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

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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 [1]. Thus, increasing the importance of having analytical method for determination of residual pesticides present in cumin seeds.

Figure 1. Cumin seeds

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 [2].

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 [3].

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

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.

Sample and standard preparation

Cumin seeds were grounded and used as sample for extraction. Acetonitrile was used as extraction solvent along with magnesium sulphate ($MgSO_4$) 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 $MgSO_4$ 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

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

Results

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.

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.

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

* LOQ = 20 ug/kg

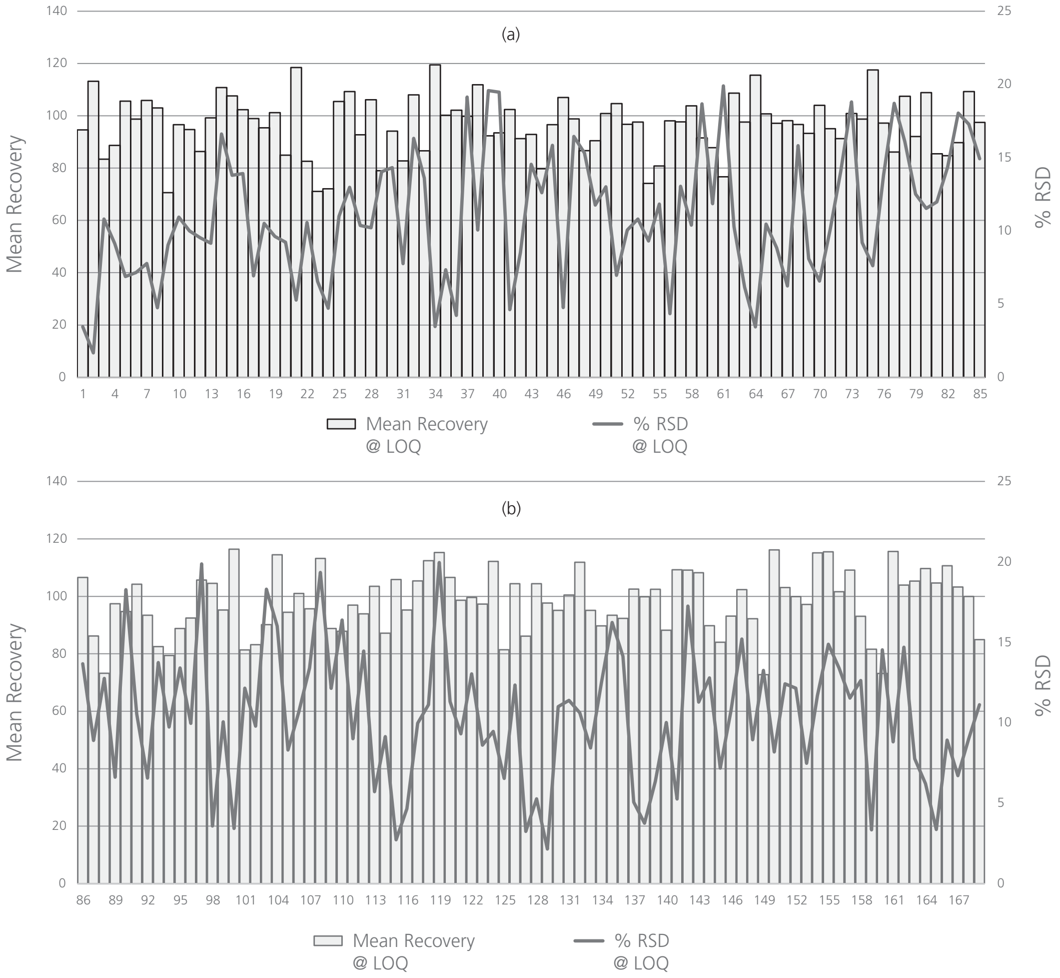

Figure 3. Trend plot of Mean Recovery and % RSD vs Compound # at LOQ level (a) 1 to 85 and (b) 86 to 169

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.

References

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3. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/12682/2019

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