DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR DETERMINATION OF PESTICIDESBY USING LC-ATMOSPERIC PRESSURE CHEMICAL IONIZATION-ORBITRAP-MASS SPECTROMETRY

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ABSTRACT: A simple accurate and precised LC-APCI-Orbitrap-MS method for the determination of Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Allethrin and Methomyl pesticides was developed and validated. By the analysis, retention time was found to be 2.87min for Dimethoate, 3.93mins for Carbofuran, 4.15mins for Aldicarb, 3.98mins for Oxamyl, 5.57mins for Quinalphos, 6.06mins for Allethrin and 5.77mins for Methomylwith a correlation coefficient (r2) of 0.9997. The spectra obtained from most compounds consisted solely of $[M + H]^+$ in positive-ion mode.The limit of detection (LOD) was calculated and found to be 0.004µg and limit of quantification (LOQ) was found to be 0.30 µg. Mobile phase composition (20–100% acetonitrile in water) and flow rate (0.8mL/min) had small effects on sensitivity of response.In Coriandrum samples precision values % RSD values were found to be 0.120. The peaks are clearly indicated that the compounds are fairly enriched after Dispersive Solid Phase Extraction which is the main point of extraction. The low detection limits, high degree of linearity, the ability to produce diagnostic fragment ions using cone voltage fragmentation. We suggest that APCI is a valuable technique for multiresidue confirmations of pesticides in various matrices.

Keywords: Pesticides, Dispersive Solid Phase Extraction, LC, APCI, Orbitrap-MS, Coriandrum.

INTRODUCTION

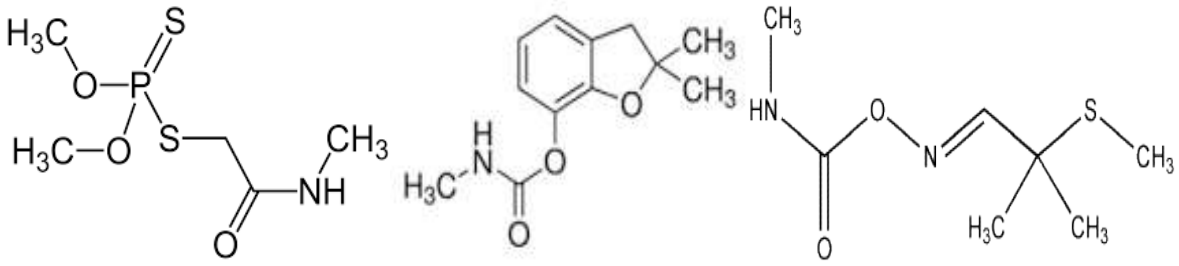
Pesticides are chemicals widely used to control a variety of pests, such as insects, plant pathogens, weeds, etc[1,2]. The use of pesticides may result in residues in crops, therefore, strict regulations are in place to control the use of these chemicals and to ensure that concentrations do not exceed statutory maximum residue levels (MRLs)

Pesticides are measured almost exclusively by liquid chromatography (LC) and gas chromatography (GC) analytical methodologies. GC coupled to a mass spectrometer (MS) as a detector is widely used in many pesticide residue laboratories, because many pesticides are not amenable to LC-MS or ionize poorly under soft ionization techniques. GC offers good separation efficiency and a choice of MS detectors, including single or triple quadrupoles. Quadrupole mass analyzers are selective, sensitive, and cost-effective instruments that operate at nominal mass resolution. When using quadrupole MS, the selectivity required to separate target pesticides from chemical background is achieved using either selected ion monitoring (SIM) or selected reaction monitoring (SRM). Both SIM and SRM are used in targeted experiments in which the mass spectrometer is pre-programmed using a list of preselected pesticides. How-ever, targeting specific compounds during acquisition limits the scope of analysis and can result in false negative results (non-detection) for both unknown and untargeted compounds, which may be of concern with respect to food safety[3-10].

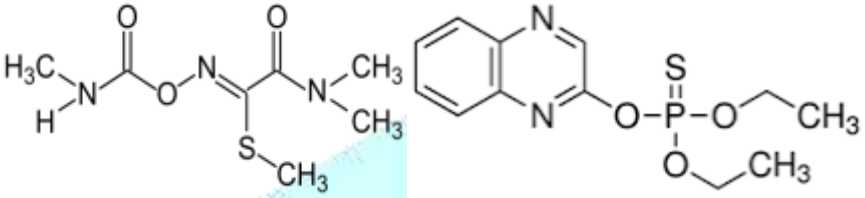
Thislimitation has led to increased interest in developing methods using MS analyzers that can operate in full scan with a higher mass resolving power than triple quadrupoles, but provide similar levels of selectivity and quantitative performance. Until now, high-resolution, accurate-mass GC-MS instruments have not gained wide acceptance due to their limited ability to provide full scan selectivity and quantitative performance comparable to triple quadrupole instruments operated in SRM.

In this work, we demonstrate the use of LC coupled to Orbitrap™ MS technology for fast, high throughput pesticide residues analysis in food samples, with an almost unlimited scope in the analysis through full scan acquisition.

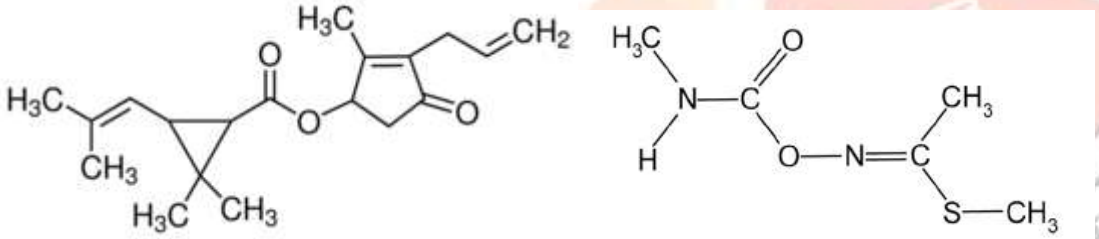
Dimethoate **Carbofuran** **Aldicarb**



Oxamyl **Quinalphos**



Alletrin **Methomyl**



MATERIALS AND METHODS

Chemicals:

Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl analytical standards (purity >99%), DSC C18, Triethyl Phosphate, Milli-Q water and Methanol of pesticide residue analysis grade were purchased from Sigma Aldrich.

EXPERIMENTAL PROCEDURE

Apparatus

Thermo Scientific-Exactive LC- Orbitrap,Centrifuge Model: RC -8C,Micro Spatula, 15ml Centrifuge Tubes, Vortex, Sonicator, Rotary Evaporatorwas used for analysis.

Mobile phase preparation

Mobile Phase A:

0.1% Formic Acid in water is Prepared. The mixture is filtered, Sonicated, and degassed for 15mins.

Mobile Phase B:

0.1% Formic Acid in methanol is Prepared. It is filtered, Sonicated, and degassed for 15mins.

Standard preparation:

Accurately weight and transfer each about 1mg of Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl into 2mL vial, add 1mL of mobile phase and sonicate to dissolve.

Preparation of stock solutions and working standards:

Individual standard stock solutions were prepared at 1 mg/mL by dissolving 10 mg of each pesticide in 10 mL of acetonitrile and were stored at -20^0C until use. Working standard solutions at different concentration levels were prepared in ethyl acetate

Chromatographic conditions

Column C-18 (33 mmx4.6 mm I.D; particle size 3µm) was used for analysis at 300°c column temperature. The mobile phase was pumped through the column at a flow rate of 800 µL/min. The sample injection volume was 1µL. Mass Spectrometer was used as detector and Chromatographic runtime was 10 minutes.

Data Processing

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software. TraceFinder software allows the analyst to build acquisition and processing methods for high throughput screening and quantitative analysis and incorporates library searching capabilities as well as easy data reviewing and data reporting. Results and Discussion The objective of this study was to evaluate the utility of Orbitrap-based GC-MS technology for fast pesticides screening and quantification to increase sample throughput and laboratory productivity.

RESULTS AND DISCUSSION

Method development

To develop a suitable and robust LC-MS method for the determination of target Analytes, different mobile phases were employed to achieve the best separation and resolution. Finally, the method development was achieved with Symmetry C-18 (33 mmx4.6 mm I.D; particle size 3µm) with the 0.1% of formic acid in water as mobile phases A and 0.1% of formic acid in methanol as mobile phase B. Gradient mode was initially maintained as 20-95% mobile phase B for 0-5mins, 95% for 5-7mins and 20% mobile phase B for 7.1-10mins. The flow rate was maintained 800µL/min. For APCI, Sheath gasflow rate is 60µL/min, Auxillary gas flow rate is 30µL/min, Capillary temperature is 350^0 C, Heater temperature is 300^0 C.

The targeted analyte mixture standards were injected and achieved good separation by the method parameters. The retention time for Dimethoate is 2.87mins, Carbofuran is 3.93mins, Aldicarb is 4.15mins, Oxamyl is 3.98mins, Quinalphos is 5.57mins, Alletrin is 6.06mins and Methomyl is 5.77mins were achieved. Finally, the method was good enough to separate targeted analytes by using proposed method. Chromatograms are represented in Figure 1-7.

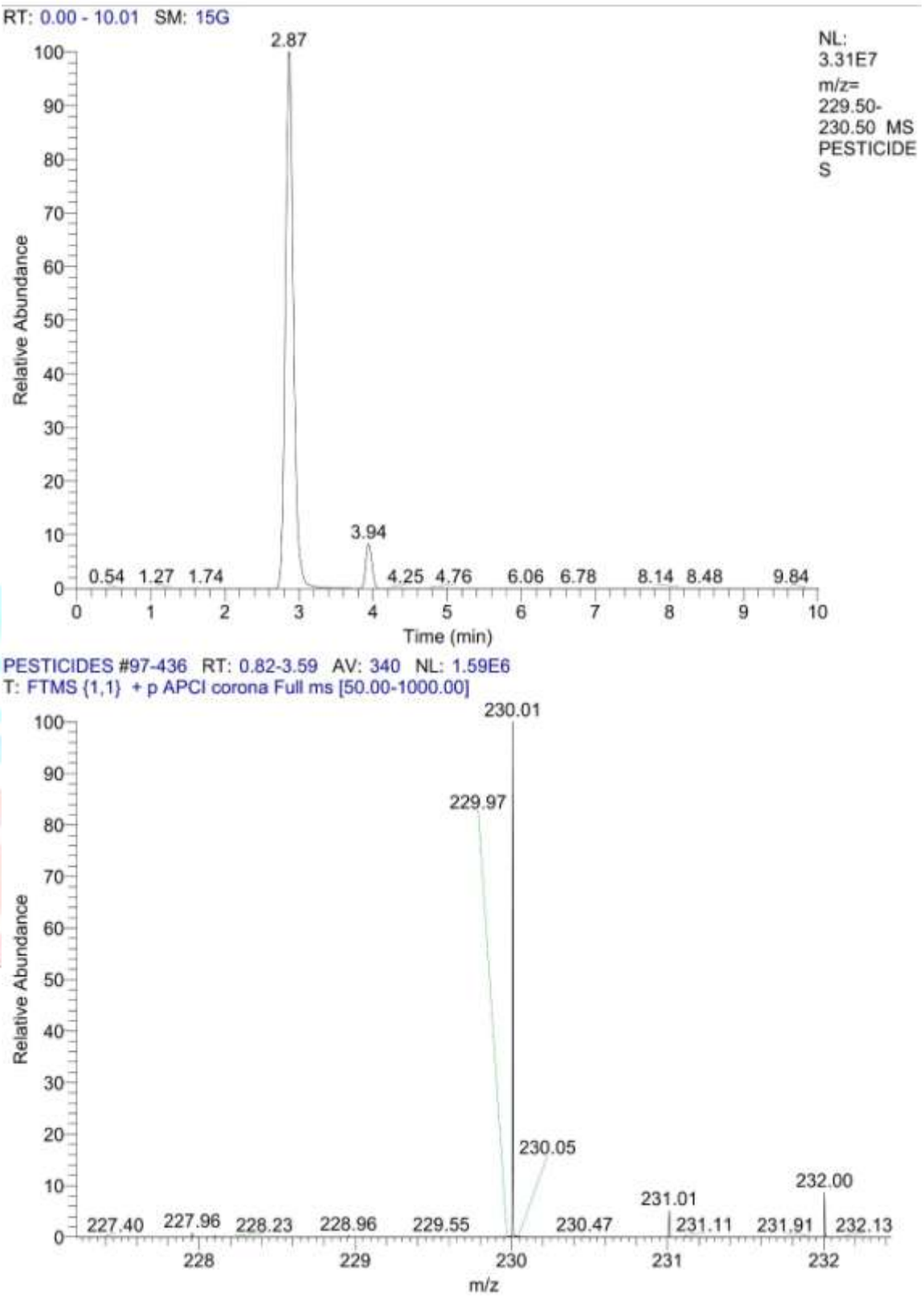


Figure 1: Chromatogram for Dimethoate

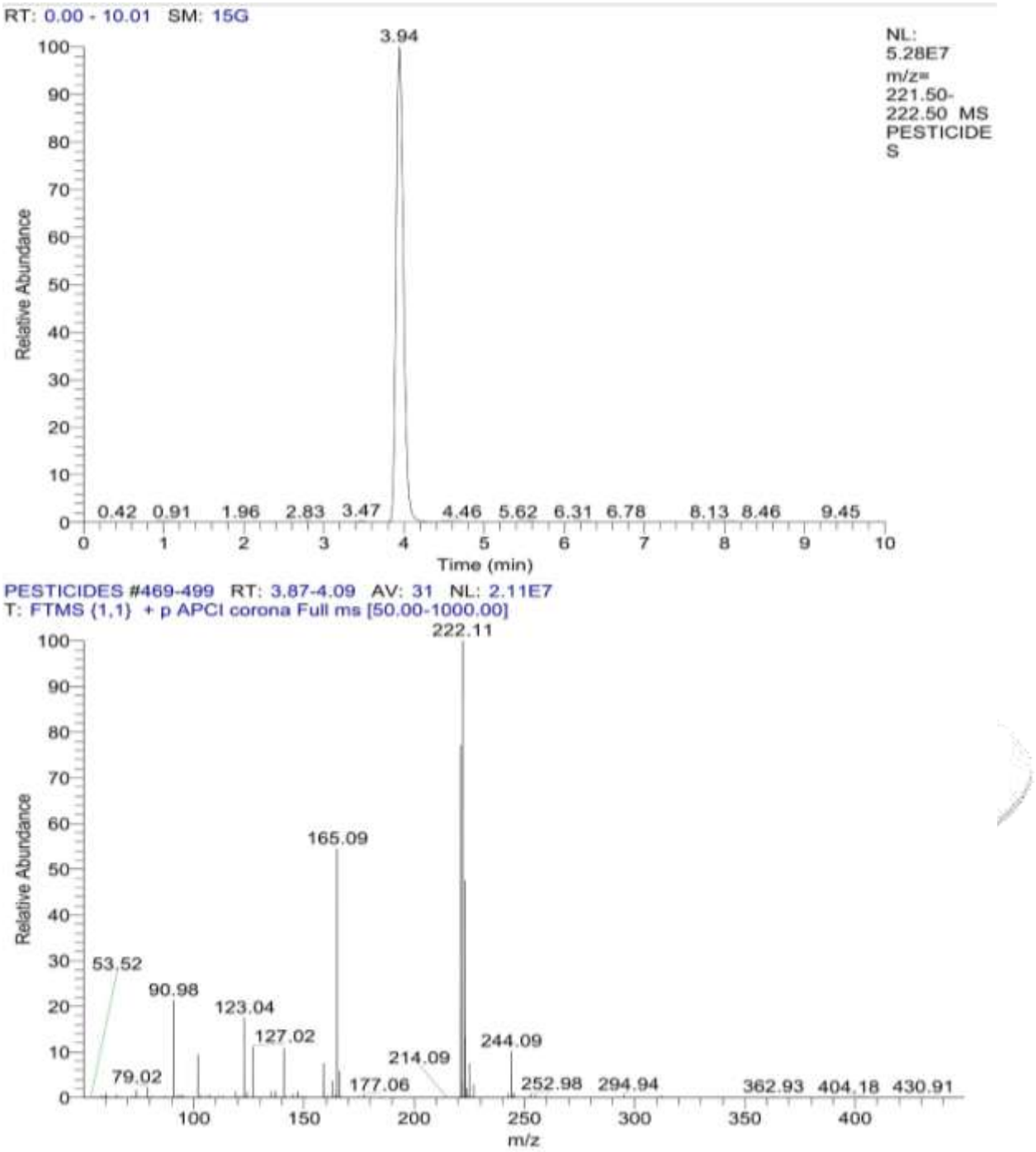


Figure 2: Chromatogram for Carbofuran

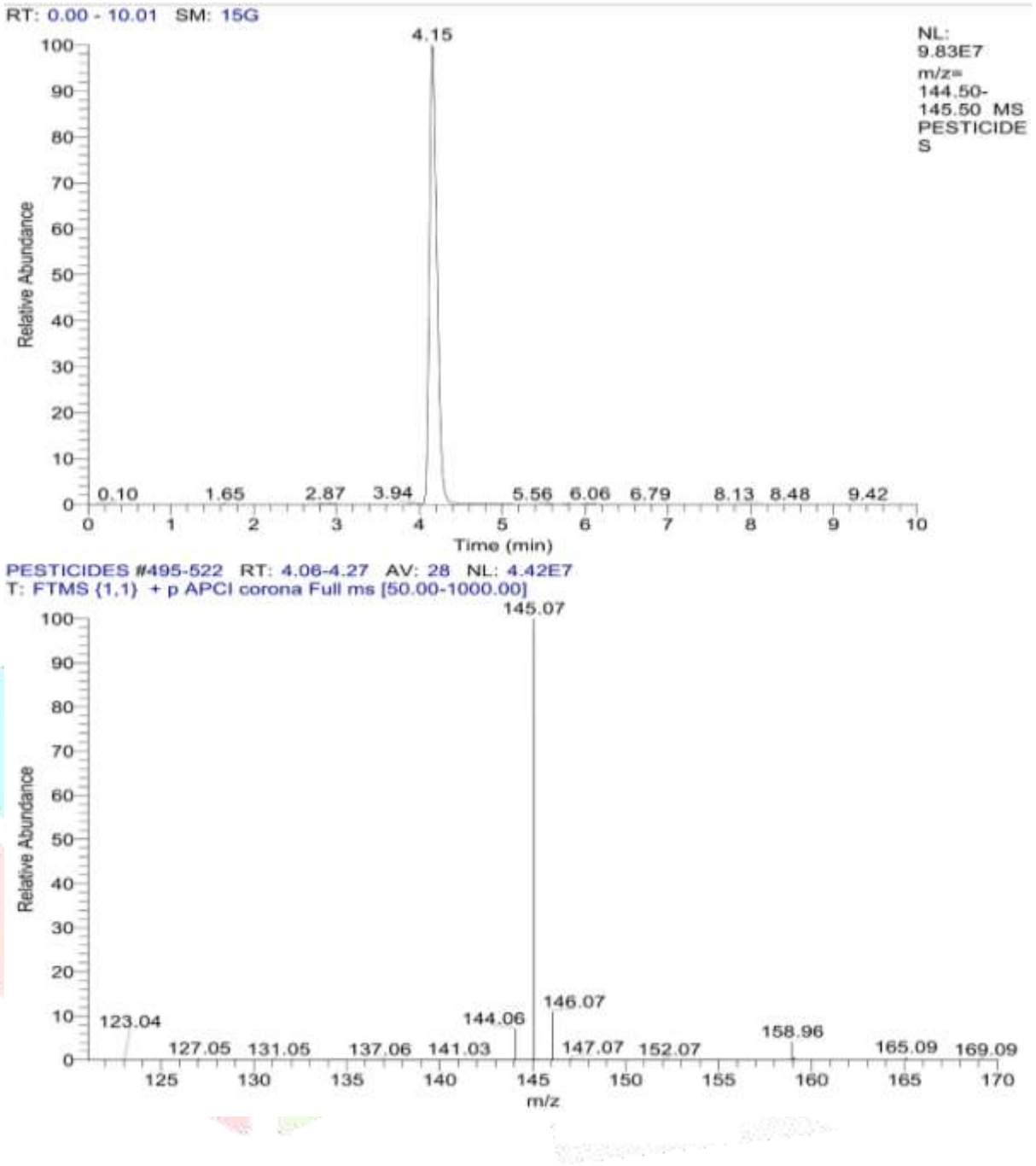


Figure 3: Chromatogram for Aldicarb

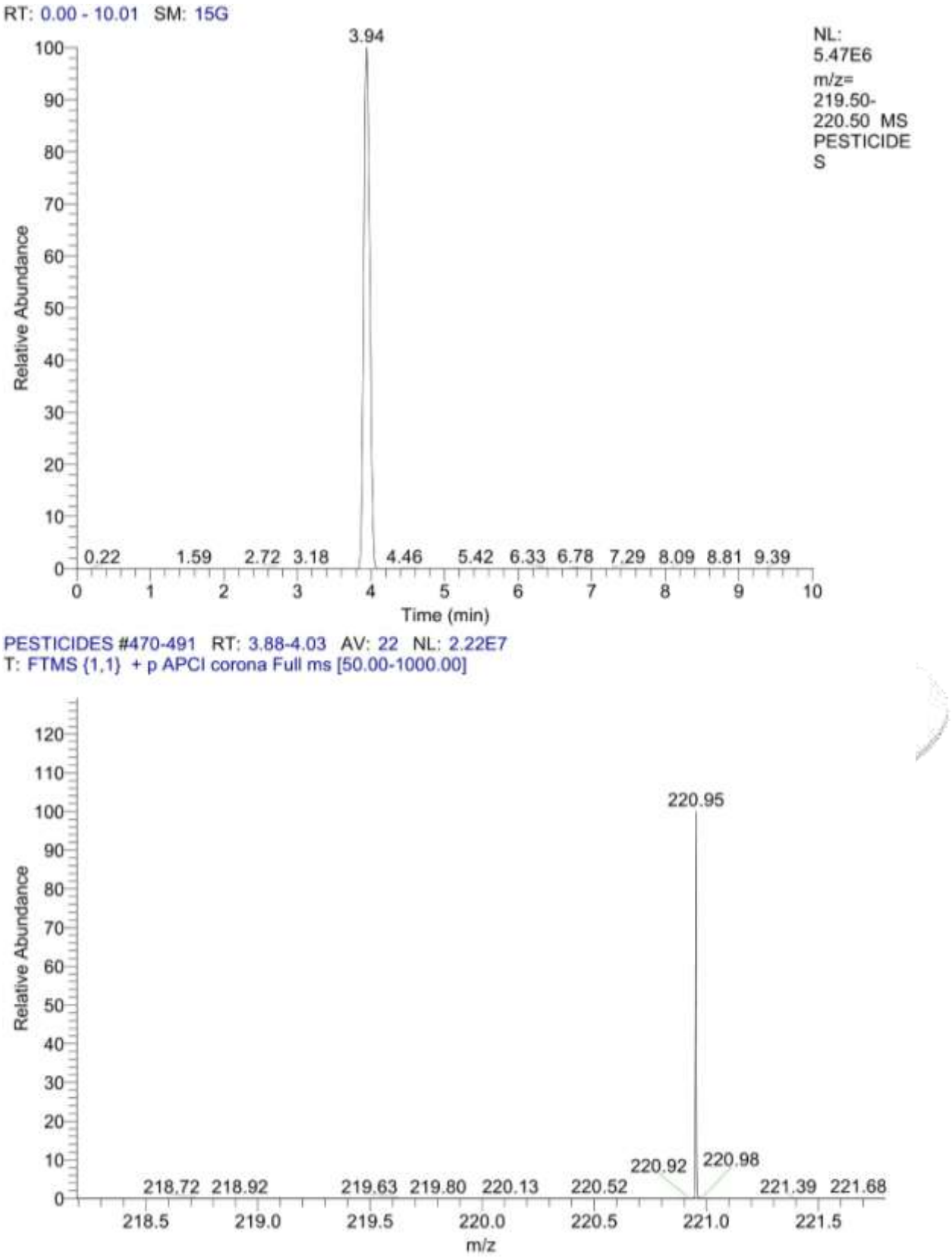


Figure 4: Chromatogram for Oxamyl

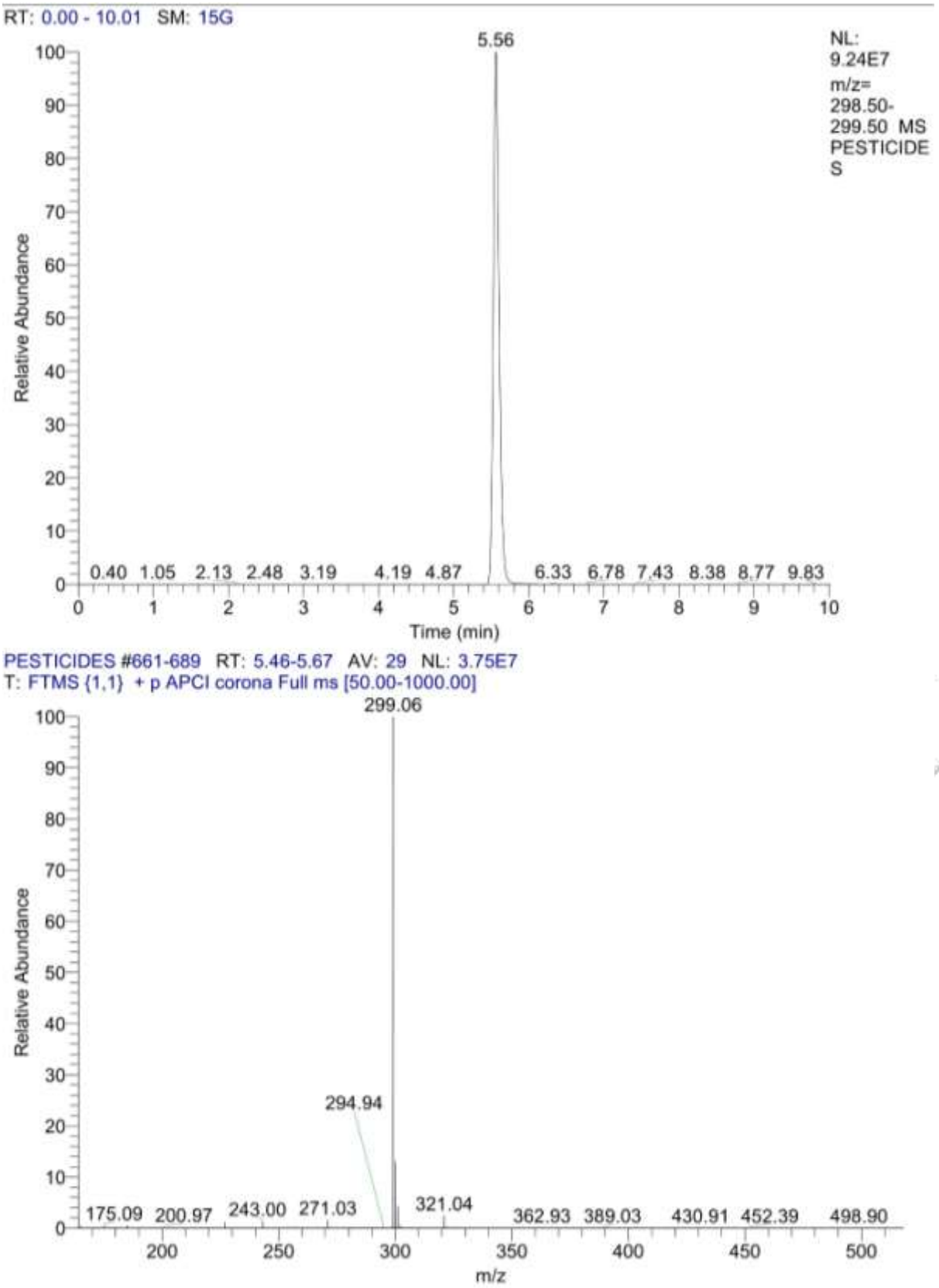


Figure 5: Chromatogram for Quinalphos

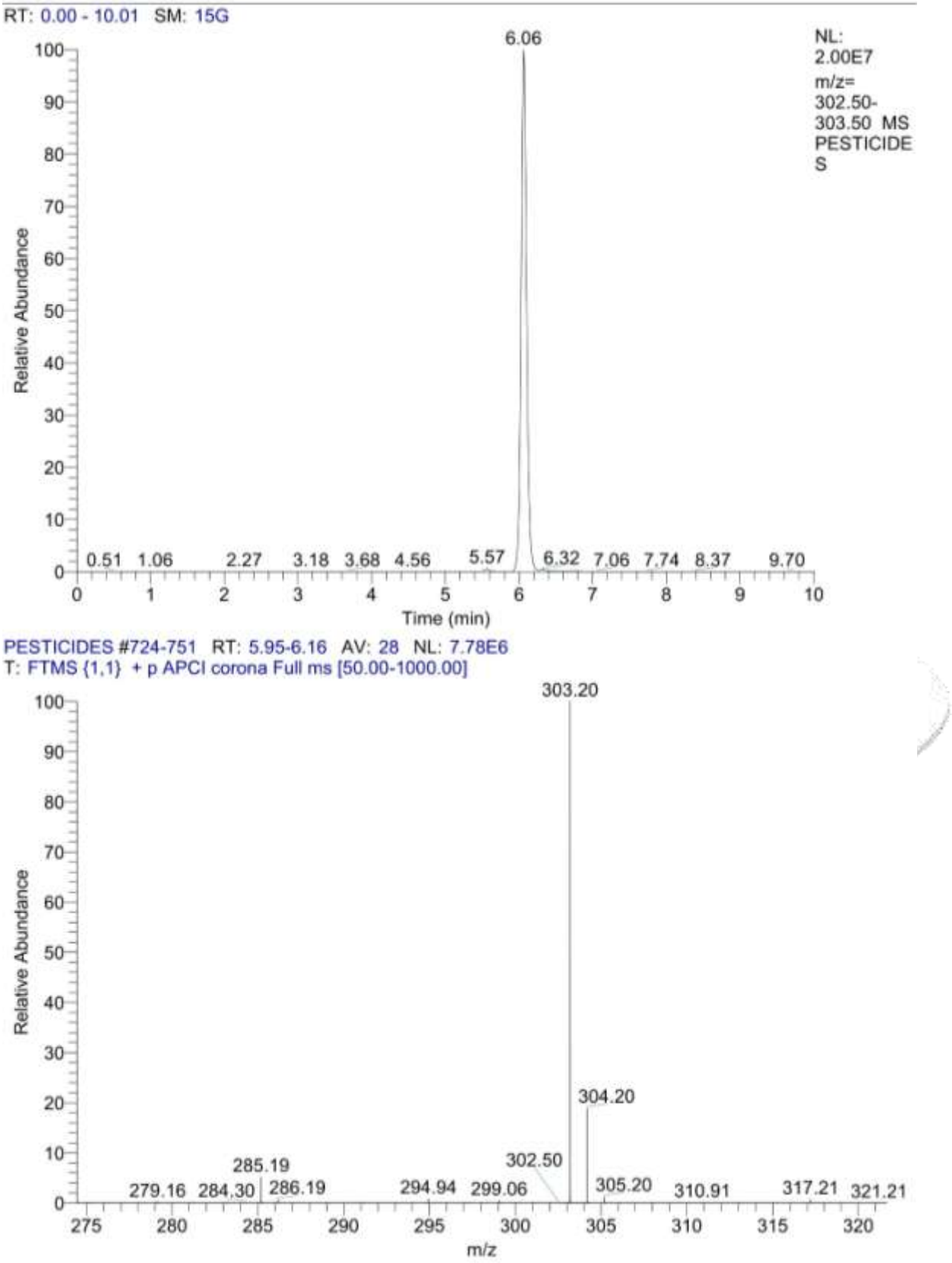


Figure 6: Chromatogram for Alletrin

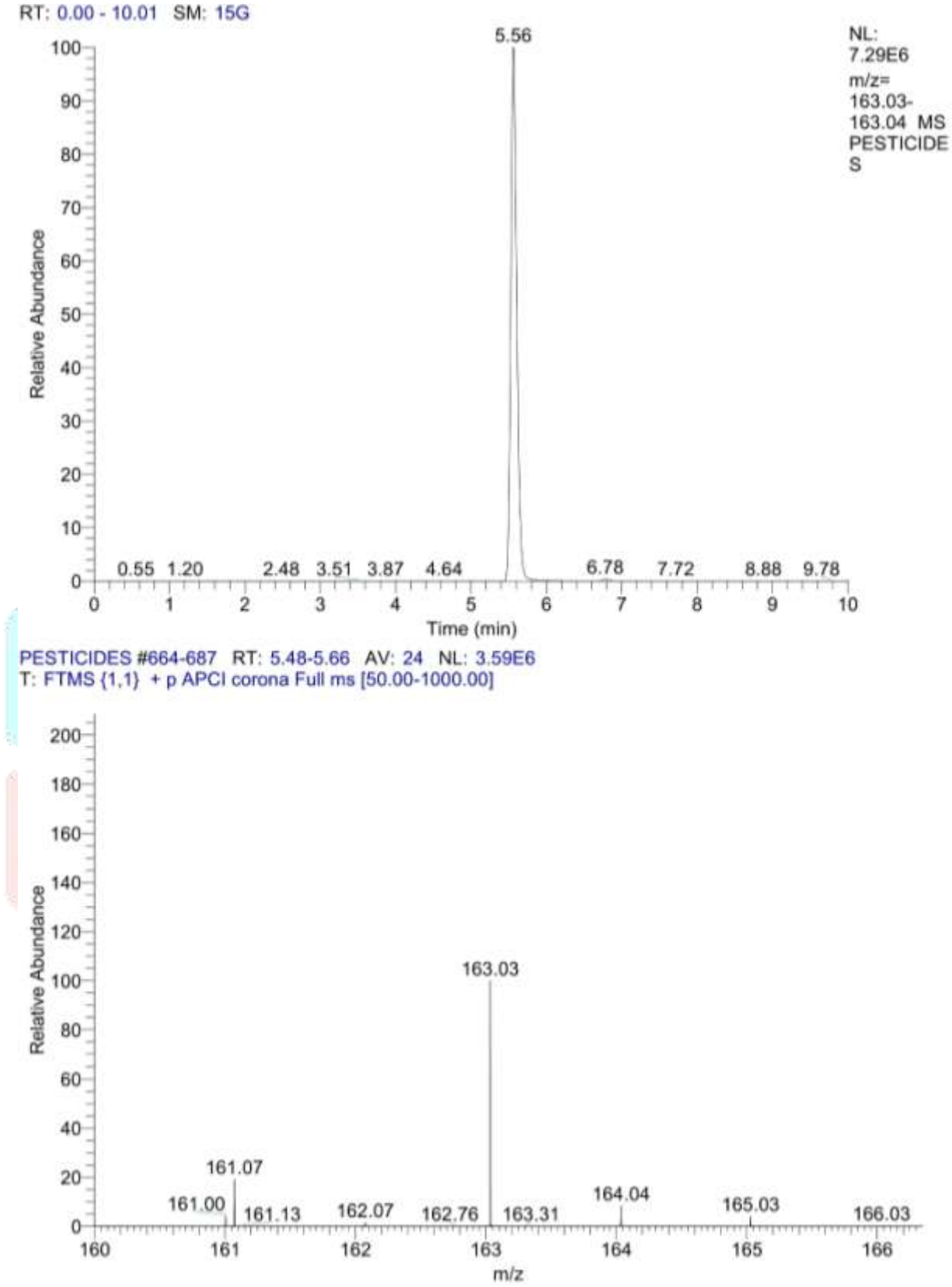


Figure 7: Chromatogram for Methomyl

METHOD VALIDATION

For method validation, from the stock solutions serial dilutions were done ranging from 100ppm-0.01pm. Triethyl Phosphate is used as an Internal Standard (IS) to normalize the signal. IS were taken about 1ppm Concentration.

SPECIFICITY

Blank Interference

1µL of blank sample wasinjected into the LC-MS system as per the proposed test method. Evaluated the interference of blank at the retention time of targeted analyte peaks and foundno peaks at the retention time of targeted peaks. The results are Figure-8.

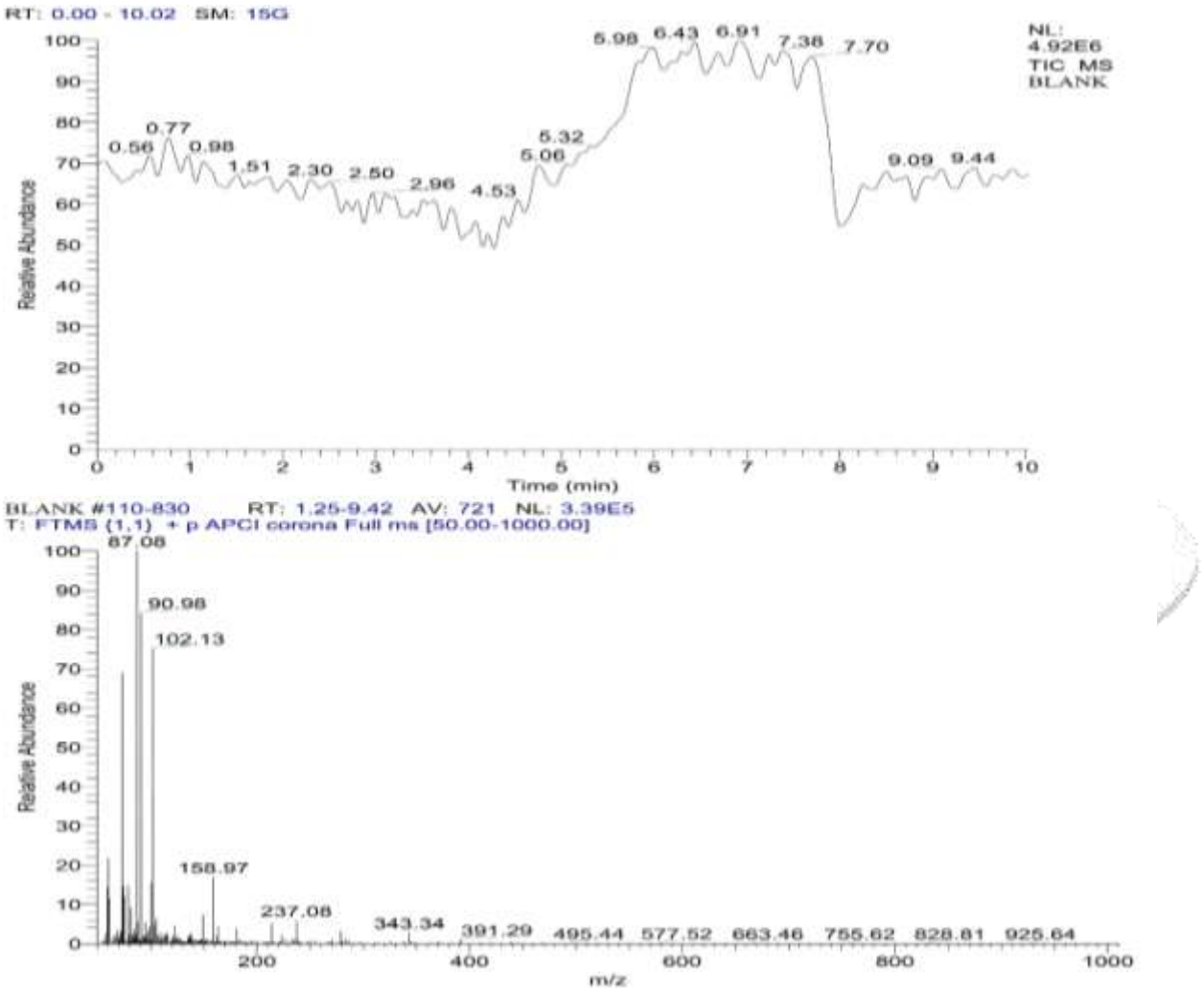


Figure 8: Chromatogram for Blank showing no interferences

ESTABLISHMENT OF LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

A study was conducted to establish the limit of detection (LOD) and limit of quantification (LOQ) of Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomylbased on slope method. Prepared a series of solutions from 10ppm to 0.01ppm of standard concentration of Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl. These solutions were injected into the LC-MS system as per methodology.

Plotted a graph by taking concentration on X-axis and area on Y-axis, calculated the standard error and slope of the calibration curve. The predicted LOQ concentration and LOD concentration are calculated by using formula given below..

$$LOQ = \frac{10 \times \sigma}{S} \qquad LOD = \frac{3.3 \times \sigma}{S}$$

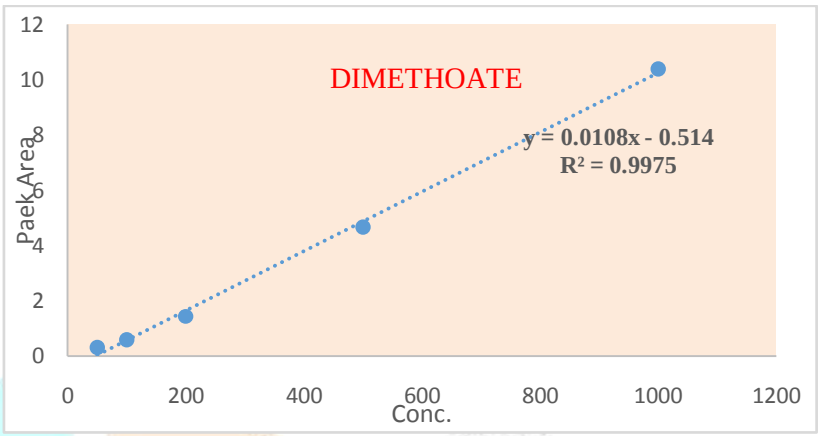
σ = Standard Error of the calibration curve

S = Slope of the calibration curve

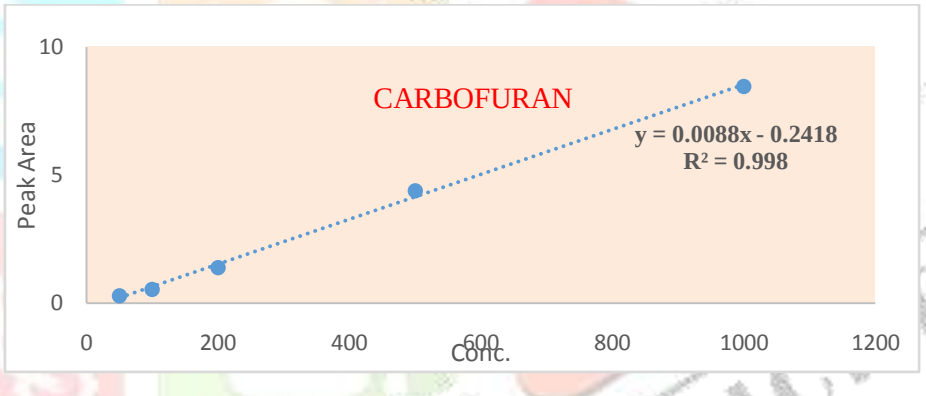
LINEARITY

Linearity is carried out under LOD-LOQ establishment experiment, the same linearity establishment data can be used to deduce the linearity from LOQ level to 200% (0.01ppm) specification level. A graph was plotted to concentration in ppm on X-axis versus response on Y-axis. Calculated % y-intercept and correlation coefficient which were shown in Graph 1-6.

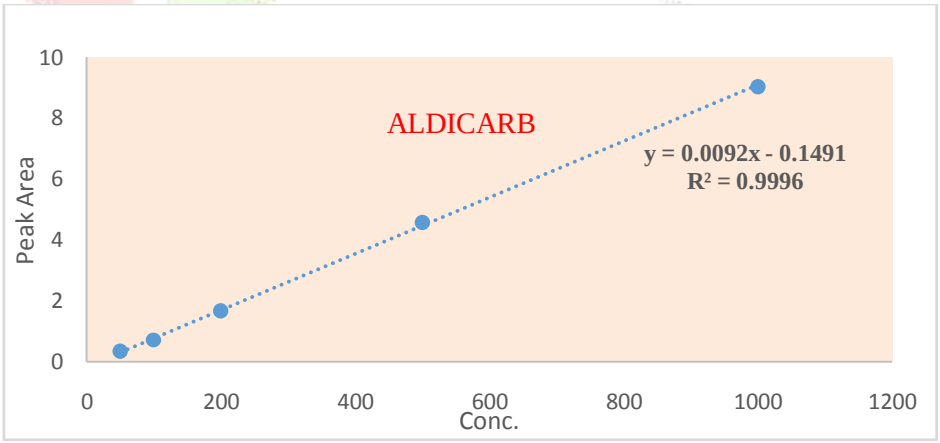
Linearity Graphs are represented as follows.



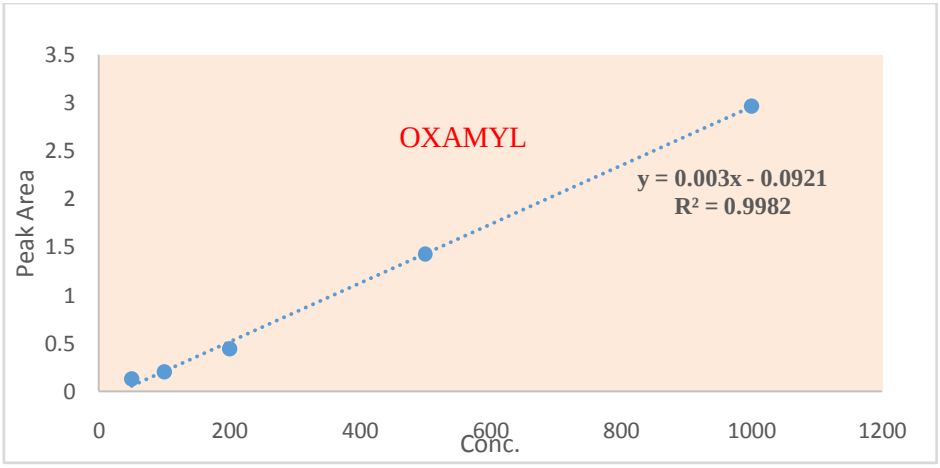
Graph 1: Linearity for Dimethoate



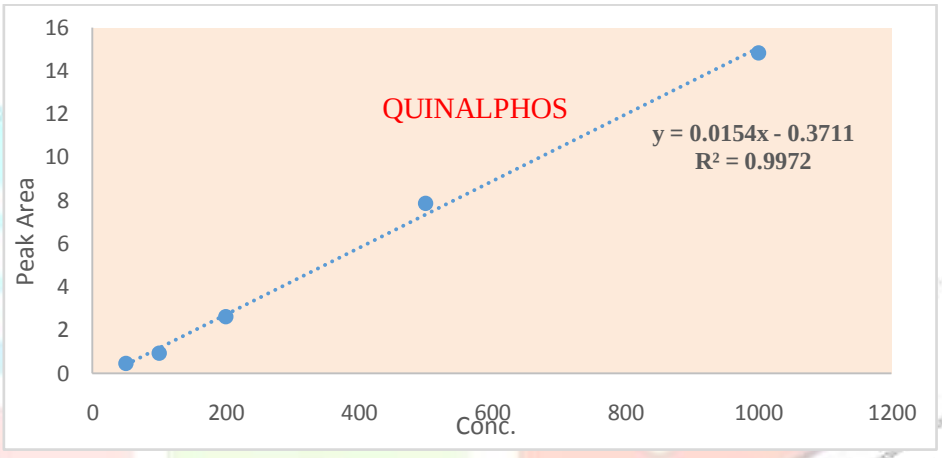
Graph 2: Linearity for Carbofuran



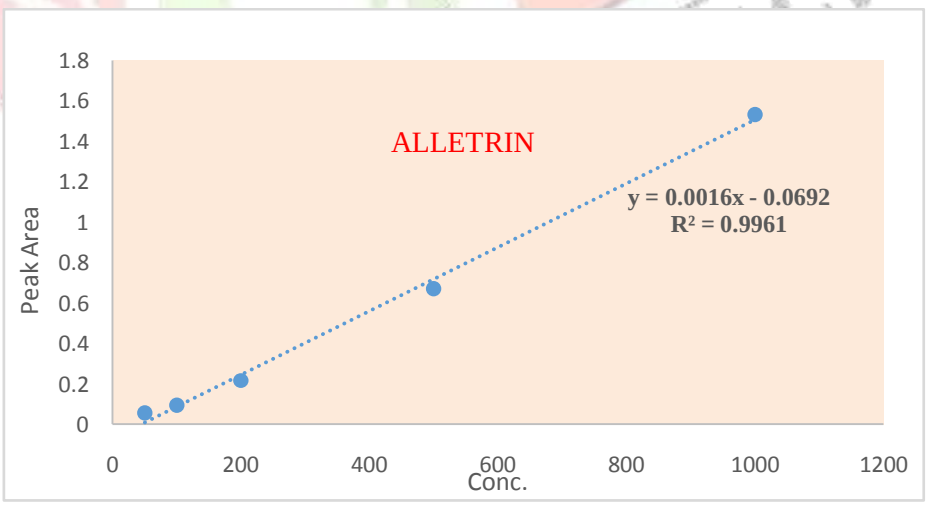
Graph 3: Linearity for Aldicarb



Graph 4: Linearity for Oxamyl



Graph 5: Linearity for Quinalphos



Graph 6: Linearity for Alletrin

PRECISION

Intra-day precision, or within-day reproducibility, is expressed as the average of the relative standard deviation (RSD%) of the areas obtained for each analyte after the replicate (n=6) analysis. The results were shown that within the acceptable limits as per ICH guidelines.

ROBUSTNESS

Similarly, Robustness also evaluated and found that the method is robust enough for various robustness parameters such as flow variation, column temperature variation, mobile phase composition variation.

All the system suitability criteria are meeting in all the robust parameters, this indicates that the proposed analytical method is robust enough for the estimation of Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl by using the analytical method.

APPLICATION TO REAL-SAMPLE ANALYSIS

SAMPLE PREPARATION

The developed method was applied successfully to the Coriandrum samples collected from local market for monitoring multiresidue analysis. Coriandrum leaves were soaked in Milli Q water for 15mins and sonicated for 30mins. The residue was centrifuged for 15mins at 10,000rmp speed.

EXTRACTION PROCEDURE OPTIMIZATION

2mg dispersive solid phase (DSC-C18) is added in the residue solution and sonicated it for 5mins, shake it by using horizontal shaker at the speed of 250ppm for 15mins and centrifuged it for 15mins. Remove the supernatant, add 10ml of Milli Q water and centrifuged it and add 10ml of methanol and again centrifuge for 15mins and collect the supernatant. Evaporate the solvent by using rotary evaporator. Add 20µLTriethyl Phosphate as an Internal Standard of 1ppm concentration. 1µL is injected in the LC-MS and run the whole method as discussed above. The results obtained is that 0.04ppm of Dimethoate pesticide is detected in the coriandrum residue. The chromatogram is represented as follows.

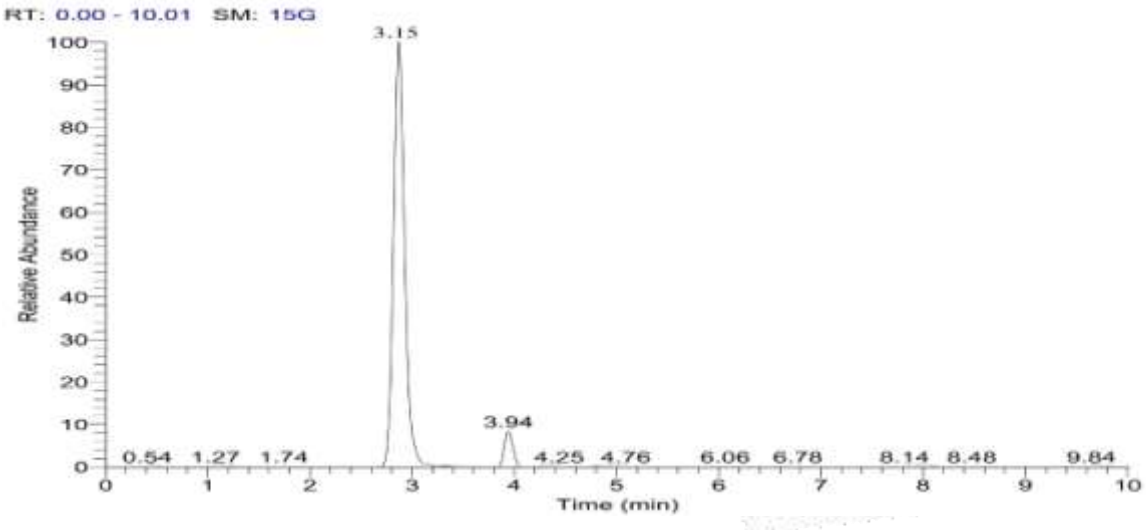


Figure 9: Chromatogram of Coriandrum leave extract showing Dimethoate Peak.

RESULTS AND DISCUSSION

A simple, economic, accurate and precise LC-MS method was successfully developed. In this method, it was carried out by using Column C18, (33× 4.6mm) with 3µm particle size. Injection volume of 1µl is injected and eluted with the mobile phase A and B over gradient program, which is pumped at a flow rate of 0.8 ml/min. Detection, was carried out by mass spectrometry. All the compounds are well resolved from blank peak and there is no interference from blank. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of Selectivity, accuracy, linearity, precision, robustness.

For Selectivity, the chromatograms were recorded for standard and sample solutions of Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl. Selectivity studies reveal that the peak is well separated from each other. Therefore, the method is selective for the determination of Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl.

The limit of detection (LOD) and limit of quantitation (LOQ) was found to be for 0.001µg/ml, 0.003µg/ml, 0.006µg/ml, 0.001µg/ml, 0.002µg/ml, 0.001µg/ml and 0.004 µg/ml respectively for Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos,

Alletrin and Methomyl. The linearity results for Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.95. Calibration curve was plotted and correlation co-efficient for Dimethoate is 0.9975, Carbofuran is 0.998, Aldicarb is 0.9996, Oxamyl is 0.9982, Quinalphos is 0.9972, Alletrin is 0.9961 and Methomyl is 0.9997.

The accuracy studies were shown as % recovery for Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl, at 50%, 100% and 150%. The limit of % recovered shown is not less than 80% and the results obtained were found to be within the limits. Hence the method was found to be accurate. The accuracy studies showed % recovery of the Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl, in the range 95-98% respectively.

For Precision studies six (6) replicate injections were performed. %RSD was determined from the peak areas of Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl. The acceptance limit should be not more than 10 %RSD, and the results were found to be within the acceptance limits. For intermediate precision, the bias is not more than ± 1.0.

In the real sample, Dimethoate is detected in coriandrum about 0.04ppm trace levels. Hence, the chromatographic method developed for Dimethoate, Carbofuran, Aldicarb, Oxamyl, Quinalphos, Alletrin and Methomyl are rapid, simple, sensitive, precise, and accurate. Therefore, the proposed method can be successfully applied for the routine analysis of the pesticide residue analysis for assurance of its presence in matrices.

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