Determination of Chlorantraniliprole (Pesticide) in Okra of Bilaspur(CG) Open Market

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Abstract: Okra from various sale points of the market of Bilaspur city (Chhattisgarh) are collected, preserved and transported to Research Laboratory Extraction, clean up and analysis processed with the use of Liquid chromatography with Mass spectrometry (LC-MS/MS). Spot diameter of chlorantraniliprole (pesticide residue) of sample are identified, reported and results and need of inspection on pesticide residues in agricultural products in order to prevent the contamination and secure human safety also suggested.

Key Words: Bilaspur open market, Okra, Pesticides, LC-MS/MS, Chlorantraniliprole, MRL values

Introduction:

Pesticide residue is a major concern for the stakeholders of the food industry, since the quality regulations and food safety standards are becoming more stringent in most countries. The management of pesticide residues in agri products is challenging because, besides direct application, pesticide residues may also appear in fruits and vegetables from indirect sources like soil, contaminatedagro-inputs (e.g.Manures, fertilizers, growth regulators, irrigation water etc.,), drift
From adjoining fields of other agricultural crops etc. A rapid analytical method is followed to determine the target pesticides in any sample by a single effort with equivalent or superior overall efficiency.

Material and Methodology

Okra from various sale points of the market of Bilaspur(Chhattisgarh) are collected, preserved and transported to Research Laboratory, Extraction, clean up and analysis then processed with the use of Liquid chromatography with Mass spectrometry (LC-MS/MS). Using following Methodology of USEPA.

Sample handling and Preparation

Freshly collected Okra sample kept in cold condition before and during transportation to the laboratory. Once received at laboratory, samples were kept at -20°C for minimum 30minutes prior to blending.

~2kg of Okra sample was blended and ~200g of crushed sample taken homogenized for one minute.

Extraction & clean up

Weighed 10.0 ± 0.5 gm of the homogenized sample in a 50ml centrifuge tube (For Pomegranate add 10 ml water).
Added 10ml ethyl acetate, 100 μl acetic acid and 10 gm. of sodium sulfate, 0.5 gm sodium acetate in it.
Note: immediately after addition of the mixture of salt, vigorously shaken by hand the centrifuge tube for at least 5 seconds, to disperse the solid phase and prevent the formation of a salt block.Homogenized at 15000 RPM for 2 mins.
Centrifuged the extract at 7000 rpm for 5 mins.
The supernatant is taken for the further cleanup for LCMSMS and GCMS.

Clean-up for LCMSMS:

Taken 4ml of the extract in centrifuge tube containing 25 mg PSA, vortex for 10 sec and then taken 2 ml extract in an evaporation tube and added 10 μl of 10% DEG.
Vortexed well and evaporate to dryness under N2 stream at 35ºC.
Dissolved the residue in 1ml 5mM Ammonium Formate in 0.1% formic acid in Methanol and 1 ml 5mM Ammonium Formate in 0.1% formic acid in water.
Transferred in to the Eppendorf tube and Centrifuge at 12000 RPM for 5 mins at 4∘C.
Filtered the extract with 0.2μm membrane Nylon filter.
Inject in LCMSMS.

Identification and Determination of Analytes

LC Conditions:

Column: C184.6 X 100mm X 2.7μm
Flow rate: 0.5 ml/min
Column temperature: 40°C
Injection volume: 4 μl
        Post Time: 2:0 min
Detector: Mass Spectroscopy
Stop time: 17.5 minutes

Mobile phase: A) 5mM Ammonium Formate in 0.1%formic acid in Water
B) 5mM Ammonium Formate in 0.1%formic acid in Methanol

Gradient program:

Table -1: Gradient program of LC

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	80	20
5	30	70
15	5	95
16	5	95
16.5	5	95
17.5	80	20

Preparation of Calibration Curve Standards

Standard stock silution

Chlorantraniliprole stock solution was prepared in ethyl acetate at a concentration level of 1000 ppb: Weighed individual raw reference standard around 10mg± 1.0mg in 10 ml volumetric flask and dissolved in Ethyl acetate. and stored in a freezer at -18°C, this stock standard solution can be used up to 3 months. Suitable concentration of working standards are prepared from stock standard solution by dilution using Ethyl acetate, immediately prior to sample preparation. As below.

Table 2: Preparation of Calibration curve standards

Concentration of Stock solution(ppb)	Volume taken from stock solution(ml)	Final Volume made (ml)	Final concentration(ppb)
1000	2	10	200
1000	1	10	100
1000	0.5	10	50
100	1	10	10
100	0.5	10	5
10	2	10	2

Mass Spectrum & Linearity:
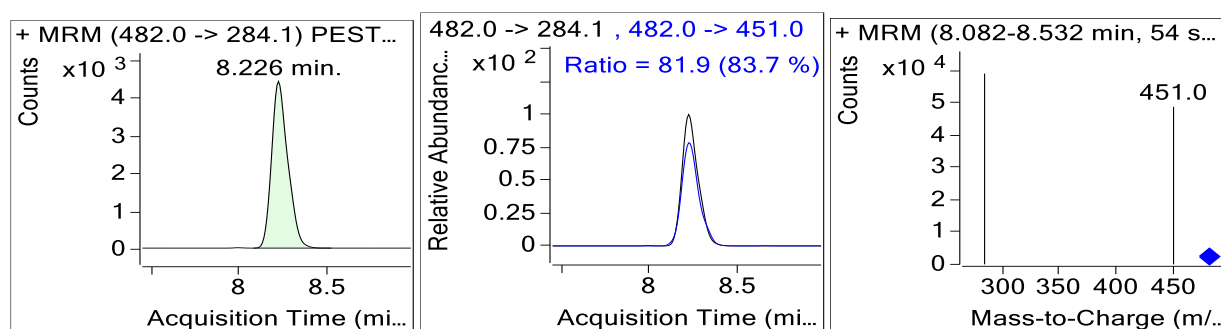
Fig.1: Mass Chromatogram of 2PPB Standard
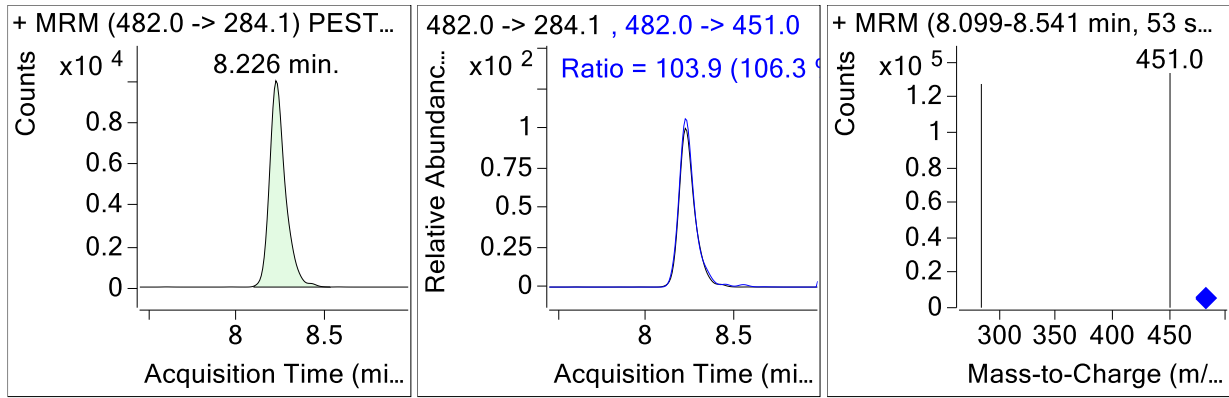
Fig.2: Mass Chromatogram of 5PPB Standard
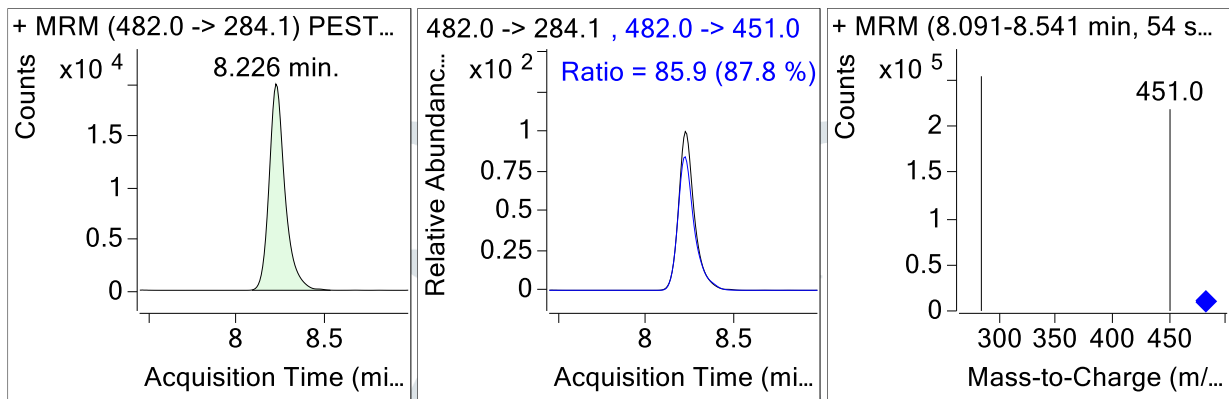

Fig.3: Mass Chromatogram of 10PPB Standard

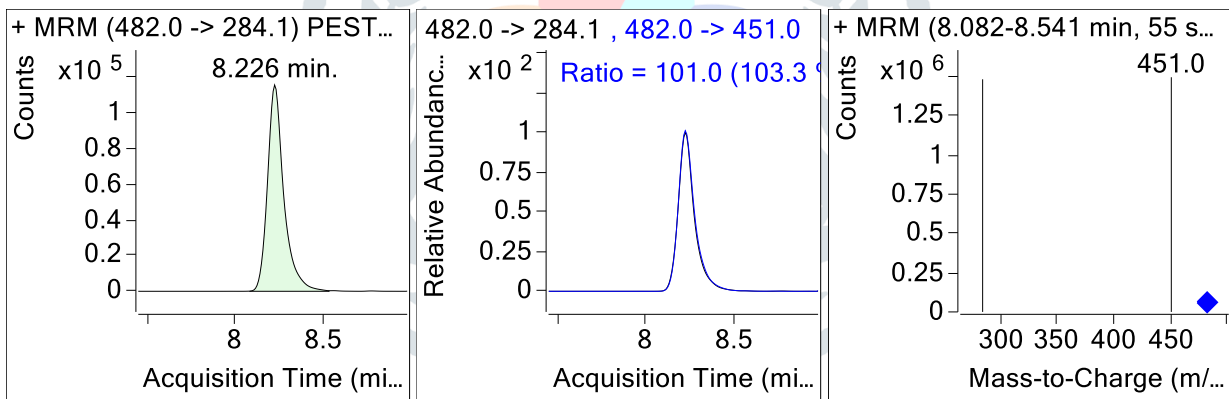Fig.4:Mass Chromatogram of 50PPB Standard

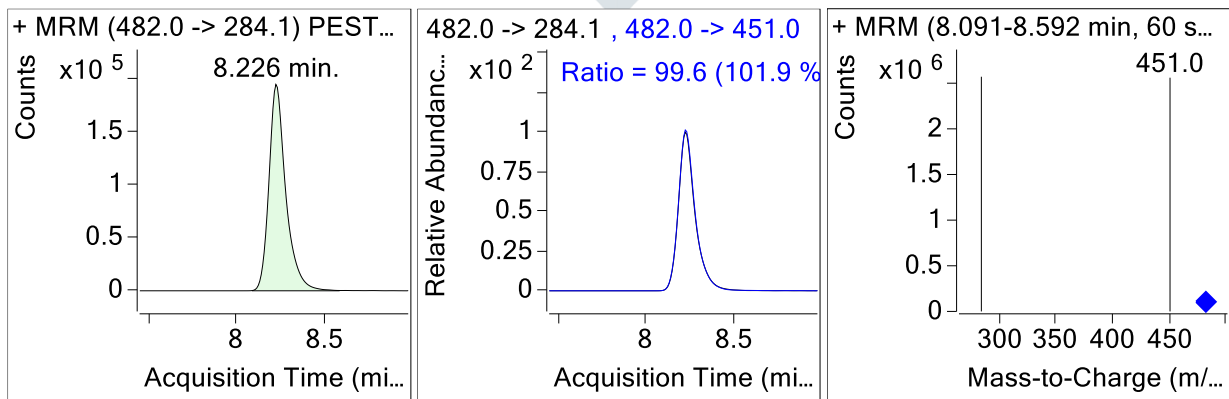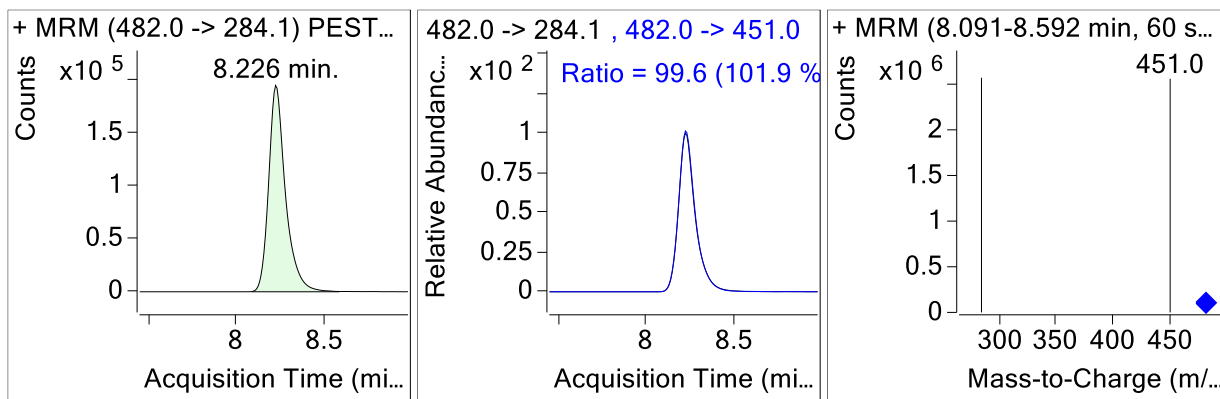Fig.5:Mass Chromatogram of 100PPB Standard

Fig.6:Mass Chromatogram of 200PPB Standard

Linearity

Table 3 : Response of calibration curve standard s with LCMS/MS

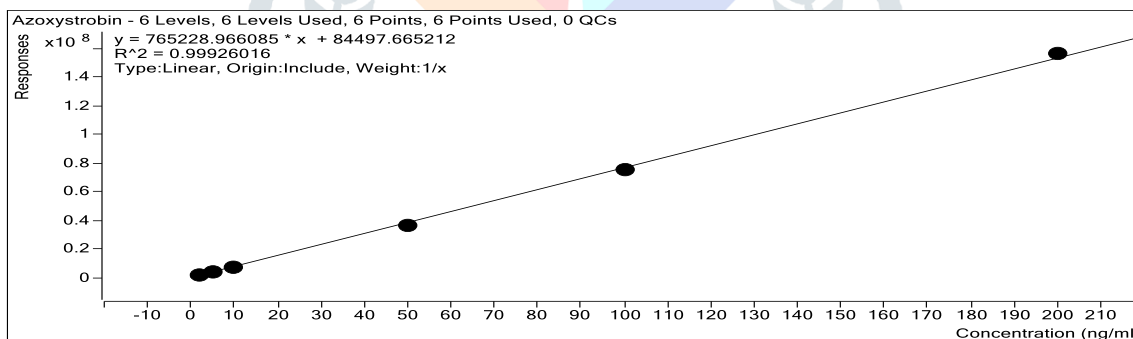| S.No.	Standard Conc(ppb)	RT	Response
1	2.0000	8.226	28827
2	5.0000	8.226	63647
3	10.0000	8.226	128192
4	50.0000	8.226	753012
5	100.0000	8.226	1307741
6	200.0000	8.226	2422730

Fig.7: Calibration curve (Linearity)
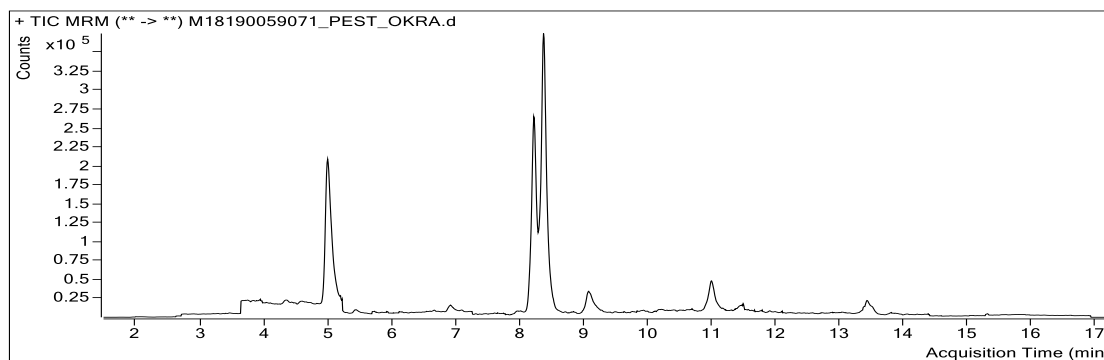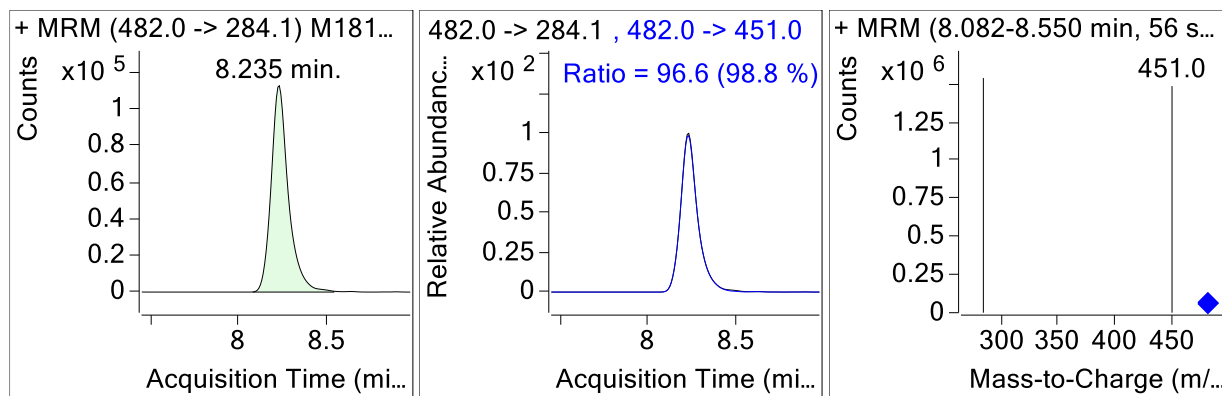

Fig.8:Chromatogram of sample

Fig.9:Mass Chromatogram of Sample

Result, Calculation & Conclusion:

Result

Table 4 : Response of Sample in LCMS/MS

S.No.	RT	Response	Dilution Factor	Final Conc(PPb)
1	8.235	780577	2	121.793

Calculation

The Concentration of Chlorantraniliprole in sample analyzed by LC-MS/MS was determined directly from the standard curve.

$$Y=mx+C$$

Where,
Y = Peak area of Standard
M=Slope of line from the calibration curve
X= Concentration of analyta in injected sample
C= 'y' intercept of the calibration curve

Recovered concentration (ppb) will be converted in to mg/kg (1ppm = 1000ppb)

Table 5: Final Concentration of Chlorantraniliprole in sample

S.No.	Name of the Compound	Unit	Result(mg/kg)
01	Chlorantraniliprole	mg/kg	0.12

In above analysis Chlorantraniliprole is identified in very high concentration.

Conclusion

In above analysis Concentration level of Chlorantraniliprole is identified in very high concentration, and it is very dangerous to human being. Hence we need more stringent guidelines to educate formers for utilization of pesticides in their crops and to restrict this type of vegetables to enter in to market in order to prevent the contamination and secure human safety.

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