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

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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.

Figure 1. Dried tobacco

Method of Analysis

Extraction of pesticides from tobacco

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.

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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.

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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).

Figure 2. GCMS-TQ8030 Triple quadrupole system by Shimadzu

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

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Table 1. List of pesticides

Sr. No.	Pesticide	Sr. No.	Pesticide	Sr. No.	Pesticide	Sr. No.	Pesticide
1	2,6-Dichlorobenzamide	52	Cyfluthrin-3	103	Fipronil sulphone	154	Permethrin-1
2	2-Phenylphenol	53	Cyfluthrin-4	104	Flucythrinate-1	155	Permethrin-2
3	3,4-Dichloraniline	54	Cyhalofop-butyl	105	Flucythrinate-2	156	Pethoxamid
4	3-Chloroaniline	55	Cypermethrin-2	106	Flufenacet	157	Phosalone
5	4-Bromo 2-Chloro phenol	56	Cypermethrin-3	107	Flumoixazine	158	Phosmet
6	4,4-Dichlorobenzophenone	57	Cypermethrin-4	108	Fluquinconazole	159	Pirimicarb
7	Acetochlor	58	Cyprodinil	109	Flurochloridone-1	160	Pretilachlor
8	Acrinathrin	59	Delta-HCH	110	Flurochloridone-2	161	Procymidone
9	Alachlor	60	Demeton-s-methyl	111	Flutolanil	162	Profenofos
10	Aldrin	61	Demeton-S-methyl sulphone	112	Flutriafol	163	Propanil
11	Azinphos-ethyl	62	Dialifos	113	Fluxapyoxad	164	Propaquizafop
12	Azinphos-methyl	63	Diazinon	114	Folpet	165	Propazine
13	Azoxystrobin	64	Dichlobenil	115	Fuberidazole	166	Propham
14	Barban	65	Dichlofluanid	116	Heptachlor	167	Propiconazole-1
15	Beflubutamid	66	Diclofop	117	Hexaconazole	168	Propisoclor
16	Benfluralin	67	Dicloran	118	Iprobenfos	169	Propyzamide
17	Benoxacor	68	Dieldrin	119	Isoprocarb	170	Proquinazid
18	Beta-endosulfan	69	Diethofencarb	120	Isoprothiolane	171	Pyraflufen-ethyl
19	Bifenox	70	Difenoconazole-1	121	Isopyrazam	172	Pyrazophos
20	Bifenthrin	71	Difenoconazole-2	122	Isoxaben	173	Pyrimethanil
21	Bitertanol	72	Diflubenzuron	123	Lactofen	174	Pyriprooxyfen
22	Boscalid	73	Diflufenican	124	Lambda-cyhalothrin	175	Pyroquilon
23	Bromacil	74	Dimethipin	125	Malaoxon	176	Quinoxyfen
24	Bromophos-ethyl	75	Dimethomorph-1	126	Malathion	177	Simazine
25	Bromopropylate	76	Dimethomorph-2	127	Mepanipyrim	178	Spirodiclofen
26	Bromuconazole-1	77	Dimoxystrobin	128	Mepronil	179	Sulfotep
27	Bromuconazole-2	78	Diniconazole	129	Metalaxyl	180	Swep
28	Butralin	79	Dinoseb	130	Metalaxyl M	181	Tebufenpyrad
29	Butylate	80	Dinoterb	131	Metazachlor	182	Tebupirimfos
30	Carbaryl	81	Dioxathion	132	Metconazole	183	Tebuthiuron
31	Carbofuran	82	Edifenfos	133	Methabenzthiazuron	184	Tefluthrin
32	Carfentrazone	83	Endosulfan sulphate	134	Methacrifos	185	Terbacil
33	Chlordane-trans	84	Endrin	135	Methidathion	186	Tetraconazole
34	Chlordecone	85	Epoxiconazole	136	Methiocarb	187	Tetradifon
35	Chlorfenvinphos	86	Ethalfluralin	137	Metholachlor-s	188	Thiobencarb
36	Chlormephos	87	Ethoprophos	138	Methoxychlor	189	Tolylfluanid
37	Chlorobenzilate	88	Etoxazole	139	Metribuzin	190	Tralkoxydim
38	Chloroneb	89	Etridiazole	140	Mevinphos	191	Triadimefon
39	Chlorothalonil	90	Etrimfos	141	Monolinuron	192	Tri-allate
40	Chlorpyriphos-ethyl	91	Famoxadone	142	Myclobutanyl	193	Triazophos
41	Chlorpyriphos-methyl	92	Fenamidone	143	Napropamide	194	Tricyclazole
42	Chlorpyriphos-oxon	93	Fenarimol	144	Nitrapyrin	195	Trifloxystrobin
43	Chlorthal-dimethyl	94	Fenbuconazole	145	Oxadiargyl	196	Triflumizole
44	Cinidon-ethyl	95	Fenchlorphos	146	Oxadiazon	197	Triflumuron
45	Cis-1,2,3,6 tetrahydrophthalimide	96	Fenchlorphos oxon	147	Oxycarboxin	198	Trifluralin
46	Clodinafop propargyl	97	Fenhexamid	148	p,p-DDE	199	Triflusulfuron
47	Clomazone	98	Fenobucarb	149	Parathion-ethyl	200	Triticonazole
48	Crimidine	99	Fenoxycarb	150	Parathion-methyl	201	Valifenalate
49	Cyanophos	100	Fenthion sulphoxide	151	Penconazole	202	Vinclozolin
50	Cyfluthrin-1	101	Fenvalerate	152	Pencycuron (Deg.)	203	Zoxamide (Deg.)
51	Cyfluthrin-2	102	Fipronil	153	Pendimethalin		

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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 (°C /min)	Temperature (°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 °C
• Interface Temp.	: 280.0 °C
• Ionization Mode	: EI
• Acquisition Mode	: MRM

Results

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).

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.

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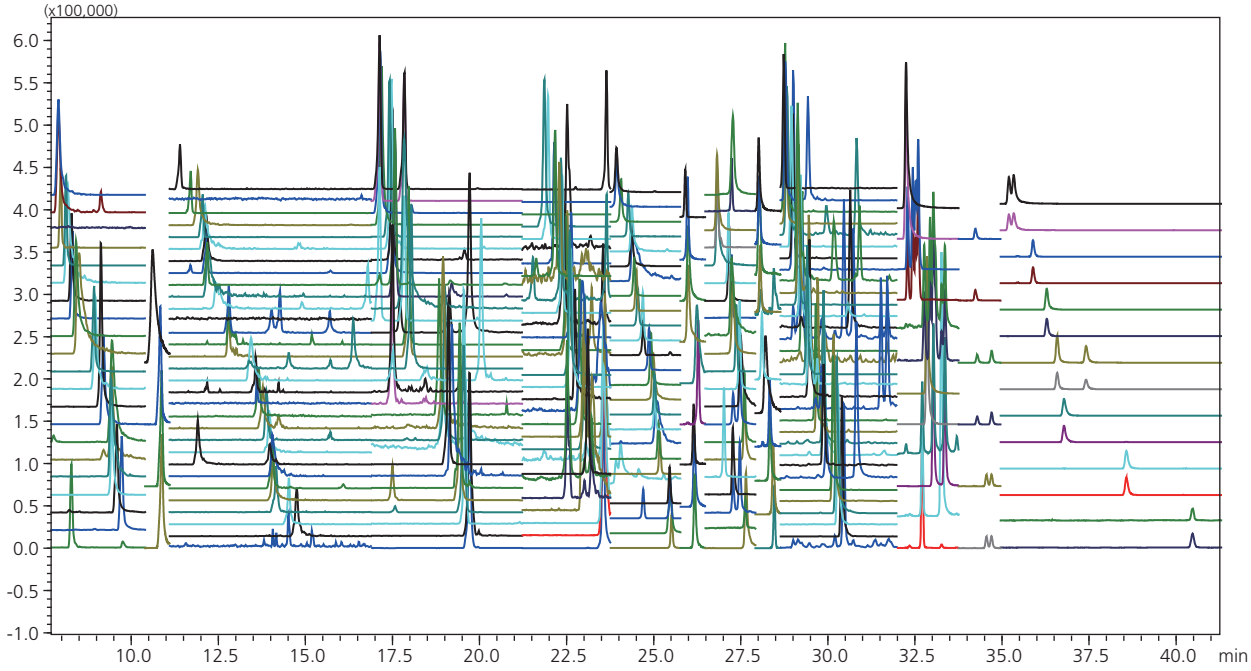
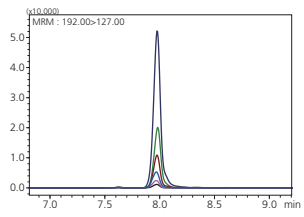
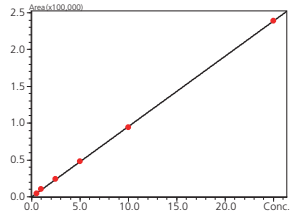
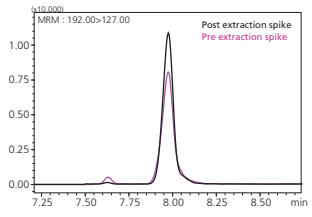
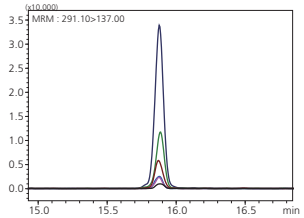
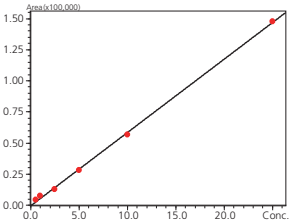
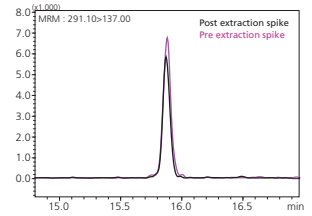


Figure 3. MRM Chromatogram for 203 pesticides mixture

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

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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

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[3] Zareen S Khan, Kaushik Banerjee, Rushali Girame, Sagar C Utture et al., Journal of Chromatography A, Volume 1343, (2014), 3.

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