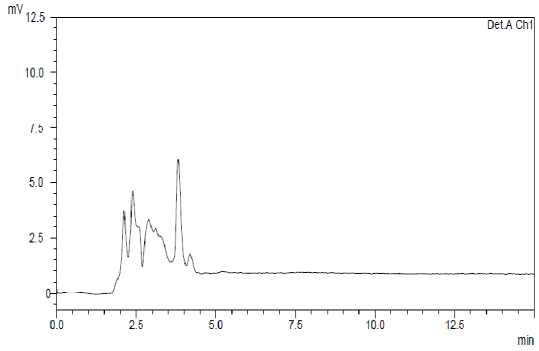

Fig.1. Representative Chromatogram at cotten seed oil control

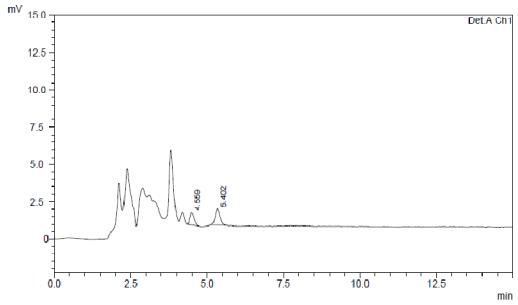

Fig.2. Representative Chromatogram at fortification level of 0.05 µg/mL

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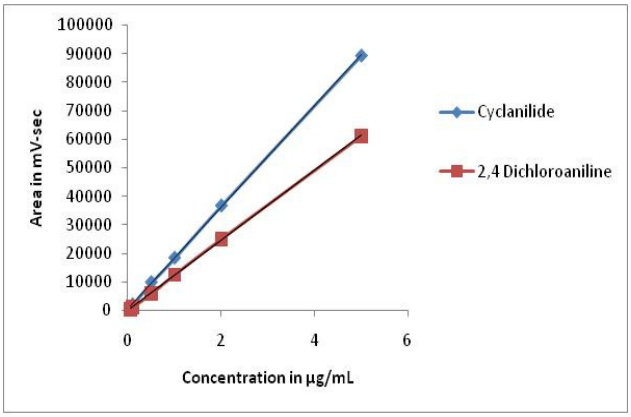

Fig.3. Representative Calibration curve of Cyclanilide and 2, 4 dichloroaniline.

Fortification Concentration in µg/mL	Replication	Recovery (%)	
		Cyclanilide	2, 4 dichloroaniline
0.05	R1	87	88
	R2	89	89
	R3	89	89
	R4	89	88
	R5	88	90
	R6	90	89
	Mean	**89**	**89**
	RSD	**1.16**	**0.85**
0.5	R1	94	96
	R2	97	94
	R3	95	97
	R4	96	97
	R5	95	98
	R6	94	93
	Mean	**95**	**96**
	RSD	**1.23**	**1.74**

Table1.Recoveries of the Cyclanilide and 2, 4 dichloroaniline from fortified cotton seed oil control sample (n=6)

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Fortification Concentration in µg/mL	Storage Period in Days	Recovery in %	
		Cyclanilide	2,4 dichloroaniline
0.1	0	92	91
		94	94
		93	95
		92	96
		94	94
		93	94
		93	94
		0.96	1.78
	30	90	94
		91	95
		91	95
		92	96
		90	94
		93	93
		91	95
		1.28	1.11

Table2. Storage stability Details (n=6)

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