Determination of Chlormequat (Pesticide) in Keera (salad vegetable) of Chhattisgarh Open Market

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Abstract: Keera (salad vegetable) 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 Chlormequat(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: Chattisgarh open market, Keera(salad vegetable), Pesticides, LC-MS/MS, Chloromequat, MRL values

Introduction:

Pesticides are the only toxic substances released intentionally into our environment to kill living things
The use of toxic pesticides to manage pest problems has become a common practice around the world. Pesticides are used almost everywhere not only in agricultural fields, but also in homes, parks, schools, buildings, forests, and roads. It is difficult to find somewhere where pesticides aren't used
world is filled with pesticides. Pesticides have been linked to a wide range of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm, and endocrine disruption
Acute dangers - such as nerve, skin, and eye irritation and damage, headaches, dizziness, nausea, fatigue, and systemic poisoning - can sometimes be dramatic, and even occasionally fatal.
Chronic health effects may occur years after even minimal exposure to pesticides in the environment, or result from the pesticide residues which we ingest through our food and water.
Pesticides can cause many types of cancer in humans. Some of the most prevalent forms include leukemia, lymphoma, brain, bone, breast, ovarian, prostate, testicular and liver cancers
There is also mounting evidence that exposure to pesticides disrupts the endocrine system, wreaking the complex regulation of hormones, the reproductive system, and embryonic development. Endocrine disruption can produce infertility and a variety of birth defects.
Children are particularly susceptible to the hazards associated with pesticide use.Children have not developed their immune systems, nervous systems, or detoxifying mechanisms completely, leaving them less capable of fighting the introduction of toxic pesticides into their systems.

Material and Methodology

Keera(salad vegetable) 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 samples 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 keera sample was blended and ~200g of crushed sample taken homogenized for one minute.

Extraction & clean up

Homogenized the ~2 Kg sample and weighed 10±0.1in 50 mL polypropylene centrifuge tube Added an internal standard (CCC d4), mixed thoroughly by vortexing for 1 min. Added 20 mL of methanol (1% formic acid). Homogenized and vortexed the sample for 2 min and centrifuged at 4000 rpm for 5 minute. Drawn 0.5 mL supernatant and diluted with 0.5 mL methanol , Injected into LC-MS/MS.

Identification and Determination of Analytes

Injected 10 µl from the extract in to LC-MS/MS with following specifications.
Column: Phenomenex, Luna, 5µm, Hilic 200 A, 4.6 x 150 mm.
Mobile Phase A: 10 mm Ammonium formate in Water
Mobile Phase B: 100 % Acetonitrile
Injection volume: 10 µl
Detector: Mass Spectroscopy

Stop time: 12 minutes

Preparation of Calibration Curve Standards

Standard stock silution

Chloromequat stock solution was individually prepared in acetonitrile at a concentration level of 1000 ppb 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 acetonitrile, immediately prior to sample preparation. As below.

Table 1: Preparation of Calibration curve standards

Concentration of Stock solution(ppb)	Volume taken from stock solution(ml)	Final Volume made (ml)	Final concentration(ppb)
1000	1	10	100
1000	0.75	10	75
1000	0.5	10	50
100	2	10	20
100	1	10	10

Mass Spectrum & Linearity:

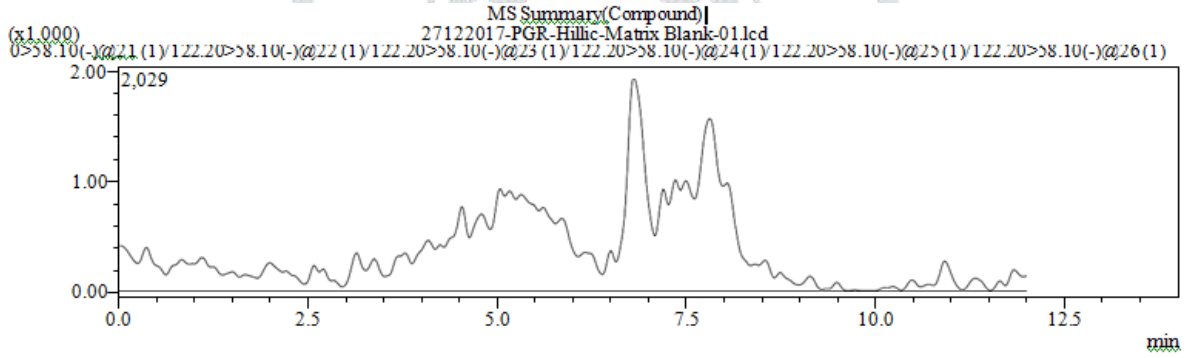

Fig.1:Chromatogram of Blank

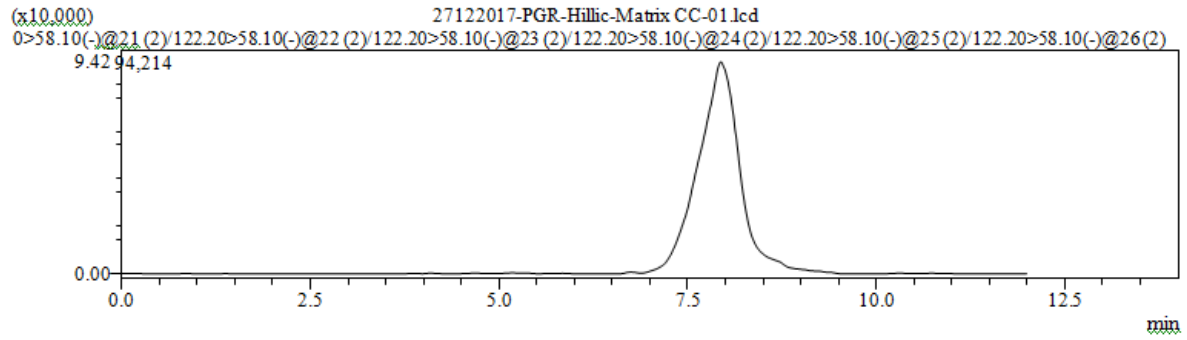

Fig.2:Chromatogram of 10PPB Standard

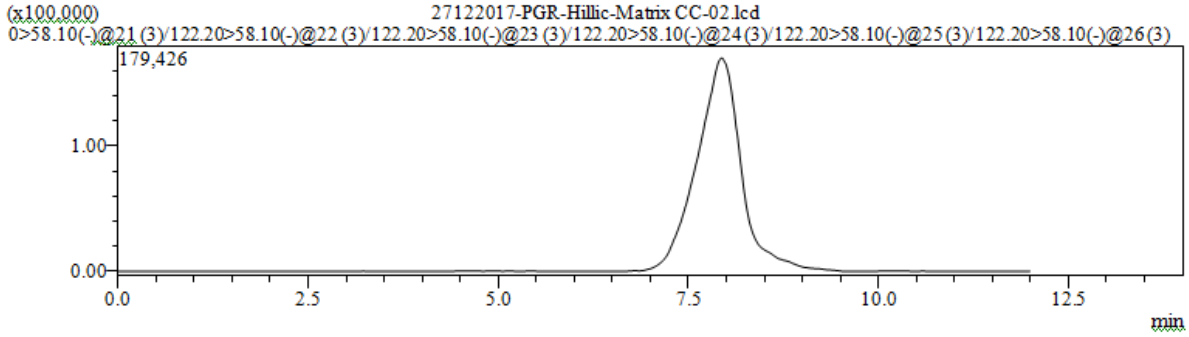

Fig.3:Chromatogram of 20PPB Standard

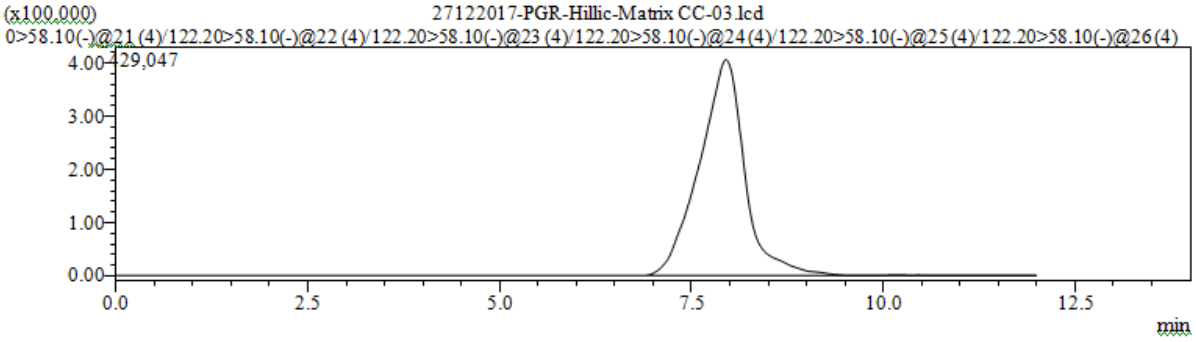

Fig.4:Chromatogram of 50PPB Standard

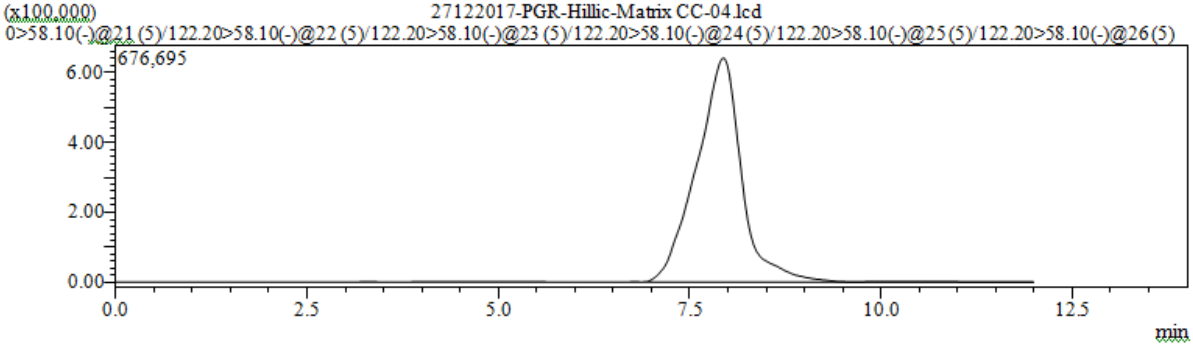

Fig.5:Chromatogram of 75PPB Standard

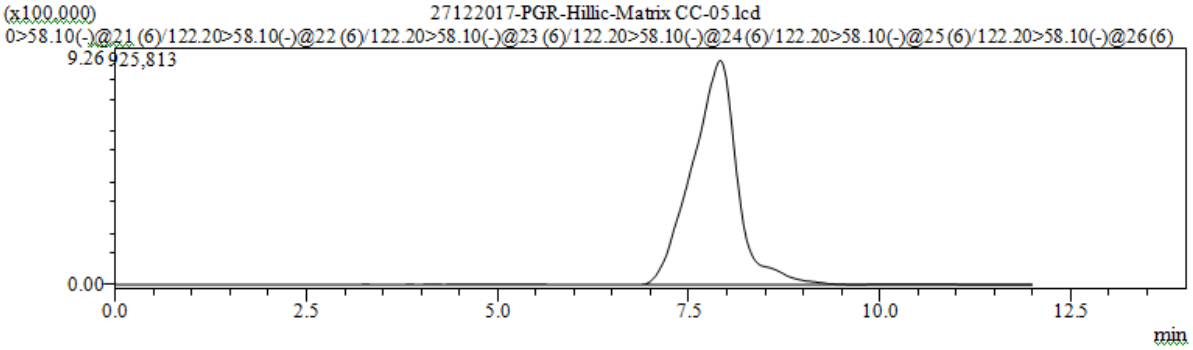

Fig.6:Chromatogram of 75PPB Standard

Linearity

Table 2 : Response of calibration curve standard s with LCMS/MS

S.No.	Standard Conc(ppb)	RT	Response
1	10.0	7.943	3308912
2	20.0	7.941	6600661
3	50.0	7.949	15981665
4	75.0	7.940	25131804
5	100.0	7.895	35263061

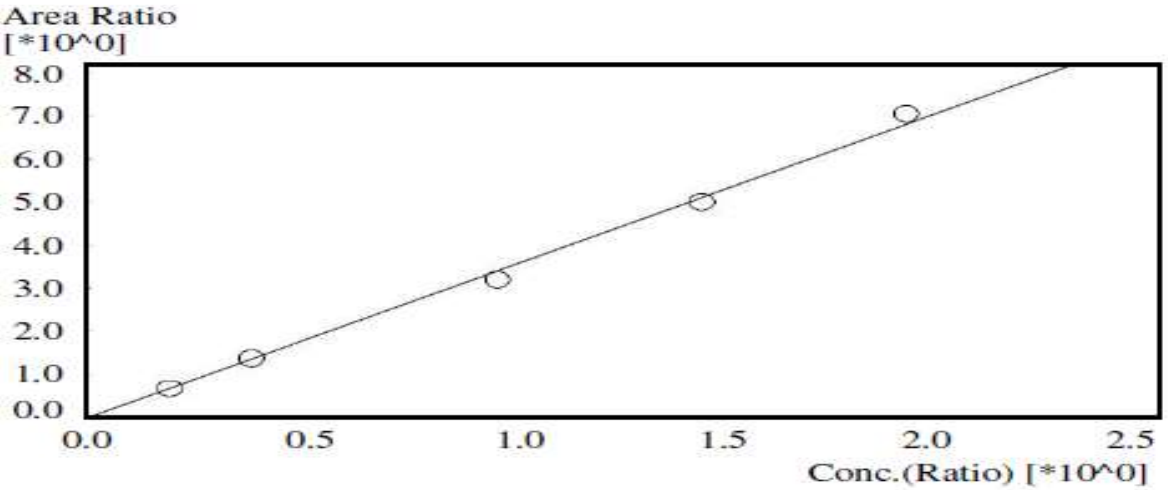

Fig.7: Calibration curve (Linearity)

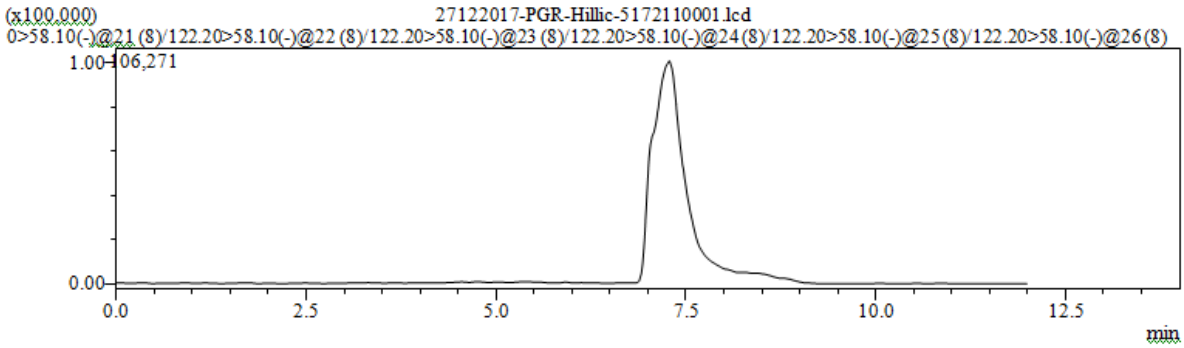

Fig.9:Chromatogram of sample

Result,Calculation & Conclusion:

Result

Table3 : Response of Sample in LCMS/MS

S.No.	RT	Response	Final Conc(PPb)
1	7.608	85730840	224.365

Calculation

The Concentration of Acephate 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 4: Final Concentration of Chloromequat in sample

S.No.	Name of the Compound	Unit	Result(mg/kg)
01	Chloromequat	mg/kg	0.22

In above analysis Chlormequat is identified in very high concentration.

Conclusion

In above analysis Concentration level of Chloromequat 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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