

**Study of Pesticide Residue from two common fruits of Nashik area using HPLC.****Dr. K.H. Kapadnis**

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**Abstract**

Pesticide Residue from Guava and Pomegranate collected from Malegaon, Manmad and Chandwad villages from Nashik district has been identified Using HPCL technique, the identification was done by comparing their retention times with respect to technical grade standard reference. Two common used pesticides Carbendazim and Dimethanoate were detected in all samples frequently but below the limit of WHO, similarly few other pesticides were detected below the range of hazard. Results are interpreted in tabular form found by HPLC spectra.

**Key words**

HPCL, residual pesticide, Guava, Pomegranate and pathogens.

**Introduction**

A pesticide is any substance intended for preventing, destroying, repelling or mitigating any pest. A pesticide may be a chemical substance, biological agent such as virus or bacteria, antimicrobial, disinfectant or a device used against any pest. A pesticide is substance or mixture of substances used to kill a pest. Pest includes insect plant pathogens, weeds, mollusks, birds, fish, nematodes, microbes etc. Although there are benefits to the use of pesticides, there are also drawbacks, such as potential toxicity to humans and animals.

In India, the commercial cultivation of crops receives frequent application of a large number of pesticides throughout the cropping seasons to control a variety of pests and diseases. Pesticides residue is a major concern for the quality regulations and food safety standard are becoming more stringent in most countries. The management of pesticide residue in crops challenging because, besides direct application, pesticide residue may also appear in crops from indirect sources like soil, contaminated agro-inputs. Drift from adjoining fields of other agriculture crops, etc. this is the reason why many chemicals are regularly monitored in all the fruits samples of Indian origin for export.

Considering the importance of food safety, the Government of India has made the certificate of residue analysis a mandatory pre-requisite for issuance of a phytosanitary certificate for export. The pesticides in the monitoring list belong to diversified chemical classes and testing laboratories in India used to analyze them in multiple groups by GC and HPCL-based methods, requiring a long time to complete the analysis but the present situation demands a rapid turn-around time where the analysis need to be completed within 24-48 hrs. after a sample is submitted to a laboratory. Thus, it is necessary to have an analytical method by which we can simultaneously determine the target pesticides in any sample by a single effort with equivalent or superior overall efficiency.

**SAMPLE COLLECTION**

Fruits	Area	Pesticide use	Mode of Action	Contents
Guava	Malegoan	Hamla (500)	Insecticides	Chloropyriphos + Cypermethrine(50%+50)
Guava	Chandward	Roko – 70	Insecticides	Thiopenate methyl 70%
Guava	Manmad	Roko – 70	Insecticides	Thiopenate methyl 70%

Fruits	Area	Pesticide use	Mode of Action	Contents
Pomegranate	Malegoan	Karate	Insecticides	Lamda-Cyhalothrin
Pomegranate	Chandward	Nuvan	Insecticides	Dichorous 76%
Pomegranate	Manmad	Laryigo-z	Pesticide	Organic Phosphate

List of pesticides generally used for Pomegranate and Guava :

- Pomegranate : Endosulfan, Lamda-cyhelotrin, dichlorvous, p-quate di chloride.
- Guava : Chlorpyrifos, Cypermethrin, Thiopenate methyl

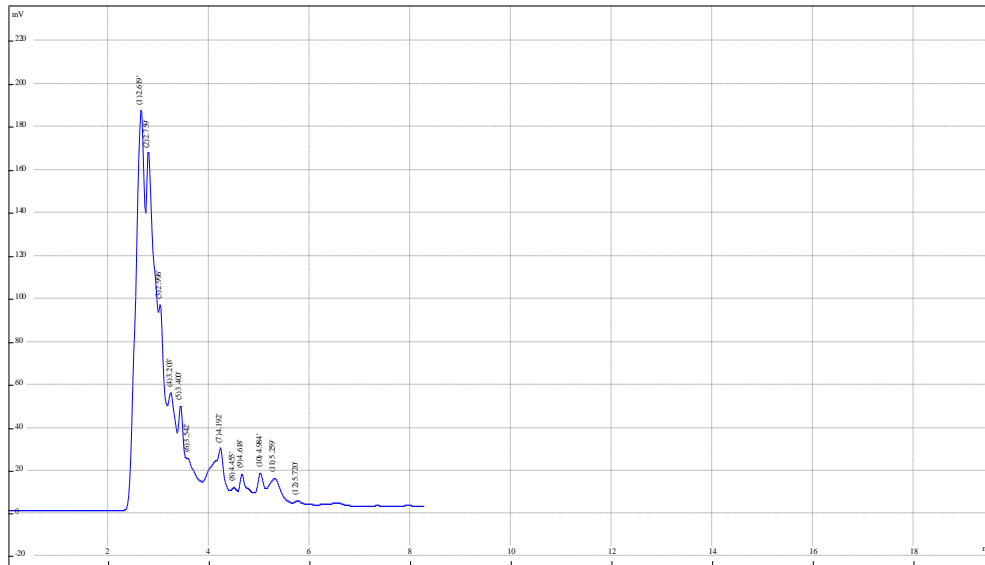
Determination of residual pesticide in guava and pomegranate by HPLC using Acetonitrile:-

**Sample Preparation and Analysis:** -Add 1gm NaCl, homogenized 5min. Transfer 1.3ml to 1.5ml acetonitrile to tube. Add 0.3gm sodium sulphate, shake well to remove water. Transfer 10ml aliquot to 15ml tube. Use this sample for HPLC analysis.

HPLC CONDITION:-US made Analytical HPLC system having four compartments. It was gradient binary system type. 1<sup>st</sup> is injector, 2<sup>nd</sup> detector, 3<sup>rd</sup> pump A, 4<sup>th</sup> is pump B. UV/Visible detector is used for and quantification of pesticide. Separation was performed on C18 is 4.6cm in diameter. Sample was injected manually injector. Detector was connected to computer for data processing. The working condition of HPLC was binary gradient, mobile phase was acetonitrile: water(70:30), flow rate was 0.5ml min<sup>-1</sup>, injection volume was 20ul and the wavelength of UV/visible detector was fixed at 254nm for residual analysis of pesticides.

HPLC Spectra of guava sample:-

- Sample Name: Guava Sample 1 Wavelength: 254nm
- Mobile Phase: ACN:Water(70:30) Injection volume: 20µl
- Flow Rate: 0.8ml/min Pressure: 6-7 MPa



Time	Conc.	Area	Resolut.	T.PlateNum	k	Asymmetry	Pesticide
2.619	29.1654	2259343	0.44	1107	0	1.00	-----
2.759	25.7572	1995296	0.89	1273	0.053	1.17	-----
2.996	10.1810	788681	0.84	3132	0.144	0.84	-----
3.203	7.3711	571003	0.82	2220	0.223	0.93	-----
3.400	4.9471	383227	0.49	4517	0.298	0.93	-----
3.542	4.4072	341406	1.38	1482	0.352	0.86	Imidacloprid
4.192	7.9397	615051	0.66	901	0.600	0.63	-----
4.455	1.1280	87378	0.59	6300	0.701	0.90	-----
4.618	2.6555	205707	1.23	3243	0.763	1.58	-----
4.984	2.1476	166362	0.71	5921	0.903	1.17	-----
5.259	3.4541	267576	0.89	1764	1.008	0.88	-----
5.720	0.8461	65547	1.24	1969	1.184	0.91	Dichlorovous

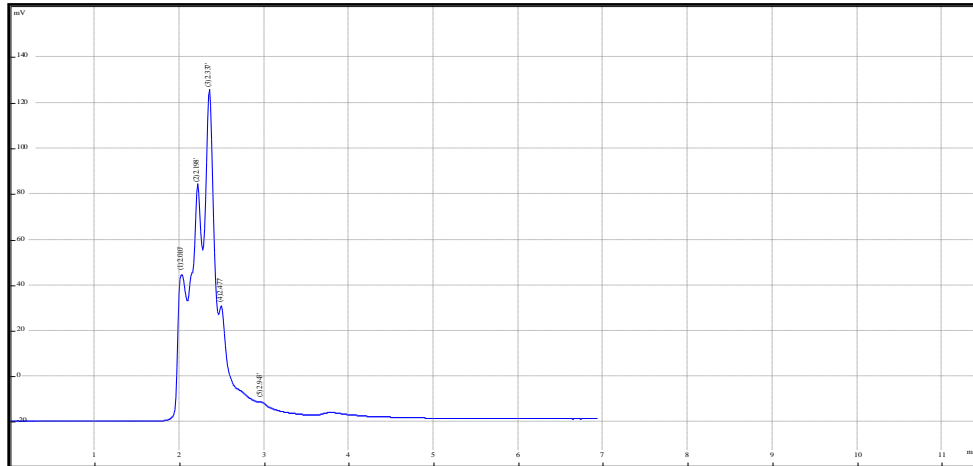
With that of the same method HPLC spectra and corresponding values of two more guava sample carried and the results were interpreted.

### HPLC Spectra of Pomegranate sample

SampleName: Pomegranates Sample 1 (0.5ml) Wavelength: 254nm  
 Mobile Phase: ACN:Water (70:30) Injection volume: 20µl

Flow Rate: 0.8ml/min

Pressure: 6-7 MPa



Time	Conc.	Area	Resolut.	T.PlateNum	k	Asymmetry	Pesticide
2.010	14.3421	441877	0.95	1998 0	1.39	-----	
2.198	27.2014	838065	0.67	1747	0.0941.24		Monocrotophos
2.337	35.0742	1080628	0.58	2332	0.163 0.96	-----	
2.477	17.8543	550086	1.11	1215	0.232 0.87	-----	
2.943	5.5280	170315	1.20	474	0.464 0.74		Dementon-s-methyl

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With that of the same method HPLC spectra and corresponding values of two more Pomegranate sample carried and the results were interpreted.

### Calculations:

Peak area of standard of carbendazim = 10mg/kg, 3624293 = 10mg/kg

Peak area of carbendazim by graph, 248499=?

Therefore,Concentration of carbendazim is 0.685mg/kg

Similarly,according to above calculations,ForChloropyriphos is 0.05mg/kg

Residual pesticide for different pesticides:-

Name of pesticide	Residual conc.	Name of pesticide	Residual conc.
Mevinphos	0.76mg/kg	Dementon-s-methyl	0.42mg/kg
Dimethanoate	0.73mg/kg	Imidacloprid	0.38mg/kg
Pencanazole	1.05mg/kg	Bitertenol	1.59mg/kg
Monocrotophos	0.069mg/kg		

**Observations:-**

Guava and pomegranate sample collected from different area shows different residual. It has been seen that some pesticides are used specifically in particular area for different fruits like carbendazim pesticide is used more commonly in Malegaon for both Guava & Pomegranate while in Chandwad Dimethanoate and Monocrotophos are used for Guava & Pomegranate respectively. In case of Manmad Mev in phose & Imidacloprid, Dementon-s-methyl is used for Guava & Pomegranate respectively. The use of pesticide varies from area to area and fruits to fruits.

**Conclusion:-**

Conclusion has been drawn from above calculations & observation that concentration of residual pesticide is in the range of 0.3-1.05ug/ul which is just above the safety scale. According to safety range of residual pesticide is below 1ug/ul. If concentration varies then it will be lethal to human health as well as environment.

The concentration of all the pesticides like Monocrotophos, Mevinphos Carbendazim, Dimethanoate, Imidacloprid Bitertenol analyzed by HPLC are in the safety range i.e. 0.1-0.7ug/ul, but the pesticide Pencanazole shows conc. 1.053ug/ul which is little exceeds in range & effect on non-targeting parts sometimes.

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