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Application Note

No. GC-05-ADI-005

GCMS-TQ8030

Multi pesticide residue analysis in tobacco by GCMS/MS using QuEChERS as an extraction method

❑ Introduction

India is the world's second largest producer (after China) and consumer (after Brazil) of tobacco with nearly $ 1001.54 million revenue generated annually from its export.[1] In countries like India, with tropical-humid climate, the incidences of insect attacks and disease infestations are frequent and application of pesticides for their management is almost obligatory. Like any other crop, tobacco (*Nicotiana tabacum Linn.*), one of the world's leading high-value crops, is also prone to pest attacks, and the farmers do apply various pesticides as a control measure.

The residues of pesticides applied on tobacco during its cultivation may remain in the leaves at harvest that may even sustain post harvest processing treatments and could appear in the final product. Thus, monitoring of pesticide residues in tobacco is an important issue of critical concern from public health and safety point of view demanding implementation of stringent regulatory policies.[2] To protect the consumers by controlling pesticide residue levels in tobacco, the Guidance Residue Levels (GRL) of 118 pesticides have been issued by the Agro-Chemical Advisory Committee (ACAC) of the Cooperation Center for Scientific Research Relative to Tobacco (CORESTA).

Tobacco is a complex matrix and hence requires selective extraction and extensive cleanup such as QuEChERS (Quick Easy Cheap Effective Rugged Safe) to ensure trace level detection with adequate precision and accuracy. The objective of the present study was to develop an effective, sensitive and economical multi-pesticide residue analysis method for 203 pesticides in tobacco as listed in Table 1.

❑ Experimental

Extraction of pesticides was done using QuEChERS method, as described below.[3]

Take 2 g of dry powdered tobacco leaves (Figure 1).
Add 18 mL of water containing 0.5 % acetic acid.
Homogenize the sample and Keep it for 30 min.

↓

Add 10 mL ethyl acetate. Immediately, put 10 g sodium sulfate.

↓

Homogenize it thoroughly at 15000 rpm for 2 min.

↓

Centrifuge at 5000 rpm for 5 min for phase separation.

↓

Draw 3 mL of ethyl acetate upper layer from the extract for further cleanup.

↓

Add 1 mL toluene to it and vortex for 0.5 min.

↓

Add cleanup mixture [PSA (150 mg), C18 (150 mg), GCB (75 mg) and anhydrous $MgSO_4$ (300 mg)] and vortex for 2 min.

↓

Centrifuge the mixture at 7000 rpm for 7 min.

↓

Collect the supernatant and filter through a 0.2 µm PTFE membrane filter.

↓

Inject 2.0 µL of the clean extract into GCMS-TQ8030 (Figure 2).

Key Features of GCMS-TQ8030

- ASSP™ (Advanced Scanning Speed Protocol) enables high-speed scan and data acquisition for accurate quantitation at 20,000 u/sec
- Capable of performing simultaneous Scan/MRM
- UFsweeper® technology efficiently sweeps residual ions from the collision cell for fast, efficient ion transport ensuring no cross-talk
- Two overdrive lenses reduce random noise from helium, high-speed electrons and other factors to improve S/N ratio
- Flexible platform with EI (Electron Ionization), CI (Chemical Ionization), and NCI (Negative Chemical Ionization) techniques
- Full complement of acquisition modes including MRM, Scan/MRM, Precursor Ion, Product Ion and Neutral Loss Scan

Figure 2. GCMS-TQ8030 Triple quadrupole system by Shimadzu

GCMS/MS Analytical Conditions

The analysis was carried out on Shimadzu GCMS-TQ8030 as per the conditions given below.

Chromatographic parameters

- Column : Rxi-5Sil MS (30 m L x 0.25 mm I.D.; 0.25 µm)
- Injection Mode : Splitless
- Sampling Time : 2.0 min
- Split Ratio : 5.0
- Carrier Gas : Helium
- Flow Control Mode : Linear Velocity
- Linear Velocity : 40.2 cm/sec
- Column Flow : 1.2 mL/min
- Injection Volume : 2.0 µL
- Injection Type : High Pressure Injection
- Total Program Time : 41.87 min
- Column Temp. Program

Rate (^{0}C /min)	Temperature (^{0}C)	Hold time (min)
	70.0	2.00
25.00	150.0	0.00
3.00	200.0	0.00
8.00	280.0	10.00

Mass Spectrometry parameters

- Ion Source Temp. : 230.0 ^{0}C
- Interface Temp. : 280.0 ^{0}C
- Ionization Mode : EI
- Acquisition Mode : MRM

❑ Results and Discussion

For MRM optimisation, well resolved pesticides were grouped together. Standard solution mixture of approximately 1 ppm concentration was prepared and analyzed in Q3 scan mode to determine the precursor ion for individual pesticides. Selected precursor ions were allowed to pass through Q1 & enter Q2, also called as Collision cell. In Collision cell, each precursor ion was bombarded with collision gas (Argon) at different energies (called as Collision Energy-CE) to produce fragments (product ions). These product ions were further scanned in Q3 to obtain their mass to charge ratio. For each precursor ion, product ion with highest intensity and its corresponding CE value was selected, thereby assigning a characteristic MRM transition to every pesticide. Based on MRM transitions, the mixture of 203 pesticides was analyzed in a single run (Figure 3).

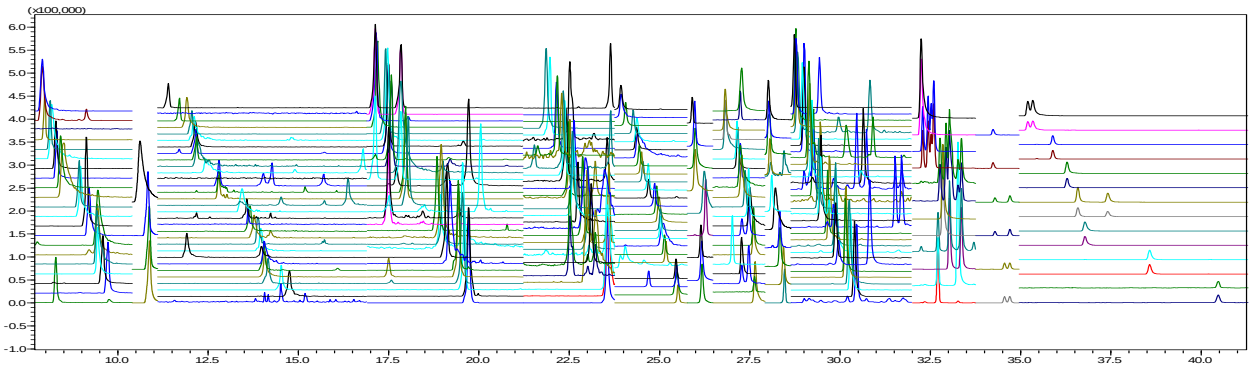


Figure 3. MRM Chromatogram for 203 pesticides mixture

Method was partly validated for each pesticide with respect to linearity (0.5 to 25 ppb), reproducibility, LOQ and recovery. The validation summary for two pesticides namely Mevinphos and Parathion-ethyl (Sr. Nos.140 and 149 in Table 1) is shown in Figures 4 and 5. The summary data of linearity and LOQ for 203 pesticides is given in Table 2 and 3 respectively.

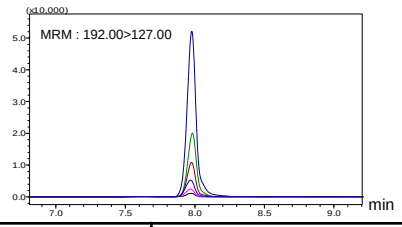
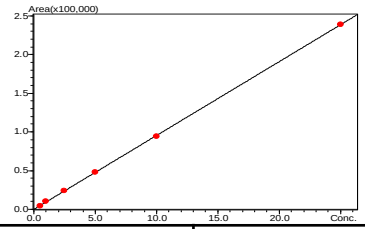
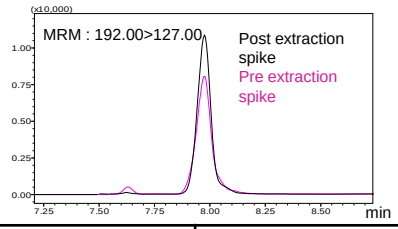
Calibration overlay		Linearity curve		Recovery overlay	
					
Linearity (R^2)	**LOD (ppb)**	**LOQ (ppb)**	**S/N at LOQ**	**% RSD at LOQ (n=6)**	**% Recovery at LOQ**
0.9999	0.3	1	173	6.93	89.28

Figure 4. Summary data for Mevinphos

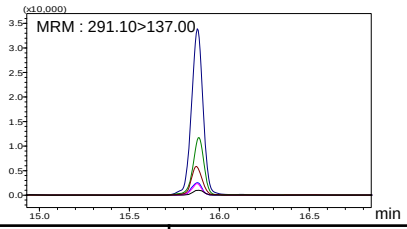
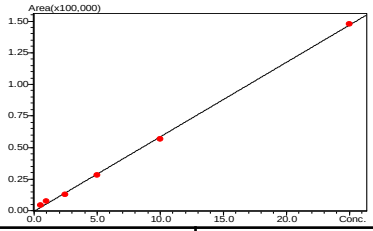
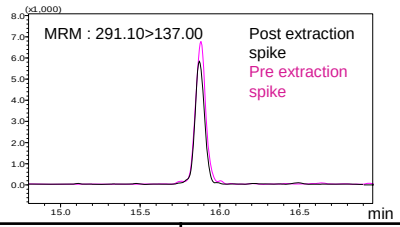
Calibration overlay		Linearity curve		Recovery overlay	
					
Linearity (R^2)	**LOD (ppb)**	**LOQ (ppb)**	**S/N at LOQ**	**% RSD at LOQ (n=6)**	**% Recovery at LOQ**
0.9993	1.5	5	93	4.05	109.10

Figure 5. Summary data for Parathion-ethyl

Table 2. Linearity Summary

Sr. No.	Linearity (R^2)	Number of pesticides
1	0.9950 - 1.0000	193
2	0.9880 - 0.9950	10

Table 3. LOQ Summary

Sr. No.	LOQ (ppb)	Number of pesticides	% RSD range (n=6)	S/N Ratio range	% Recovery range
1	1	15	6 – 15	16 – 181	70 – 130
2	5	18	3 – 15	19 – 502	
3	10	158	0.95 – 15	10 – 14255	
4	25	12	1 – 10	19 – 660	

❑ Conclusion

A highly sensitive method was developed for quantitation of 203 pesticides in complex tobacco matrix by using Shimadzu GCMS-TQ8030.

The MRM method developed for 203 pesticides can be used for screening of pesticides in various food commodities. For 90 % of the pesticides, the LOQ of 10 ppb or below was achieved.

Ultra Fast scanning, UFsweeper® and ASSP™ features enabled sensitive, selective, fast, reproducible, linear and accurate method of analysis.

❑ Reference

1. Tobacco Board (Ministry of Commerce and Industry, Government of India), Exports performance during 2013-14, (2014), 1. http://tobaccoboard.com/admin/statisticsfiles/Exp_Perf_Currentyear.pdf
2. CORESTA GUIDE N^0 1, The concept and implementation of cpa guidance residue levels, (2013), 4. http://www. Coresta.org/Guides/Guide-No01-GRLs%283rd-Issue-July13%29.pdf
3. Zareen S Khan, Kaushik Banerjee, Rushali Girame, Sagar C Utture et al., Journal of Chromatography A, Volume 1343, (2014), 3.
4. Tobacco Board (Ministry of Commerce and Industry, Government of India), Exports performance during 2013-14, (2014), 1. http://tobaccoboard.com/admin/statisticsfiles/Exp_Perf_Currentyear.pdf
5. CORESTA GUIDE N^0 1, The concept and implementation of cpa guidance residue levels, (2013), 4. http://www. Coresta.org/Guides/Guide-No01-GRLs%283rd-Issue-July13%29.pdf
6. Zareen S Khan, Kaushik Banerjee, Rushali Girame, Sagar C Utture et al., Journal of Chromatography A, Volume 1343, (2014), 3.

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