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Determination of kresoxim-methyl residues in different types of Indian tropical soils

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ABSTRACT

A undemanding and economical method was developed using liquid - liquid extraction, simultaneously with high pressure liquid chromatographic method with UV detection for quantification of Kresoxim-methyl residues in four different tropical soils. The four different tropical soils (Sandy clay, Sandy loam, Loamy sand and clay) were collected from different areas. These soils were identified by soil characteristics approximating Sand content, pH, and Organic carbon content. The method was validated using in soils fortified with known concentration of Kresoxim-methyl reference standard solution at different concentration levels (0.05 and 0.5 mg/kg). Mean recoveries (using each concentration six replicates) with acceptable range (82-93%) and the relative standard deviations for two concentration levels were less than 2%. The linearity solution concentrations in the range of 0.5-5 mg/L. The limit of detection (LOD) and limit of quantification (LOQ) were 0.05 mg/L and 0.5 mg/L respectively. The estimated method can be applied successfully for the quantification of Kresoxim-methyl residues in different tropical soils.

KEYWORDS

Kresoxim-methyl;
HPLC-UV;
LOD;
LOQ;
Residues.

INTRODUCTION

Fungicides are the essential part of agriculture crop management for better yields. In this process several new molecules have been introduced for the potential control of pests and diseases. Fungicides are divided into two types, first one is protectant fungicides and second one is specific fungicides. Protectant fungicides were older type and includes copper and sulfur based products. They form a protective film on the plant surface and restrain the germination of fungal spores. Specific type fungicides were reacted with fungus chemically. Strobilurin compounds, they inhibit the respiratory electron transport is fungus and thereby killing fungus[1,2]. They act as efficient inhibitors. kresoxim methyl is mostly used for the control of powdery mildew and scab in apples, pears, grapes, strawberries and vegetables[3,4]. It is one of the majority frequently used fungicides in Indian viticulture, where application is done by foliar spray and also through drip irrigation[5,6].

Soil consists of living and non-living components which exist in complex and heterogeneous mix-

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tures. Microorganisms play an important role in break-down and transformation of organic matter in fertile soils with many species contributing to different aspects of soil fertility[7,8]. The site chosen for soil collection should be one which allows long term use. Permanent pastures, fields with annual cereal crops (except maize) or densely sown green manures are suitable. The selected sampling site should not have been treated with crop protection products for a minimum of one year before sampling. Also, no organic fertilizer should have been applied for at least six months[9,10]. For ploughed soil samples were collected from a depth of 0 down to 20 cm. For grass land or other soils where ploughing does not occur over longer periods (at least one growing season), the maximum depth of sampling may be slightly more than 20cm (e.g. to 25 cm). Soil samples were transported using containers and under temperature conditions such that the initial soil properties are not significantly altered[11]. The use of soils freshly collected from the field is preferred. If storage in the laboratory cannot be avoided; soil was stored in the dark at 4 ± 2°C for a maximum of three months. During the storage of soils, aerobic conditions must be ensured.

Soil collection

The tropical soils were collected from three different regions in Vizianagaram district- Andhra Pradesh state, India. The geographical distribution was found to be

Twelve set of soils were collected from these regions and soil physicochemical parameters have been performed. Based on the results, it was observed that different regions have different soils. Based on the percentage of clay, sand, silt it was concluded that:

- Mugada – contains clay and sandy clay soil.
- Badangi – contains sandy loam soil.
- Vadada – contains loamy sand.

EXPERIMENTAL

Materials and methods

Reference analytical standards of kresoxim methyl (purity 99%) were obtained from Sigma Aldrich. The test item kresoxim methyl 50% Wettable granules (WG) was purchased from local market. HPLC grade Acetonitrile and HPLC grade Water and charcoal were procured from the Merck India limited.

Preparation of Standard stock solution

Accurately 10.56 mg of Kresoxim-methyl reference standard, purity (99.0 %) was weighed into 20 mL volumetric flask. The content was dissolved in 10 mL of acetonitrile, sonicated and made up to the mark with the same solvent. The concentration of the stock solution was 522.72 mg/L. The standard stock solution was used for analysis up to 3 months. Suitable concentrations of reference standards were prepared from the stock solution by diluted with acetonitrile, immediately prior to sample preparation.

Sample preparation

Representative 50.0 gram portions of soil spiked with 0.1 mL of reference standard stock solution. The sample was allowed to stand at ambient temperature for one hour, before it was kept at refrigerator condition, until analysis.

Extraction Procedure

50 g of soil was taken into a 100 mL capacity amber colored bottles. To this, 30 mL of extraction solvent i.e., acetonitrile: HPLC water (90:10 % v/v) was added and then kept in orbital shaker incubator for homogenization for about 25 min. The samples were filtered and 20 mL of extraction solvent was again added to the samples and filtered again. The filterate was collected into the same bottles. The samples were decolorized with activated charcoal and filtered. Final filterate were injected into HPLC with respective to standard and control sample. Control samples were maintained without fortifying with test item.

Instrumentation

The concentration of Kresoxim-methyl was determined by HPLC-UV analysis. The quantization

S.No.	Location	Latitude	Longitude
1	Mugada	18.494685	83.368235
2	Badangi	18.494686	83.368234
3	Vadada	18.4940742	83.3669121

was performed using peak area measurements and analyzing against a calibration curve. The following HPLC-UV conditions were used in the analysis.

Chromatograph : Shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC Solution software

Column :	PhenomenexLuna-C18 (250mm x 4.6mm x 5.0µm)
Detector wavelength :	230 nm
Oven temperature :	30°C
Injection volume :	20µL
Mobile phase :	Acetonitrile :0.1% ortho phosphoric acid (80:20 (v/v)).
Flow Volume :	1.0 mL/Min
Retention Time (Approximate) :	5.2 minutes

Method validation

The validity of the analytical method was performed prior to start of the study, the parameters accuracy, precision, linearity and Limits of Detection (LOD) and Quantification (LOQ) were considered[12,13,14]. The Recoveries of the samples fortified at the 1xLOQ level = 0.05 mg/kg and 10xLOQ =0.5 mg/kg were determined using six samples for the validation of the method. Linearity was checked by different known concentrations (0.05, 0.1, 0.5, 1.0 and 2.0, 5.0 µg/mL) which were prepared by diluting the standard stock solution. The Limit of Detection (LOD, mg/L) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control sample. The Limit of Quantification (LOQ, mg/L) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

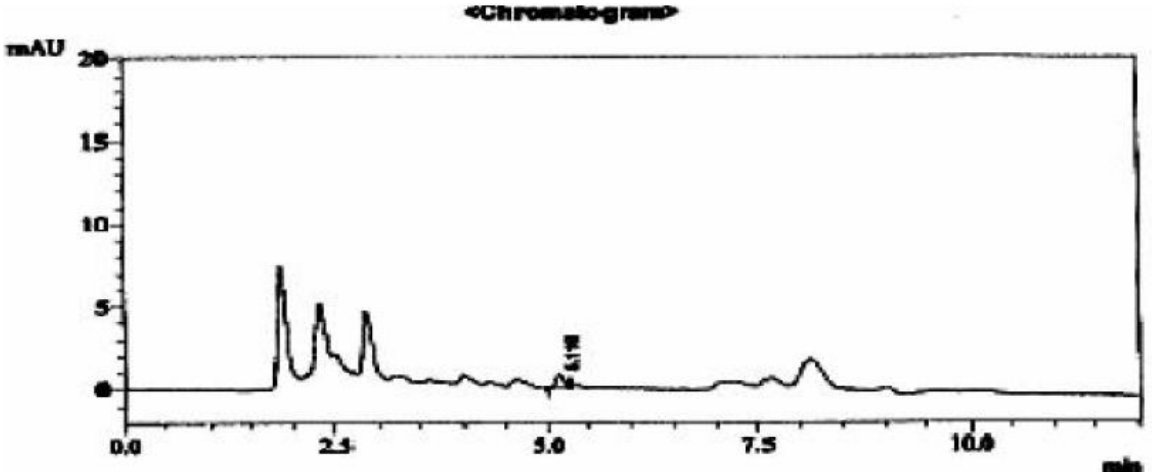

Figure 1 : Representative chromatogram of kresoxim methyl -sandy loam

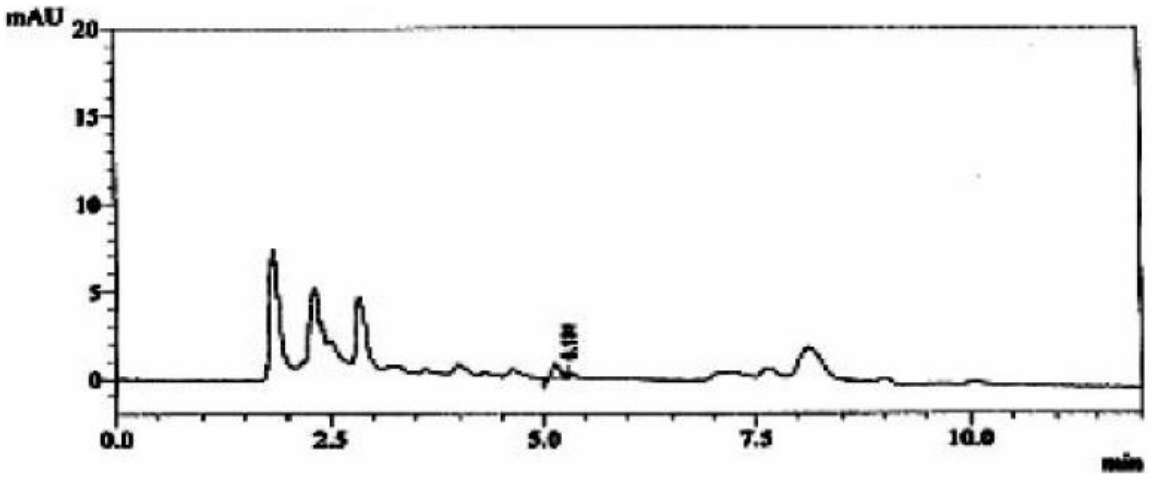

Figure 2 : Representative chromatogram of kresoxim methyl - loamy sand

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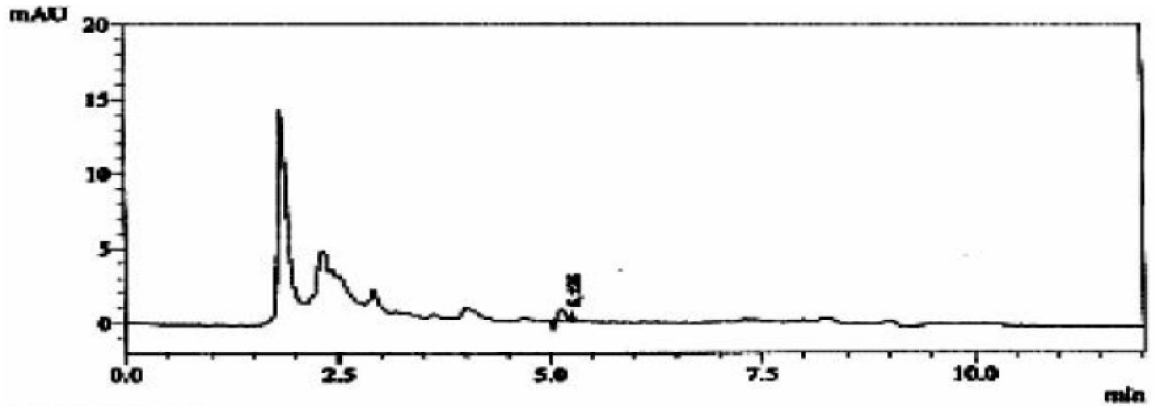

Figure 3 : Representative chromatogram of kresoxim methyl – sandy clay

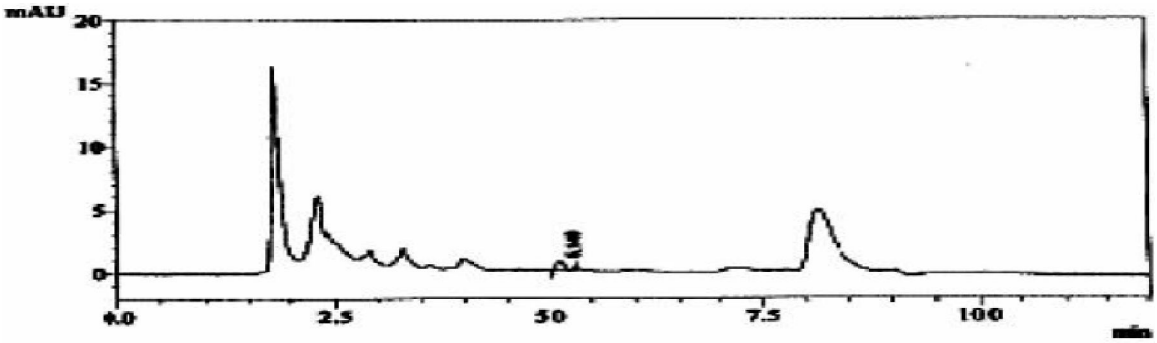

Figure 4 : Representative chromatogram of kresoxim methyl – sandy clay

Specificity

Specificity was confirmed by injecting the Mobile phase solvents i.e., Acetonitrile and 0.1% Orthophosphoric acid, HPLC water, sample solution standard solution and soil controls (acidic, neutral, basic) There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues shown in Figure 1, Figure 2, Figure 3 and Figure 4. Furthermore, the retention time of Kresoxim-methyl was constant at 5.2± 0.2 min.

Linearity

Different known concentrations of fungicides (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) were prepared into a different 10 mL volumetric flasks by diluting the stock solution. The serial dilution details were presented in TABLE 1. These standard solutions were directly injected into a HPLC. A linear curve was plotted for the concentration of standards versus observed peak area and the correlation coefficient was determined (Tang et al., 2003 and Zuin V.G et al., 2003). The value of correlation coefficient was 0.9998. A calibration curve is showed in (Figure 5).

Accuracy and precision

The analytical method was validated for the recovery of the test item at two concentration levels with sandy loam, loamy sand, sandy clay and clay.

Preparation of 0.05 mg/kg fortification level

0.5 mL aliquot of 5.0 mg/L linearity solution was fortified into a 50g of each soils (sandy loam, loamy sand, sandy clay and clay) and extracted by extraction procedure This was followed for 6 replications.

Preparation of 0.5 mg/kg fortification level

5.0 mL aliquot of 5.0 mg/L linearity solution was fortified into a 50g of each soils (sandy loam, loamy sand, sandy clay and clay) and extracted by extraction procedure. This was followed for 6 replications.

The samples were assayed for accuracy and repeatability in HPLC. Accuracy was calculated as %recovery and precision as %RSD and the results are mentioned in TABLE 2.

Detection and quantification limits

The limit of quantification was determined to be 0.05 mg/kg. Limit of quantification was determined

TABLE 1 : Serial dilutions for linearity standard solutions

Stock solution concentration (µg/mL)	Volume taken from stock solution (mL)	Final make up volume (mL)	Obtained concentration (µg/mL)
522.72	1.910	10	100
100	0.500	10	5
100	0.200	10	2
100	0.100	10	1
5	1.000	10	0.5
5	0.200	10	0.1
1	0.500	10	0.05

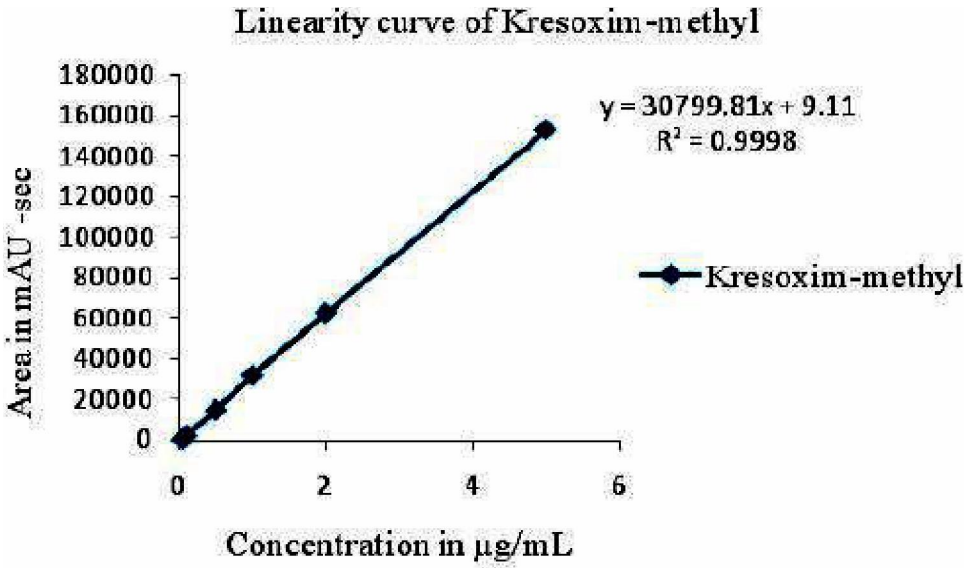

Figure 5 : Representative calibration curve of kresoxim methyl standard

TABLE 2 : Recoveries of the Kresoxim-methyl from soil samples samples (n=6)

Stock solution concentration (µg/mL)	Volume taken from stock solution (mL)	Final make up volume (mL)	Obtained concentration (µg/mL)
522.72	1.910	10	100
100	0.500	10	5
100	0.200	10	2
100	0.100	10	1
5	1.000	10	0.5
5	0.200	10	0.1
1	0.500	10	0.05

based on the lowest fortification level in the recovery study. This quantification limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram[9, 10]. The limit of detection was determined to be 0.02 mg/kg at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

CONCLUSION

This paper describes a fast, simple inexpensive analytical method based on HPLC-UV to determine the kresoxim methyl residues in three different type of tropical soils. The mobile phase combination of Acetonitrile and 0.1% ortho phosphoric acid was showed good separation in between main peak and

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unknown peaks. Satisfactory validation parameters such as linearity, recovery, precision and LOQ values were established by following South African National Civic Organization (SANCO) guidelines[15]. Therefore, the proposed analytical procedure could be useful for regular monitoring, residue labs and research scholars to determine the kresoxim - methyl residues in different commodities (crop, water and soil samples).

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