

# Multi-Residue Pesticide Analysis in Cumin using GC-MS/MS

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## 1. Overview

Cumin (*Cuminum cyminum*) has been used as a natural medicine for over 2000 years. It shows anti-inflammatory property and supports the immune system. Cumin seeds (Figure 1) have been widely used as a spice in many food preparations. In order to fulfil the high-demand of such spices, pesticides are widely used. But their overuse may cause acute and permanent health problems in humans. Therefore, to protect human health, the European Union has set maximum residue limits (MRLs) for the presence of insecticides in cumin seeds<sup>[1]</sup>. Thus, increasing the importance of having analytical method for determination of residual pesticides present in cumin seeds.



Figure 1. Cumin seeds

## 2. Introduction

This study reports a validated method for the determination of 169 pesticides in cumin seeds of Indian origin using Shimadzu GCMS-TQ8040 NX. The multi-residue extraction was performed using modified QuEChERS' extraction method<sup>[2]</sup>.

## 3. Materials and methods

The reference standards for this study were procured from Restek with below catalogue number:

GC multi-residue pesticides kit – 32562

Cumin seeds procured from local market, were used to prepare matrix-matched calibration standards and spiked samples. This method is validated for criteria as mentioned in SANTE Guidelines<sup>[3]</sup>.

GCMS-TQ8040 NX (Figure 2), manufactured by Shimadzu Corporation Japan, was used to quantify residual pesticides in cumin seeds sample.

### 3-1. Method development

Instrumental method was developed based on chromatographic and mass spectrometric parameters. Smart Pesticides Database Ver.2 for GC-MS/MS enabled quick instrumental method optimization for higher throughput. For most of the pesticides, 1 target and 2 reference MRM transitions were included in the method. Shimadzu's 'LabSolutions Insight' software was used for data processing, which helped in evaluating validation parameters with ease. This greatly reduced the development and optimization time of method parameters. Pretreatment method was optimized based on modified QuEChERS to give better and consistent recoveries.

### 3-2. Sample and standard preparation

Cumin seeds were grounded and used as sample for extraction. Acetonitrile was used as extraction solvent along with magnesium sulphate (MgSO<sub>4</sub>) salt in optimized proportion to maximize recoveries of pesticides. After extraction, the aliquot of acetonitrile was used for further clean up.

After extraction, clean up was performed using optimum combination of C18, GCB (Graphitized Carbon Back), PSA (Primary secondary amine) and anhydrous MgSO<sub>4</sub> to minimize matrix interference. The extract was filtered through 0.22 µm PTFE filter. Final reconstitution volume was adjusted such that recovery samples' concentration is diluted by two times. All samples were analyzed as per conditions shown in table 1.

### • Preparation of solvent standard concentration levels

GC multi-residue pesticides mixture obtained from Restek® was diluted using ethyl acetate to prepare stock solution of about 1000 ppb standard mixture for more than 170 pesticides. From this, concentration levels of 10 ppb, 25 ppb, 50 ppb, 250 ppb and 500 ppb were prepared.

### • Preparation of matrix matched standard linearity levels

Locally purchased Cumin seeds were used as a sample. It was extracted as per the sample extraction method (3-2) to prepare matrix blank. Further it was spiked with above solvent standard levels to prepare matrix match linearity of 1 ppb, 2.5 ppb, 5 ppb, 25 ppb, 50 ppb and 100 ppb.

### • Preparation of spike samples (Recovery samples)

In order to determine the extraction efficiency of the method, recovery study was conducted. For this, 2 g sample was spiked with pesticide standard mixture to prepare recovery samples of 10 ppb and 20 ppb. The spiked pesticides were then extracted, analyzed and quantified against matrix matched linearity to study their recoveries.



Figure 2. Shimadzu GCMS-TQ8040 NX

## 3-3. Analytical Conditions

Table 1 Instrument configuration and Analytical Conditions: GC-MS/MS

System Configuration	
Instrument	: GCMS-TQ8040 NX
Auto-injector	: AOC-20i + s
Column	: SH-I-Rxi-5Sil MS (30 m × 0.25 mm I.D., df = 0.25 µm)
Liner	: Restek Topaz Liner, Splitless (with wool)

GC	
Injector temp.	: 250 °C
Column oven temp	: 80 °C (2 min), 20 °C/min to 180 °C (0 min), 5 °C/min to 300 °C (3.00 min)

Run time	: 34 min
Injection mode	: Splitless (High pressure at 250kPa)
Injection volume	: 2 µL
Carrier gas	: He
Linear Velocity	: 40.4 cm/sec (Constant mode)

MS	
Interface temp.	: 280 °C
Ion source temp.	: 230 °C
Ionization mode	: EI
Solvent cut time	: 3.5 min
Loop Time	: 0.3 sec

## 4. Results

### 4-1. Linearity

For linearity study and quantifying spiked samples, matrix matched calibration standards were used. Multilevel calibration curve included 1, 2.5, 5, 25, 50 and 100 µg/L concentration levels. All calibration standards were found within 80 to 120% accuracy range as per SANTE guidelines.

### 4-2. Recovery

Recovery was evaluated by analyzing spiked samples at 10 and 20 µg/kg (six spiked samples at each level) against matrix matched linearity plotted between 1 to 100 µg/L. Mean recoveries were found to be within 70-120%. The bar chart of mean recoveries at LOQ level is shown in figure 3.

### 4-3. Reproducibility (% RSD)

Reproducibility experiment for recoveries was performed on six different spiked samples at 10 and 20 µg/kg concentration levels. The % RSD for recovery of six spiked samples at their respective LOQ levels was found to be less than 20%. Trend plot of reproducibility (% RSD) for spiked samples at LOQ level is presented in figure 3.

The method successfully achieved 10 and 20 µg/kg LOQ for 131 and 38 pesticides, respectively. The list of pesticides is shown in table 2. The pesticides having 20 µg/kg LOQ are marked with asterisk (\*) in the table. Some pesticides (like Chlorpyrifos, Profenofos, Ethion, Tricyclazole etc.) were detected as incurred residues in the blank sample and hence could not be included for recovery studies.

Table 2 List of Pesticides

#	Pesticides Name	#	Pesticides Name	#	Pesticides Name
1	Dichlobenil	58	Pirimiphos-methyl	115	p,p'-DDD
2	Biphenyl	59	Prodiamine*	116	p,p'-DDT
3	Mevinphos	60	Fenitrothion*	117	Chlorthiophos-3
4	Etridiazole	61	Pentachlorothioanisole	118	Triazophos
5	Pebulate*	62	Malathion	119	Sulprofos
6	Methacrifos	63	Metolachlor (S-Metolachlor)	120	Carfentrazone-ethyl
7	Chloroneb	64	Fenthion*	121	4,4'-methoxychlor olefin
8	2-Phenylphenol	65	Chlorthal-dimethyl	122	Carbophenothion*
9	Pentachlorobenzene	66	Antraquinone	123	Edifenphos
10	Tecnazene	67	Parathion*	124	Norfurazon
11	Propachlor	68	Triadimefon	125	Endosulfan sulfate*
12	2,3,5,6-Tetrachloroaniline	69	4,4'-Dichlorobenzophenone	126	Lenacil*
13	Diphenylamine	70	Fenson	127	p,p'-DDT
14	Cycloate	71	Pirimiphos ethyl	128	Hexazinone
15	Ethalfuralin	72	Bromophos	129	2,4'-Methoxychlor
16	Chlorpropham	73	MGK 264-1	130	Piperonyl butoxide
17	Trifluralin	74	Isopropalin	131	Nitralin*
18	Benfluralin	75	Isodrin	132	Pyridaphenthion
19	Sulfotep	76	Pendimethalin	133	Iprodione*
20	Di-allate	77	Cyprodinil	134	Tetramethrin-1*
21	Phorate*	78	Metazachlor	135	Endrin ketone*
22	alpha-BHC	79	MGK 264-2	136	Phosmet
23	Hexachlorobenzene	80	Fipronil*	137	Bifenthrin
24	Pentachloroanisole	81	Perconazole*	138	EPN*
25	Dicloran	82	Chlozolinate*	139	Bromopropylate
26	Atrazine*	83	Bromfenvinfos-methyl*	140	Tetramethrin-2
27	beta-BHC	84	(Z)-Chlorfenvinphos	141	Methoxychlor
28	Clomazone	85	Quinalphos	142	Fenpropathrin
29	Quintozene*	86	Procymidone	143	Tebufenpyrad
30	Pentachlorobenzonitrile	87	Triflumizole*	144	Tetraclifon*
31	gamma-BHC (Lindane)	88	Folpet*	145	Phosalone
32	Profluralin	89	Bromophos-ethyl	146	Leptophos
33	Terbutylazine	90	trans-Chlordane	147	Azinphos-methyl*
34	Terbufos*	91	Chlorbenseide	148	Pyriproxyfen
35	Fonfios	92	p,p'-DDE	149	Mirex
36	Propyzamide	93	Tetrachlorvinphos*	150	Acinathrin
37	Diazinon	94	cis-Chlordane*	151	Pyrazophos*
38	Fluchloralin	95	trans-Nonachlor*	152	Fenarimol
39	Pyrimethanil	96	Iodofenphos	153	Azinphos-ethyl
40	Isazofos	97	Flutolanil	154	Pyraclifos
41	Telluthrin	98	Chlorfenson	155	Coumaphos
42	delta-BHC	99	Prothiofos	156	trans-Permethrine
43	Tri-allate	100	Fludioxonil*	157	Fluquinconazole
44	Pentachloroaniline	101	Pretlathlor	158	Pyridaben
45	Endosulfan ether	102	p,p'-DDE	159	Acequinocyl deg.*
46	Dimethachlor	103	Oxadiazon	160	Cyfluthrin-1
47	Propanil*	104	Myclobutanil	161	Cyfluthrin-2
48	Acetochlor	105	p,p'-DDD	162	Cyfluthrin-3
49	Chlorpyrifos-methyl	106	Flusilazole*	163	Cyfluthrin-4
50	Vincozolin*	107	Oxyfluorfen*	164	Flucythrinate-1
51	Parathion-methyl	108	Bupirimate	165	Flucythrinate-2
52	Toxicofos-methyl	109	Fluazifop-P-butyl	166	Fluridone
53	Alachlor	110	Nitrofen	167	tau-Fluvalinate-1
54	Transfluthrin	111	1,1-Dichloro-2,2-bis(4-ethylphenyl)ethane	168	tau-Fluvalinate-2
55	Heptachlor	112	Chlorthiophos*	169	Deltamethrin (Tlalometrin deg.)*
56	Metaxyl (Mefenoxam)*	113	Chlorobenzilate		
57	Fenchlorphos	114	cis-Nonachlor		

\* LOQ = 20 ug/kg

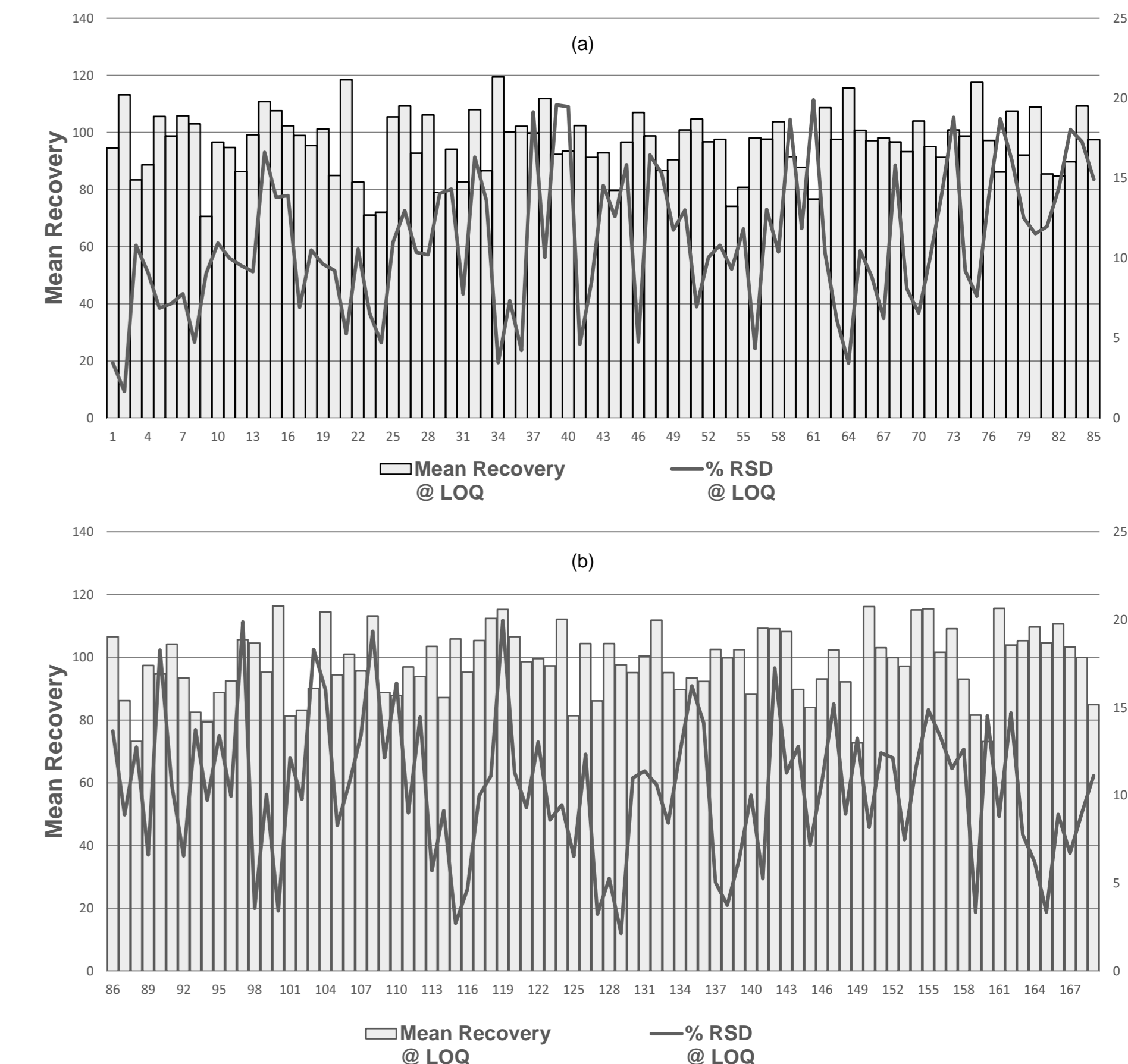


Figure 3. Trend plot of Mean Recovery and % RSD vs Compound # at LOQ level (a) 1 to 85 and (b) 86 to 169

## 5. Conclusion

- A simple, sensitive and rapid method has been developed and validated as per SANTE guidelines for determination of 169 pesticides in cumin seeds. Quantification of pesticides in cumin seeds is challenging due to presence of high sugar content. A modified QuEChERS' extraction technique was used for sample preparation.
- The method developed on Shimadzu GC-MS/MS proved to be highly sensitive and consistent as all the pesticides showed reproducibility < 20% (as per SANTE guidelines) at LOQ levels.
- The combination of sensitive instrument and reliable method enables its use in testing laboratories for multi-residue analysis of cumin seeds.

## 6. References

- GB 2763-2019 National Food Safety Standard—Maximum Residue Limits for Pesticides in Food: <https://www.codeofchina.com/standard/GB2763-2019.html>.
- M. Anastassiades, S. J. Lehotay, D. Štajnbaher, F. J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, J. AOAC Int., 86: 412–431, 2003
- Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/12682/2019