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Organophosphorus compounds are mainly highly and moderately toxic to humans and warm-blooded animals. Upon entering into the

procedure is based on the use of a sorbent, which acts as an abrasive in order to produce a modi ed “opening” of the solid matrix, facilitating the extraction process when using a suitable solvent for eluting the analytes. e 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 di erent 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 ve di erent 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

Certi cated 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. ey were brought to the laboratory and stored in plastic bag under refrigerator condition until they were processed in the laboratory.

Standard stock solutions

e 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. e 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

vortar for 30 min, to assess the homogeneity of the sample. e sample was allowed to stand at room temperature for one hour, until analysis.

Extraction procedure

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

Chromatographic separation parameters

e HPLC-UV system used, consisted Shimadzu high performance liquid chromatography with LC-20AT pump and SPD-20A interfaced with LC solution so ware, 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. e injected sample volume was 20 µL. Mobile Phases A and B were acetonitrile and Milli-Q water (75:25(v/v)). e ow- rate used was kept at 1.2 mL/min. e detector wavelength was 230 nm. e 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 quanti cation (LOQ) were considered. e accuracy of the method was determined by recovery tests, using samples spiked a concentration levels of 0.01 and 0.1 mg/kg. Linearity was determined by di erent known concentrations (0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 µg/mL) were prepared by diluting the stock solution. e limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise de ned from the analysis of control (untreated) sample. e limit of quanti cation (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

Speci city

Speci city was con rmed by injecting the honey control. ere 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

Di erent 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. e 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. e peak areas obtained from di erent concentrations of organophosphorus insecticides were used to calculate linear regression equations. ese were Y= 132256.12X+84.23, Y=105266.62X+32.18, Y=115461.52X+12.93, Y=123968.33X+25.12 and 150918.15. +51.3

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SANCO guidelines [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. erefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of organophosphorus insecticide residues on a large number of honey samples.

Acknowledgement

The authors are thankful to the Management and Dr. P. Balakrishnamurthy, Director, IIBAT, for providing necessary facility to conduct the experiment.

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2. Barker SA(2000) Applications of matrix solid-phase dispersion in food analysis. J Chromatogr A 880: 63-68.

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Conclusions

is paper describes for the rst time a fast, simple sensitive analytical method based on MSPD with HPLC-UV was developed and validated for the simultaneous determination of ve organophosphorus insecticides residues in honey. e 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.

e mobile phase acetonitrile and Milli-Q water yields good separation and resolution and the analysis time required for the chromatographic determination of the ve 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. erefore, 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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