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Determination of Acephate (Pesticide) in Grapes of Chhattisgarh Open Market

Srinivasa Rao Damma[1] Dr. Manish Upadhyay[2]
Department of Chemistry, Dr.C.V.Raman University, Kota, Bilaspur, Chhattisgarh - 495113
E-mail: *nani.srinu05@gmail.com*

Abstract: Grapes from various sale points of the market of Bilaspur city (Chhattisgarh) are collected, preserved and transported to Research Laboratory for analysis of acephate residue.The steps involved in process Extraction, clean up of extract and analysis using Liquid chromatography with tandem Mass spectrometry (LC-MS/MS). The analysis was carried out with most confirmative method with two MRM transitions and reported the results and results are compared with Maximum Residual Limit(MRL) of European union available with Agricultural & Processed Food Products Export Development Authority (APEDA-Government of India) and need of inspection on pesticide residues in agricultural products in order to prevent the contamination and secure human safety also suggested.

Keywords: Chhattisgarh open Market; Grapes; Pesticides; LC-MS/MS; Acephate; MRL Values.

1. INTRODUCTION

Pesticides are the sole noxious substances the free designedly into environment to kill living things

The use of noxious pesticides to manage pest issues has become a standard follow round the world.

Pesticides area unit used virtually all over not solely in agricultural fields, however conjointly in homes, parks, schools, buildings, forests, and roads. it's troublesome to search out somewhere wherever pesticides are not used[2] .world is crammed with pesticides. Pesticides are connected to a large vary of human health hazards, starting from short impacts like headaches and nausea to chronic impacts like cancer, procreative hurt, and endocrine disruption.

Acute dangers - like nerve, skin, and eye irritation and injury, headaches, dizziness, nausea, fatigue, and general poisoning - will generally be dramatic, and even sometimes fatal[3].

Chronic health effects could occur years once even minimal exposure to pesticides within the setting, or result from the chemical residues that we tend to ingest through our food and water. Pesticides will cause many sorts of cancer in humans. a number of the foremost prevailing forms embrace malignant neoplastic disease, lymphoma, brain, bone, breast, ovarian, prostate, gonad and liver cancers. There is conjointly mounting proof that exposure to pesticides disrupts the system, wreaking the advanced regulation of hormones, the genital system, and embryonic development [1]. Endocrine disruption will turn out physiological condition and a spread of birth defects. Children area unit notably liable to the hazards related to chemical use[2]. Kids haven't developed their immune systems, nervous systems, or detoxifying mechanisms fully, feat them less capable of fighting the introduction of noxious pesticides into their systems.

2. MATERIAL AND METHODOLOGY

Grapes 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)[7]. Using following Methodology of USEPA and Standard procedure provided by National Research Centre for Grapes.

2.1 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[6].

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

2.2. Isolation of Pesticides from samples

10±0.1g homogenized grape sample taken in to a polypropylene centrifuge tube , 10ml,of ethyl acetate is added vertexed for 1minute, 10g of anhydrous sodium sulfate is added and homogenized at 15000rpm for 1 minute and centrifuged at 5000rpm for 5minutes[8]

2.3. Extraction & clean up

For LC-MS/MS amenable compounds: 5ml supernatant is taken in to 15ml polypropylene centrifuge tubes containing 25mg primary secondary amine (PSA), shaken for 30sec. and centrifuged for 5minutes at 10000rpm[5].

2ml of supernatant drawn in to the test tube containing 200µl of 10% diethylene glycol(DEG) solution, evaporated it to dryness under nitrogen at 35°C.

Reconstituted with 1ml Methanol and (followed by) 1ml 0.1% acetic acid in water, sonicated for 1min and vortex for 30seconds.

Centrifuged the extract at 10000rpm for 5min and filtered through 0.2µm Nylon6,6 membrane filter in to a LC vial[5].

2.4. Identification and Determination of Analytes.

injected 10 µl from the extract in to LC-MS/MS with following specifications[4] .
Column: C_{18}(Agilent-ODS-4- 150mmX2.1mm,1.7µ)
Mobile Phase: Water +Acetonitrile
Injection volume: 10 µl
Detector: Mass
Stop time: 20minutes

2.5. Preparation of Calibration Curve Standards

2.5.1. Standard stock solution:

Acephate 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[6]. Suitable concentration of working standards are prepared from stock standard solution by dilution using acetonitrile, immediately prior to sample preparation[7]. As below.

Table1 : 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	0.5	10	50
100	2	10	20
100	1	10	10
50	1	10	5
20	1	10	2

2.6. Mass Spectrum & Linearity:

2.6.1. Mass Spectrum

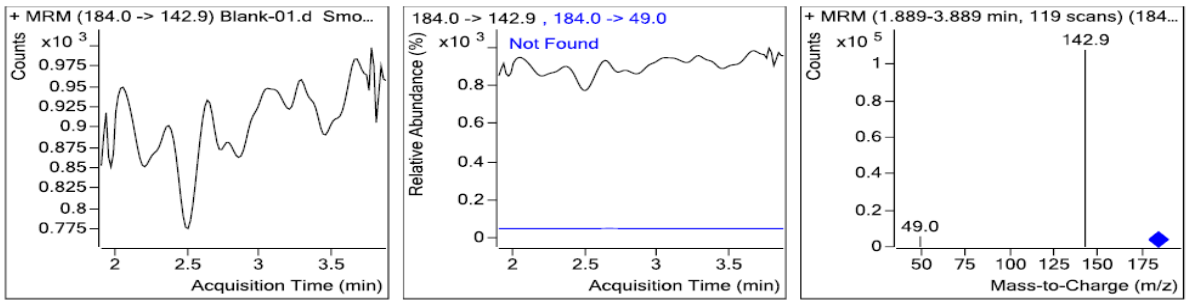

Fig.1:Chromatogram and Mass spectrum of Blank

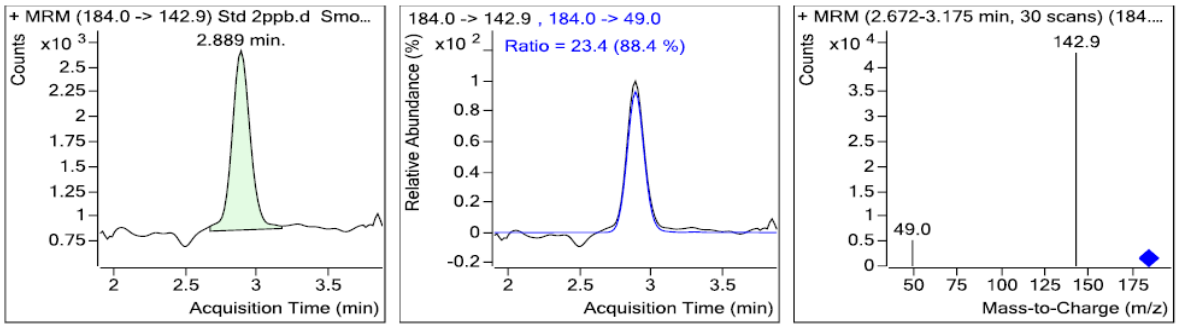

Fig.2:Chromatogram and Mass spectrum of 2PPB Standard

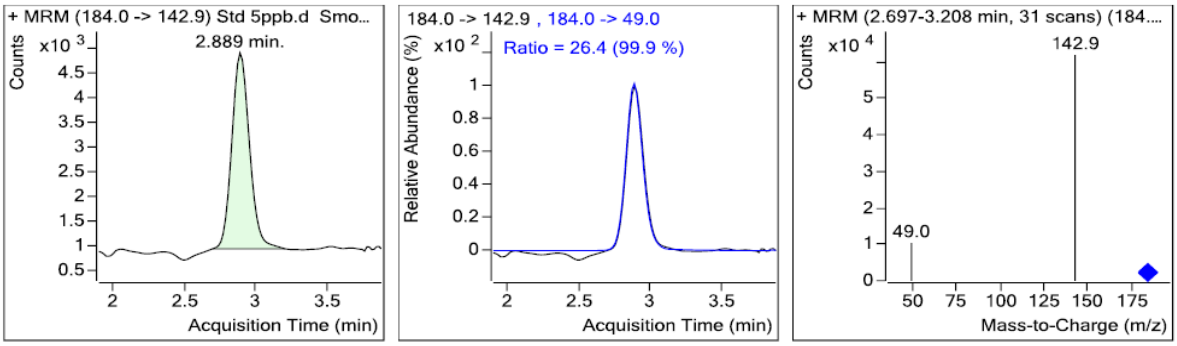

Fig.3: Chromatogram and Mass spectrum of 5PPB Standard

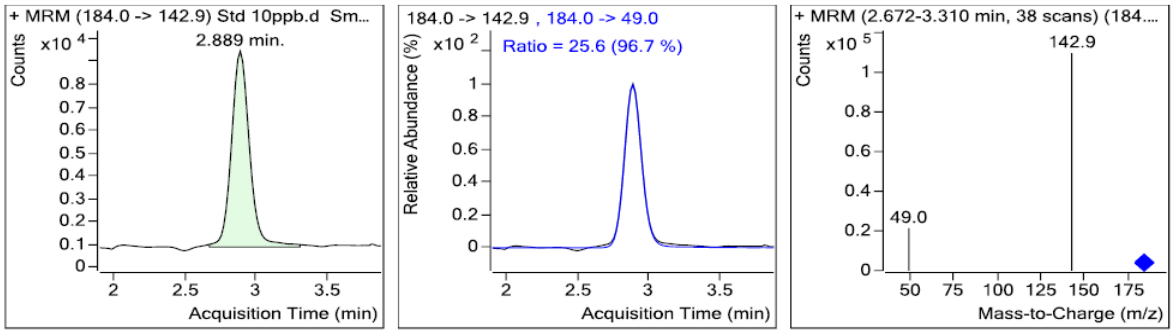

Fig.4: Chromatogram and Mass spectrum of 10PPB Standard

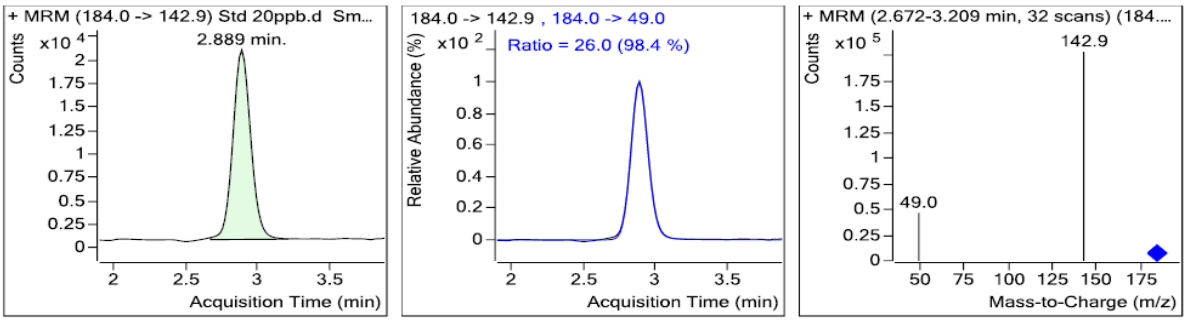

Fig.5: Chromatogram and Mass spectrum of 20PPB Standard

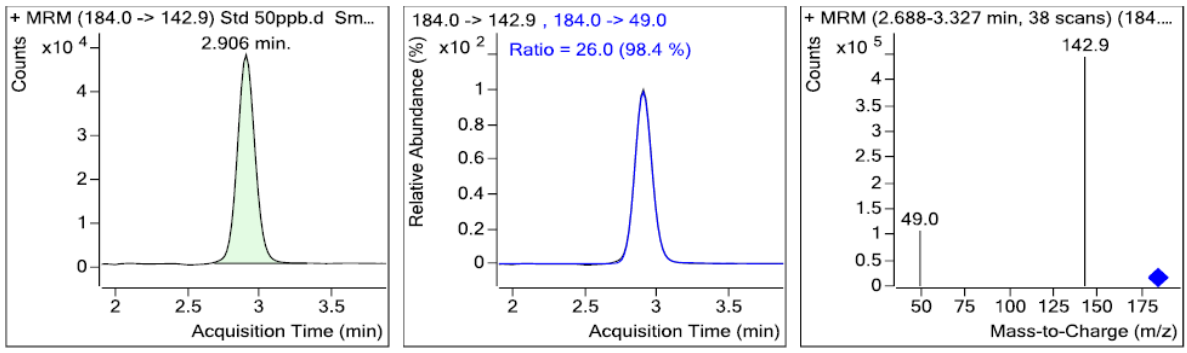

Fig.6: Chromatogram and Mass spectrum of 50PPB Standard

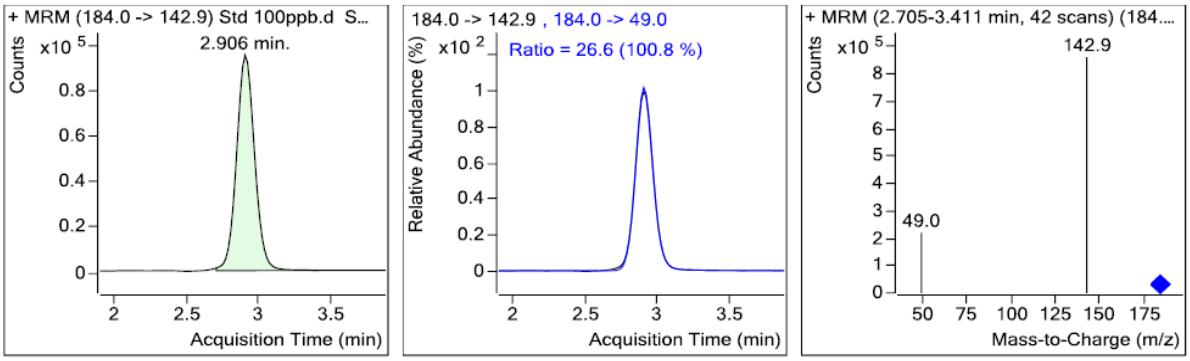

Fig.7: Chromatogram and Mass spectrum of 100PPB Standard

2.6.2. Linearity

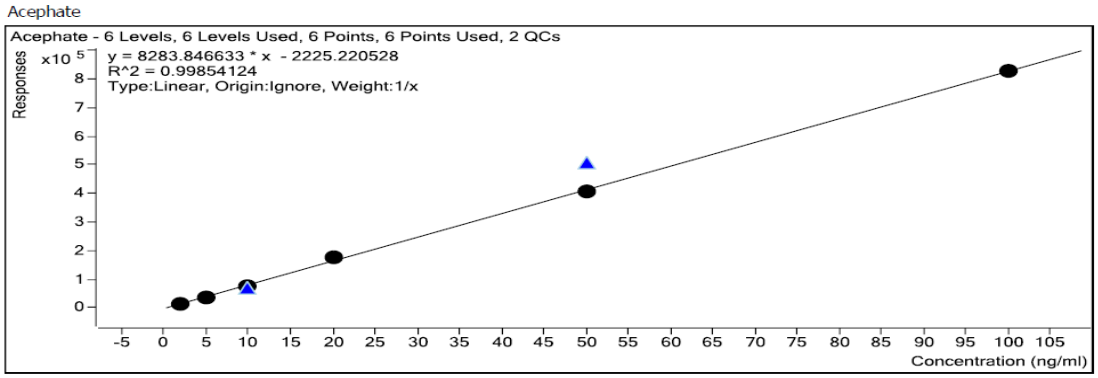

Fig.8: Calibration curve (Linearity)

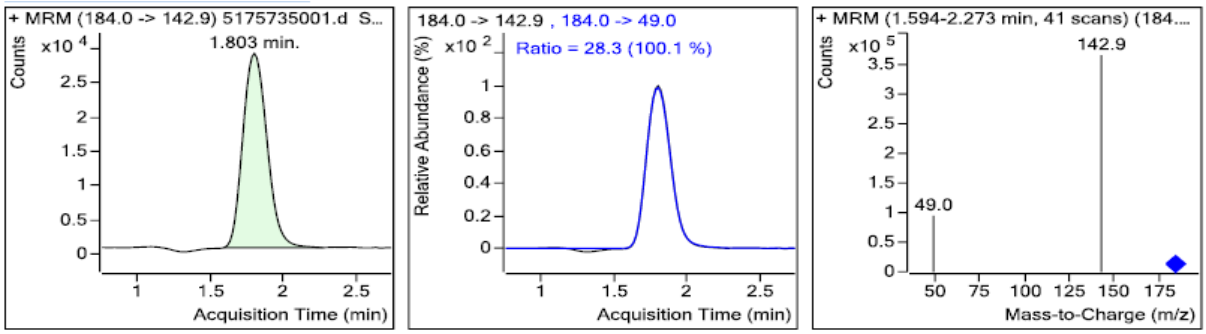

Fig.9: Chromatogram and Mass spectrum of sample

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

S.No.			Standard Conc(ppb)	RT	Response
1			2.0	2.889	16150
2			5.0	2.889	34333
3			10.0	2.889	76422
4			20.0	2.889	174675
5			50.0	2.906	408586
6	100.0	2.906	825562		

3. RESULT & CALCULATION:

5.1. Result

Table 3 : Response of Sample in LCMS/MS

S.No.	RT	Response	Final Conc(PPb)
1	2.923	229200	30.01

3.2. Calculation

The Concentration of Acephate in sample analyzed by LC-MS/MS was determined directly from the standard curve[7].

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)[7]

Table 4: Final Concentration of Acephate in sample and comparison with Harmonized EU-MRL

S.No.	Name of the Compound	Unit	Result(mg/kg)	Harmonized EU-MRL(mg/kg)
01	Acephate	mg/Kg	0.031	0.010

In above analysis Acephate is identified and compared with Maximum Residual Limit (MRL) values of European union available with Agricultural & Processed Food Products Export Development Authority (APEDA-Government of India).

4. DISCUSSION

In above identified compound Concentration level of Acephate beyond the Minimum Residual Limits of European Union. This product will be rejected to export to European Union countries, but without any restriction, road side vendors are selling in India.Hence India needs more stringent guidelines to educate formers for utilization of pesticides in their crops and to restrict this type of fruits/food material to enter in to market in order to prevent the contamination and secure human safety.

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