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Application Note

No. 93

Food & Beverages

Method Validation for Determining 513 Pesticide Residues in Cucumber Using LCMS-8060NX and 308 Residues by GCMS-TQ8040 NX

Rakan Saad Abdullah Alajmi [1], Hatim Zaid Ali Alhazmi [1], Sarah Awwadh Sultan Alotaibi [1]
Jayachandran Namasivayam [2],
Anant V. Lohar [3] , Shailesh Damale [3], Jessin Mathai [3]

Food & Beverages

1. Introduction

Cucumbers are often enjoyed raw, as their crisp texture and refreshing taste make them a popular choice for salads, sandwiches, and as a standalone snack. When consumed raw, cucumbers retain their natural crunch and vibrant flavor, making them a refreshing addition to a variety of dishes. However, to fully enjoy the benefits of raw cucumbers, it's essential to ensure they are free from harmful pesticide residues, emphasizing the importance of pesticide residue analysis to guarantee their safety.

To safeguard human well-being, the European Union has established maximum residue limits (MRLs) governing the concentration of pesticides in different commodities. For most of the pesticides the MRL was set 10 µg/L. This highlights the growing significance of developing analytical techniques for identifying a variety of pesticides that may be found in cucumbers.

This study presents a validated approach for detecting 692 different pesticides in cucumber by using of LCMS-8060NX and GCMS-TQ™8040 NX. To extract the pesticides from the sample matrix, QuEChERS extraction technique followed by dSPE clean technique were used.

Representative structures of pesticides are shown in Fig. 1.

This validation study was conducted with the help of Saudi Food and Drug Authority (SFDA) Riyadh, Kingdom of Saudi Arabia and the AnalyticaOne, Doha Qatar authorized distributor for Shimadzu in Qatar.

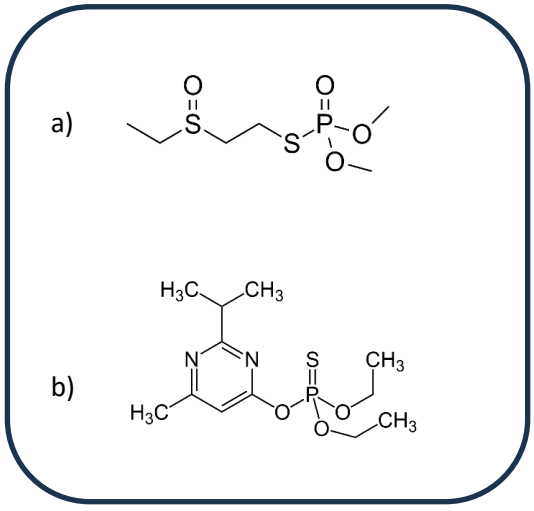


Fig. 1 Representative structures of pesticides (a) Oxydemeton-methyl & (b) Diazinon.

1 Saudi Food and Drug Authority, Riyadh, Saudi Arabia
2 AnalyticaOne, Doha, Qatar.
3 Shimadzu Middle East & Africa FZE, Dubai. UAE

2. Materials and methods

The LC multi residue pesticides kit (31971) and GC multi residue pesticides kit — 32562 were procured from Restek Corporation. Additionally, some individual reference standards were procured from AccuStandard and Dr. Ehrenstorfer GmbH.

Cucumber samples procured from local market was used to prepare matrix-matched calibration standards and spiked samples. The calibration standards were analyzed in the range of 1 to 75 µg/L for LC-MS/MS and GC-MS/MS. Calibration curves were generated by using internal standard calibration method and weighted regression of $1/C^2$. The LC/MS analysis employed Chlorpyrifos-D10 and Triphenyl phosphate as internal standards, while GC/MS analysis utilized PCB-10. Six sets of spiked samples were created, with concentrations set at 10, 20, and 50 µg/kg for LC-MS/MS, and 10 and 20 µg/kg for GC-MS/MS. Internal standards were incorporated into both the samples and matrix-matched standards.

Shimadzu LCMS-8060NX with Nexera™ LC-40 series (Fig. 2) and GCMS-TQ8040 NX (Fig. 3), manufactured by Shimadzu Corporation Japan, were used to quantify residual pesticides in cucumber sample.

Shimadzu's LC/MS/MS Method Package for Residual Pesticides Ver.3 and Smart Pesticides Database Ver.2 for GC-MS/MS enabled quick instrumental method optimization for higher throughput for most of the compounds, 1 target and 2 reference MRM transitions were included in the method. Shimadzu's data processing software LabSolutions Insight™ was used for data processing, which helped in evaluating validation parameters with ease.

2.1. Sample preparation

The modified QuEChERS[1] method involved the use of acetonitrile along with anhydrous magnesium sulfate, sodium chloride, sodium citrate dihydrate, and sodium hydrogencitrate sesquihydrate to extract pesticides. Subsequently, a cleanup step was conducted employing PSA and anhydrous magnesium sulfate. Following this cleanup, the supernatant was filtered through a 0.2 µm nylon syringe filter. This ensured the removal of any remaining impurities or particulate matter before analysis.

Moreover, the filtrate was directly transferred into a 1.5 mL HPLC vial for GC/MS analysis. For LC/MS analysis the same filtrate was diluted for 5 times with LC/MS-grade water and used for LC/MS analysis.

The acquisition method for both LC-MS/MS and GC-MS/MS analysis is a crucial aspect of this study. Detailed acquisition methods specific to LC-MS/MS and GC-MS/MS are outlined in Table 1 and 2, respectively, providing comprehensive insights into the parameters and settings employed for each analytical technique.

It was observed in LCMS/MS, that early eluting compounds were having broad or distorted peak shapes. To overcome this issue, SIL-40's "Co-Injection" feature during LC injection which enhance the peak shape of early-eluting polar compounds,

Fig. 2 Shimadzu LCMS-8060NX

2.2. Analytical Conditions

Table 1 Analytical conditions LC-MS/MS

LC

Flow rate	: 0.4 mL/min
Mobile Phase A	: 2 mM Ammonium formate in water + 0.002% Formic acid
Mobile Phase B	: 2 mM Ammonium formate in methanol + 0.002% Formic acid
Gradient program	3 to 10 %B (0.0 min to 1.0 min) 10 to 55 %B (1.0 min to 3.0 min) 55 to 100 %B (3.0 min to 10.5 min) 100 %B (10.5 min to 12.0 min) 100 to 3 %B (12.0 min to 15.0 min)
Run time	15 min
Injection volume	4 µL (Co-injection with water)
Column Oven temp.	35^{0}C

MS

Ionization	ESI
Interface temp.	350 ^{0}C
Nebulizing gas flow	3 L/min
Heating gas flow	10 L/min
Drying gas flow	10 L/min
DL temp.	250 ^{0}C
Heating block temp.	300 ^{0}C

Fig. 3 Shimadzu GCMS-TQ™8040 NX

Table 2 Analytical conditions GC-MS/MS

GC

Column	SH-I-5Sil MS (30 m x 0.25 mm I.D., 0.25µm) (PN:221-75954-30)
Injector temp	250^{0}C
Oven temp	105^{0}C (3 min), 10^{0}C/min to 130^{0}C, 4^{0}C/min to 200^{0}C to 8^{0}C/min to 290^{0}C (6 min)
Run time	40 min.
Injector mode	Splitless
Injection volume	1 µL

3. Result and Discussion

Validation parameters like matrix effect, linearity, recovery and precision was studied as per SANTE guidelines[2]. The illustrative data is presented through various plots in the figures below, specifically referring to Fig. 4, 5, 6, and 7.

Processing multi-residue data poses a significant challenge due to its sheer volume of compounds and samples, often resulting in time-consuming efforts. However, within LabSolutions Insight, the innovative QA/QC flagging feature emerges as a game-changer. This functionality streamlines the identification of potential outliers, swiftly pinpointing near-outliers or completely deviant samples and compounds. By harnessing this capability, the cumbersome task of individually inspecting each sample and compound is drastically reduced, allowing for a more efficient and effective workflow. Refer Fig. 8.

3.1. System precision and specificity

System precision was evaluated by calculating variation of the peak area and retention time of six injections of 10 µg/L pesticide mixture. The %RSD of peak area for 513 compounds on LC-MS/MS and 308 compounds on GC-MS/MS was found to be less than 20%. Specificity of the method was determined by comparing the response of six different blank samples (reagent and matrix) against reporting level of 10 µg/L.

Matrix effects are known to occur frequently in both GC and LC methods and upon assessing at the initial method validation stage. It was decided to use Matrix-matched calibration to compensate for matrix effects.

3.2. Linearity study

The linearity study utilized calibration standards matched to the matrix. Tables 3 and 4 show the compounds investigated in this research. The calibration curve spanned from 1 to 75 µg/L for LC-MS/MS and from 1 to 100 µg/L for GC-MS/MS L (2 to 200 µg/L for compounds marked with* in Table 4) . All calibration standards met the criteria of falling within the 80 to 120% accuracy range according to SANTE guidelines. In Fig. 9, the linearity graphs and chromatograms of each representative compound from the Restek mixture are presented.

3.3. Recovery study

As cucumber consists of over 80% water, our initial trials didn't involve using extra water for extraction. However, during these trials, we noticed that certain GC amenable molecules exhibited lower recovery rates. To address this, we experimented by adding an additional 10 mL of water to the extraction process alongside acetonitrile, resulting in significantly improved recovery rates.

To affirm the trueness of the method, spiked samples were spiked with concentrations of 10, 20, and 50 µg/kg for LC-MS/MS, while GC-MS/MS samples were spiked with 10 & 20 µg/kg. Six replicates of these spiked samples underwent evaluation against a matrix-matched calibration curve. The mean recoveries for most compounds fell within the 70-120% range, demonstrating the method's accuracy. In accordance with SANTE guidelines, all compounds exhibited reproducibility with a 20% RSD at their LOQ levels, confirming both accuracy and precision.

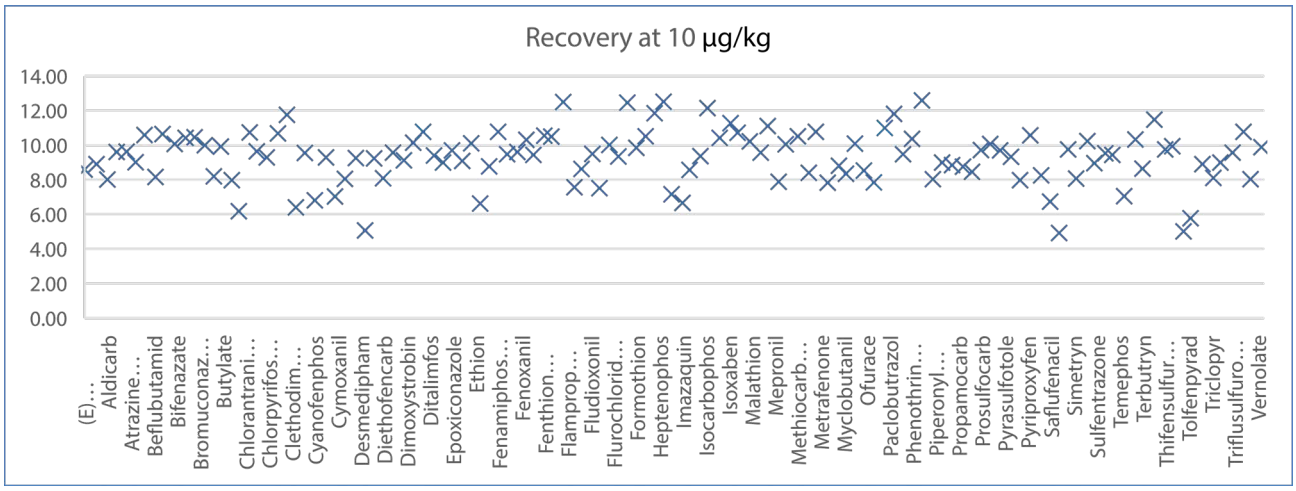


Fig. 4 Recovery plot at 10 µg/kg for all LC-MS/MS compounds

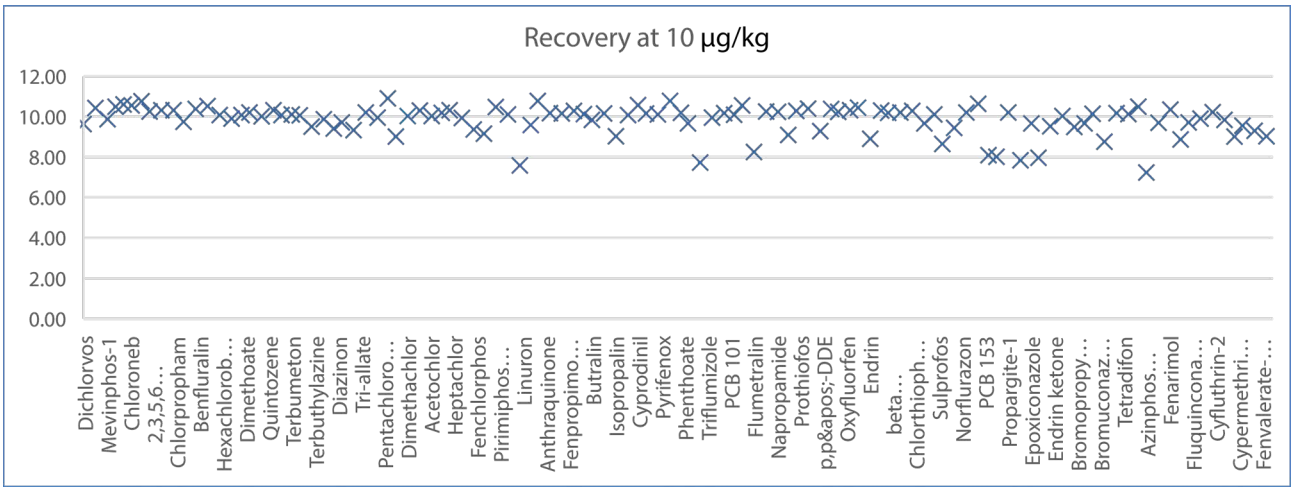


Fig. 5 Recovery plot at 10 µg/kg for all GC-MS/MS compounds

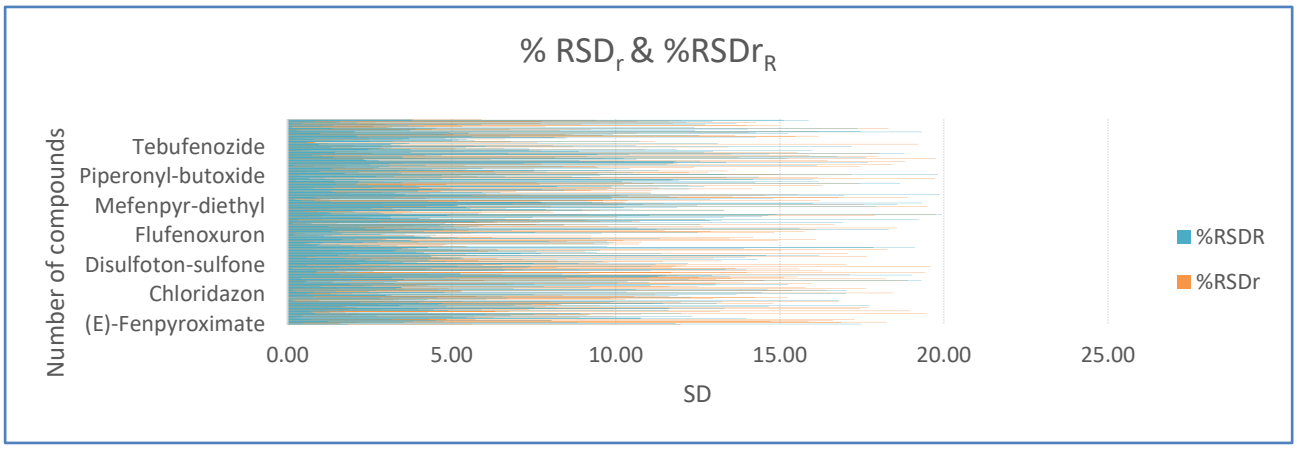


Fig. 6 Comparison of %RSD for Repeatability (RSD_r) and Reproducibility (RSD_R) during LC-MS/MS validation

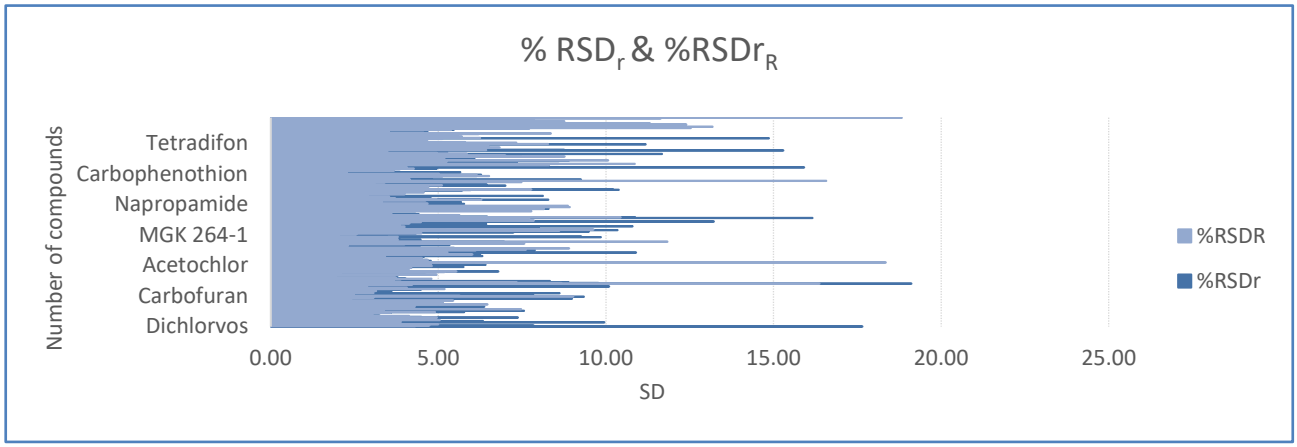


Fig. 7 Comparison of %RSD for Repeatability (RSD_r) and Reproducibility (RSD_R) during GC-MS/MS validation

3.4. Precision study

Precision, repeatability (RSD_r), and within-laboratory reproducibility (RSD_R) studies were conducted to assess accuracy. The concentrations of spiked samples were then determined by back-calculating against matrix-matched linearity plots using the internal standard calibration method.

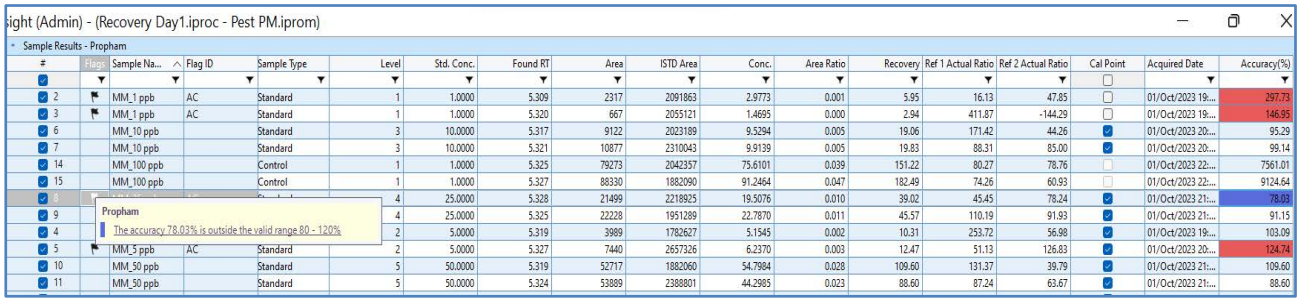
Repeatability (RSD_r):

The repeatability experiment involved injecting six replicates at concentration levels of 10 µg/kg, 20 µg/kg, and 50 µg/kg in LC-MS/MS, and 10 µg/kg and 20 µg/kg in GC-MS/MS. The percentage relative standard deviation (%RSD) for repeatability, determined from six injections at their respective Limit of Quantification (LOQ) level and the 2 X LOQ were injected, and was observed to be below 20% which is well within the acceptance criteria given by the SANTE guidelines of method validation. The detail plot of %RSD were plotted, refer to Fig. 6 & Fig. 7.

Reproducibility (RSD_R):

A reproducibility experiment for recoveries was conducted using six distinct fortified samples, with spiked concentration levels matching those specified in the repeatability study. The percentage relative standard deviation (% RSD) for the recovery of six spiked samples at their respective LOQ levels was determined to be below 20%. At the LOQ level, for the total compounds, mean recoveries of 508 on LC-MS/MS and 307 on GC-MS/MS were observed to fall within the 70-120% range. However, 5 compounds on LC-MS/MS and 1 compound on GC-MS/MS exhibited recoveries below 70%. According to SANTE guidelines, the recoveries of all compounds were found to be reproducible, with RSD values at their LOQ levels being less than the specified threshold. (Refer to Fig. 6, and Fig. 7)

The method effectively attained a Limit of Quantification of 10 µg/kg for compounds in both LC-MS/MS and GC-MS/MS. Representative chromatograms depicting a selection of compounds at their respective LOQ levels can be observed in Fig. 9.



#	Flags	Sample Na...	Flag ID	Sample Type	Level	Std. Conc.	Found RT	Area	ISTD Area	Conc.	Area Ratio	Recovery	Ref 1 Actual Ratio	Ref 2 Actual Ratio	Cal Point	Acquired Date	Accuracy(%)
2		MM_1 ppb	AC	Standard	1	1.0000	5.309	2317	2091863	2.9773	0.001	5.95	16.13	47.85		01/Oct/2023 19:...	297.73
3		MM_1 ppb	AC	Standard	1	1.0000	5.320	667	2055121	1.4695	0.000	2.94	411.87	-144.29		01/Oct/2023 19:...	146.95
6		MM_10 ppb		Standard	3	10.0000	5.317	9122	2023189	9.5294	0.005	19.06	171.42	44.26		01/Oct/2023 20:...	95.29
7		MM_10 ppb		Standard	3	10.0000	5.321	10877	2310043	9.9139	0.005	19.83	88.31	85.00		01/Oct/2023 20:...	99.14
14		MM_100 ppb		Control	1	1.0000	5.325	79273	2042357	75.6101	0.039	151.22	80.27	78.76		01/Oct/2023 22:...	7561.01
15		MM_100 ppb		Control	1	1.0000	5.327	88330	1882090	91.2464	0.047	182.49	74.26	60.93		01/Oct/2023 22:...	9124.64
8					4	25.0000	5.328	21499	2218925	19.5076	0.010	39.02	45.45	78.24		01/Oct/2023 21:...	78.03
9					4	25.0000	5.325	22228	1951289	22.7870	0.011	45.57	110.19	91.93		01/Oct/2023 21:...	91.15
4					2	5.0000	5.319	3989	1782627	5.1545	0.002	10.31	253.72	56.98		01/Oct/2023 19:...	103.09
5		MM_5 ppb	AC	Standard	2	5.0000	5.327	7440	2657326	6.2370	0.003	12.47	51.13	126.83		01/Oct/2023 20:...	124.74
10		MM_50 ppb		Standard	5	50.0000	5.319	52717	1882060	54.7984	0.028	109.60	131.37	39.79		01/Oct/2023 21:...	109.60
11		MM_50 ppb		Standard	5	50.0000	5.324	53889	2388801	44.2985	0.023	88.60	87.24	63.67		01/Oct/2023 21:...	88.60

Fig. 8 LabSolutions Insight QA/QC flagging criteria highlights results that approach or exceed your limits are automatically color-coded

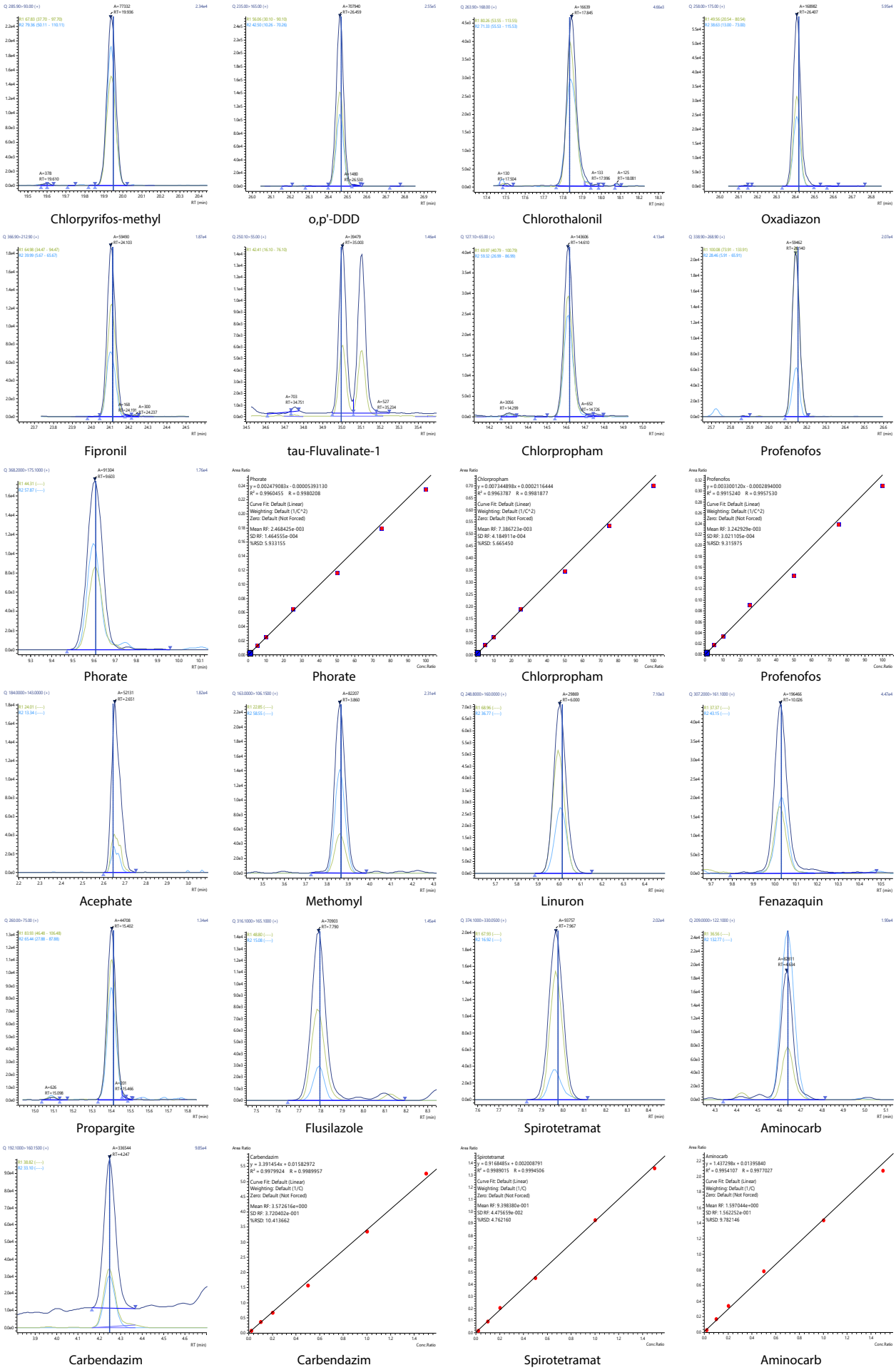


Fig. 9 Representative Chromatograms of GC-MS/MS and LC-MS/MS Compounds in a 10 µg/Kg Spiked Sample.

Table 3. List of LC-MS/MS compounds

1	(E)-Fenpyroximate	35	Bensulfuron-methyl	69	Carboxin	103	Cyanofenphos
2	2,3,5-Trimethacarb	36	Bensulide	70	Carfentrazone-ethyl	104	Cyazofamid
3	2,4-Dimethylaniline	37	Bentazone	71	Carpropamid	105	Cycloheximide
4	6-Benzyladenine	38	Benthiavalicarb-isopropyl	72	Chlorantraniliprole	106	Cyclosulfamuron
5	Acephate	39	Benzoximate	73	Chlorbromuron	107	Cycloxydim
6	Acetamiprid	40	Benzoylprop-ethyl	74	Chlorfenvinphos	108	Cycluron
7	Acibenzolar-S-methyl	41	Benzthiazuron	75	Chlorfluazuron	109	Cyflufenamid
8	Acrinathrin	42	Bifenazate	76	Chloridazon	110	Cyflumetofen
9	Alachlor	43	Bifenthrin	77	Chlorimuron-ethyl	111	Cyhalothrin (isomer)
10	Alanap/Naptalam	44	Bispyribac-sodium	78	Chlormequat-chloride	112	Cymiazole
11	Aldicarb	45	Bitertanol (diastereo isomers)	79	Chlorotoluron	113	Cymoxanil
12	Aldicarb-sulfone (Aldoxycarb)	46	Boscalid	80	Chloroxuron	114	Cypermethrin (isomer)
13	Aldicarb-sulfoxide	47	Brodifacoum	81	Chlorpyrifos	115	Cyprazine
14	Ametryn	48	Bromacil	82	Chlorpyrifos-D10	116	Cyproconazole-I
15	Amidosulfuron	49	Bromadiolone	83	Chlorpyrifos-methyl	117	Cyproconazole-II
16	Aminocarb	50	Bromoxynil	84	Chlorsulfuron	118	Cyprodinil
17	Aminopyralid	51	Bromuconazole-I	85	Chlorthiamid	119	Cyromazine
18	Amisulbrom	52	Bromuconazole-II	86	Chlorthiophos (isomer)	120	Deltamethrin
19	Anilofos	53	Bupirimate	87	Chlozolinate	121	Demeton-S-methyl
20	Asulam	54	Buprofezin	88	Chromafenozide	122	Demeton-S-methyl-sulfone
21	Atraton	55	Butachlor	89	Cinidon-ethyl	123	Desmedipham
22	Atrazine-desethyl	56	Butafenacil	90	Cinosulfuron	124	Diazinon
23	Atrazine-desisopropyl	57	Butocarboxim	91	Ciodrin	125	Dichlofluanid
24	Avermectin B1a	58	Butocarboxim-sulfoxide	92	Ciodrin_NH4	126	Dichlormid
25	Azaconazole	59	Butoxycarboxim (Butocarboxim-sulfone)	93	Clethodim (isomer)	127	Dichlorprop
26	Azamethiphos	60	Butralin	94	Clodinafop-propargyl	128	Dichlorvos
27	Azimsulfuron	61	Buturon	95	Clofentezine	129	Diclobutrazol (stereo isomer)
28	Azinphos-ethyl	62	Butylate	96	Clomeprop	130	Diclofop-methyl
29	Azoxybenzene/Fenazox	63	Cadusafos	97	Cloquintocet-mexyl	131	Diclosulam
30	Azoxystrobin	64	Carbaryl (NAC)	98	Clothianidin	132	Dicrotophos
31	Barbamate/Barban	65	Carbendazim	99	Coumatetralyl	133	Diethofencarb
32	Beflubutamid	66	Carbetamide	100	Crimidine	134	Difenoconazole (isomer)
33	Benalaxyl	67	Carbofuran	101	Crufomate	135	Difenoxuron
34	Bendiocarb	68	Carbofuran-3-hydroxy (3-Hydroxycarbofuran)	102	Cyanazine	136	Difenzoquat (Difenzoquat-methyl-sulfate)

Table 3. List of LC-MS/MS compounds

137	Diflubenzuron	171	Ethiofencarb-sulfone	205	Fenthion-sulfoxide	239	Fluxapyroxad
138	Diflufenican	172	Ethiofencarb-sulfoxide	206	Fentin	240	Foramsulfuron
139	Dimethenamid	173	Ethion	207	Fenuron	241	Forchlorfenuron
140	Dimethoate	174	Ethiprole	208	Fipronil	242	Formetanate hydrochloride
141	Dimethomorph-E	175	Ethirimol	209	Fipronil-desulfinyl	243	Formothion
142	Dimethomorph-Z	176	Ethofumesate	210	Fipronil-sulfide	244	Fosthiazate (isomer)
143	Dimoxystrobin	177	Ethoprophos	211	Fipronil-sulfone	245	Fuberidazole
144	Diniconazole	178	Etofenprox	212	Flamprop -ve	246	Furalaxyl
145	Dinitramine	179	Etoxazole	213	Flamprop-methyl	247	Furathiocarb
146	Dinocap	180	Etrimfos	214	Flamprop-M-isopropyl	248	Halofenozide
147	Dinoseb	181	Famoxadone	215	Flazasulfuron	249	Halosulfuron-methyl
148	Dinotefuran	182	Fenamidone	216	Flonicamid	250	Haloxyfop
149	Dioxacarb	183	Fenamiphos-sulfone	217	Fluazifop-P (free acid)	251	Haloxyfop-2-ethoxyethyl
150	Diphenamid	184	Fenamiphos-sulfoxide	218	Fluazifop-P-butyl	252	Haloxyfop-methyl
151	Disulfoton-sulfone	185	Fenarimol	219	Fluazinam	253	Heptenophos
152	Disulfoton-sulfoxide	186	Fenazaquin	220	Flubendiamide	254	Hexaconazole
153	Ditalimfos	187	Fenbuconazole	221	Flubenzimin	255	Hexaflumuron
154	Dithiopyr	188	Fenbutatin-oxide	222	Flucycloxuron	256	Hexythiazox
155	Diuron (DCMU)	189	Fenchlorazol-ethyl	223	Fludioxonil	257	Hydramethylnon
156	DMST	190	Fenhexamid	224	Flufenacet	258	Imazalil
157	Dodemorph-I	191	Fenobucarb	225	Flufenoxuron	259	Imazamethabenz-methyl (isomer)
158	Dodemorph-II	192	Fenothiocarb	226	Flumetsulam	260	Imazamox
159	Dodine	193	Fenoxanil	227	Fluometuron	261	Imazapic
160	Doramectin	194	Fenoxaprop-ethyl	228	Fluopicolide	262	Imazapyr
161	Emamectin B1a	195	Fenoxaprop-P-ethyl	229	Fluopyram	263	Imazaquin
162	Endosulfan-sulfate	196	Fenoxycarb	230	Fluoxastrobin	264	Imazethapyr
163	Epoxiconazole	197	Fenpropathrin	231	Flupyrsulfuron-methyl	265	Imidacloprid
164	Eprinomectin	198	Fenpropidin	232	Fluquinconazole	266	Indoxacarb
165	EPTC	199	Fenpropimorph	233	Flurochloridone	267	Iodosulfuron-methyl
166	Esprocarb	200	Fenpyrazamine	234	Fluroxypyr	268	Ioxynil
167	Etaconazole	201	Fensulfothion	235	Fluroxypyr-1-methylheptylester	269	Ipconazole
168	Ethafluralin	202	Fenthion-oxon-sulfone	236	Flusilazole	270	Iprobenfos
169	Ethametsulfuron-methyl	203	Fenthion-oxon-sulfoxide	237	Flutolanil	271	Iprovalicarb
170	Ethiofencarb	204	Fenthion-sulfone	238	Flutriafol (isomer)	272	Isocarbamid

Table 3. List of LC-MS/MS compounds

273	Isocarbophos	307	Metamitron	341	Noviflumuron	375	Pirimicarb
274	Isofenphos	308	Metconazole	342	Nuarimol	376	Pirimicarb-desmethyl
275	Isofenphos-methyl	309	Methabenzthiazuron	343	Ofurace	377	Pirimiphos-methyl
276	Isofenphos-oxon	310	Methamidophos	344	Omethoate	378	Primisulfuron-methyl
277	Isomethiozin	311	Methidathion	345	Oxadiargyl	379	Prochloraz
278	Isonoruron	312	Methiocarb	346	Oxadixyl	380	Promecarb
279	Isoprocarb	313	Methiocarb-sulfone	347	Oxamyl	381	Prometon
280	Isoprothiolane	314	Methiocarb-sulfoxide	348	Oxamyl Oxime	382	Prometryn
281	Isoproturon	315	Methomyl	349	Oxasulfuron	383	Propamocarb
282	Isopyrazam	316	Methoprotryne	350	Oxaziclomefone	384	Propaquizafop
283	Isoxaben	317	Methoxyfenozide	351	Oxycarboxin	385	Propargite
284	Isoxaflutole	318	Metobromuron	352	Oxydemeton-methyl	386	Propazine
285	Isoxathion	319	Metolachlor	353	Paclobutrazol	387	Propetamphos
286	Ivermectin	320	Metolcarb (MTMC)	354	Paraoxon-ethyl	388	Propham
287	Karbutilate	321	Metosulam	355	Paraoxon-methyl	389	Propiconazole (stereo isomer)
288	Kresoxim-methyl	322	Metoxuron	356	Penconazole	390	Propiconazole-isomer
289	Lactofen	323	Metrafenone	357	Pencycuron	391	Propoxur
290	Linuron	324	Metribuzin	358	Pendimethalin	392	proquinazid
291	Lufenuron	325	Metsulfuron-methyl	359	Penoxsulam	393	Prosulfocarb
292	Malaoxon	326	Mevinphos-I	360	Permethrin	394	Prosulfuron
293	Malathion	327	Mevinphos-isomer	361	Pethoxamid	395	Prothioconazole-desthio
294	Mandipropamid	328	Mexacarbate	362	Phenmedipham	396	Prothiophos
295	MCPA (MCP)	329	Molinate	363	Phenothrin (isomer)	397	Prothoate
296	Mecarbam	330	Monocrotophos	364	Phenthoate	398	Pymetrozine
297	Mecoprop (MCPP acid)	331	Monolinuron	365	Phorate	399	Pyracarbolid
298	Mefenacet	332	Monuron	366	Phorate-sulfone	400	Pyraclostrobin
299	Mefenpyr-diethyl	333	Myclobutanil	367	Phorate-sulfoxide	401	Pyraflufen-ethyl
300	Mefluidide	334	Naled	368	Phosphamidon	402	Pyrasulfatole +Ve
301	Mepanipyrim	335	Napropamide	369	Phoxim	403	Pyrasulfotole
302	Mephosfolan	336	Napthalene Acetamide	370	Picolinafen	404	Pyrazon/Chloridazon
303	Mepronil	337	Neburon	371	Picoxystrobin	405	Pyrazosulfuron-ethyl
304	Meptyldinocap	338	Nicosulfuron	372	Pinoxaden	406	Pyrethrin
305	Metaflumizone	339	Nitenpyram	373	Piperonyl-butoxide	407	Pyrethrin isomer
306	Metalaxyl	340	Novaluron	374	Piperophos	408	Pyributicarb

Table 3. List of LC-MS/MS compounds

409	Pyridaben	443	Sulfentrazone	477	Thiodicarb	511	Vamidothion
410	Pyridalyl	444	Sulfometuron-methyl	478	Thiofanox	512	Vamidothion-sulfone
411	Pyridate	445	Sulfosulfuron	479	Thiometon	513	Vamidothion-sulfoxide
412	Pyrimethanil	446	Tebuconazole	480	Thionazin	514	Vernolate
413	Pyriproxyfen	447	Tebufenozide	481	Thiophanate-methyl	515	Warfarin
414	Pyroquilon	448	Tebufenpyrad	482	Tolclofos-methyl	516	Zoxamide
415	Quinalphos	449	Tebupirimfos	483	Tolfenpyrad		
416	Quinclorac	450	Tebutam	484	Tolylfluanid		
417	Quinmerac	451	Tebuthiuron	485	Topramezone		
418	Quinoxyfen	452	Teflubenzuron	486	Tralkoxydim		
419	Quizalofop-P	453	Temephos	487	Triadimefon		
420	Quizalofop-P-ethyl	454	Tepraloxydim (isomer)	488	Triadimenol (isomer)		
421	Rimsulfuron	455	Terbufos	489	Triadimenol		
422	Rotenone	456	Terbufos-sulfone	490	Triasulfuron		
423	Saflufenacil	457	Terbufos-sulfoxide	491	Triazamate		
424	Sebuthylazine	458	Terbumeton	492	Trichlorfon		
425	Sebuthylazine-desethyl	459	Terbuthylazine	493	Triclopyr		
426	Secbumeton	460	Terbuthylazine-desethyl	494	Triclopyr butotyl		
427	Sethoxydim (isomer)	461	Terbutol	495	Tricyclazole		
428	Siduron	462	Terbutol_NH4	496	Tridemorph		
429	Silafluofen	463	Terbutryn	497	Tridemorph-isomer		
430	Silthiofam	464	Tetrachlorvinphos (CVMP)	498	Trietazine		
431	Simazine	465	Tetraconazole	499	Trifloxystrobin		
432	Simeconazole	466	Tetraethylpyrophosphate	500	Trifloxysulfuron		
433	Simetryn	467	Tetramethrin	501	Triflumizole		
434	Spinetoram J	468	Thiabendazole	502	Triflumuron		
435	Spinetoram L	469	Thiacloprid	503	Triflusulfuron-methyl		
436	Spinosyn A	470	Thiamethoxam	504	Triforine		
437	Spinosyn D	471	Thiazafluron	505	Triforine		
438	Spirodiclofen	472	Thidiazuron	506	Triforine-Isomer		
439	Spiromesifen	473	Thifensulfuron-methyl	507	Trinexapac-ethyl		
440	Spirotetramat	474	Thifluzamide	508	Triphenyl phosphate		
441	Spiroxamine	475	Thiobencarb	509	Triticonazole		
442	Sulcotrione	476	Thiocyclam hydrogene oxalate	510	Uniconazole		

Table 4. List of GC-MS/MS compounds

1	(E)-Chlorfenvinphos	35	Bromfenvinfos-methyl	69	Cyanophos	103	Disulfoton sulfone
2	(Z)-Chlorfenvinphos	36	Bromfenvinphos	70	Cycloate	104	Ditalimfos
3	1,1-Dichloro-2,2-bis(4-ethylphenyl)ethane	37	Bromophos	71	Cyflufenamid	105	Dodemorph-I
4	2,3,5,6-Tetrachloroaniline	38	Bromophos-ethyl	72	Cyfluthrin-1	106	Dodemorph-II
5	2,4'-Methoxychlor	39	Bromopropylate	73	Cyfluthrin-2	107	Edifenphos
6	2-Phenylphenol	40	Bromuconazole-1	74	Cyfluthrin-3	108	Endosulfan ether
7	3,4-Dichloroaniline	41	Bromuconazole-2	75	Cyfluthrin-4	109	Endosulfan sulfate
8	4,4'-Dichlorobenzophenone	42	Bupirimate	76	Cypermethrin-1	110	Endrin
9	4,4'-methoxychlor olefin	43	Buprofezin	77	Cypermethrin-2	111	Endrin aldehyde
10	Acetochlor	44	Butachlor	78	Cypermethrin-3	112	Endrin ketone
11	Acrinathrin-2	45	Butamifos	79	Cypermethrin-4	113	EPN
12	Alachlor	46	Butralin	80	Cyprazine	114	Epoxiconazole
13	Aldrin	47	Cadusafos	81	Cyprodinil	115	Epsilon-HCH
14	Allethrin-3,4 (Bioallethrin)	48	Carbofuran	82	Dazomet	116	Ethalfluralin
15	Allidochlor	49	Carbophenothion	83	delta-BHC	117	Ethion
16	alpha-BHC	50	Carfentrazone-ethyl	84	Deltamethrin-2 (Tralomethrin deg.-2)	118	Ethoprophos
17	alpha-Endosulfan	51	Chlorbenside	85	Di-allate-1	119	Ethoxyquin
18	Ametryn	52	Chlorfenapyr	86	Di-allate-2	120	Etofenprox
19	Aminocarb	53	Chlorfenson	87	Diazinon	121	Etridiazole
20	Amitraz	54	Chlorobenzilate	88	Dichlobenil	122	Famphur
21	Anilofos	55	Chloroneb	89	Dichlofenthion	123	Fenamidone
22	Anthraquinone	56	Chlorothalonil	90	Dichlofluanid	124	Fenamiphos
23	Atraton	57	Chlorpropham	91	Dichlorvos	125	Fenarimol
24	Atrazine*	58	Chlorpyrifos	92	Dicloran	126	Fenazaquin
25	Azinphos-ethyl	59	Chlorpyrifos-methyl	93	Dicrotophos	127	Fenchlorphos
26	Azinphos-methyl	60	Chlorthal-dimethyl	94	Dieldrin	128	Fenitrothion
27	Beflubutamid	61	Chlorthiophos-1	95	Diflufenican	129	Fenpropathrin
28	Benalaxyl	62	Chlorthiophos-2	96	Dimethachlor	130	Fenpropidin
29	Bendiocarb	63	Chlozolinate	97	Dimethenamid (Dimethenamid-P)	131	Fenpropimorph
30	Benfluralin	64	cis-Chlordane	98	Dimethipin	132	Fenson
31	beta-BHC	65	cis-Nonachlor	99	Dimethoate	133	Fensulfothion sulfone
32	beta-Endosulfan	66	cis-Permethrine	100	Diphenamid	134	Fenthion
33	Bifenthrin	67	Clomazone	101	Diphenylamine	135	Fenvalerate-1
34	Biphenyl	68	Coumaphos	102	Disulfoton	136	Fenvalerate-2 (Esfenvalerate)

Table 4. List of GC-MS/MS compounds

137	Fipronil	171	Lenacil	205	p,p'-DDD	239	Procymidone
138	Fluazifop-P-butyl	172	Leptophos	206	p,p'-DDE	240	Prodiamine
139	Fluchloralin	173	Linuron*	207	p,p'-DDT	241	Profenofos
140	Flucythrinate-1	174	Malaoxon	208	Paclobutrazol	242	Profluralin
141	Flucythrinate-2	175	Malathion	209	Parathion	243	Promecarb
142	Fludioxonil	176	Mecarbam	210	PCB 101	244	Prometryn
143	Flufenacet	177	Mefenpyr-diethyl	211	PCB 138	245	Propachlor
144	Flumetralin	178	Metalaxyl (Mefenoxam)	212	PCB 153	246	Propanil
145	Fluquinconazole	179	Metazachlor*	213	PCB 180	247	Propargite-1
146	Flusilazole	180	Methacrifos	214	PCB 28 (ISTD)	248	Propargite-2
147	Flutolanil	181	Methiocarb	215	PCB 52 (ISTD)	249	Propentamphos
148	Flutriafol	182	Methoxychlor	216	Pebulate	250	Propisochlor
149	Folpet	183	Metolachlor (S-Metolachlor)	217	Penconazole	251	Propoxur
150	Fonofos	184	Metribuzin	218	Pendimethalin	252	Propyzamide
151	Formothion	185	Mevinphos-1	219	Pentachloroaniline	253	Prosulfocarb
152	Furathiocarb	186	Mevinphos-2	220	Pentachloroanisole	254	Prothiofos
153	Gamma.-Cyhalothrin	187	MGK 264-1	221	Pentachlorobenzene	255	Pyraclofos
154	gamma-BHC (Lindane)	188	MGK 264-2	222	Pentachlorobenzonitrile	256	Pyrazophos
155	Haloxyfop-etotyl	189	Mirex	223	Pentachlorothioanisole	257	Pyridaben
156	Heptachlor	190	Myclobutanil	224	Phenothrin-1	258	Pyridaphenthion
157	Heptachlor-endo-epoxide	191	N-(2,4-dimethylphenyl) formamide	225	Phenothrin-2	259	Pyrifenox
158	Heptachlor-exo-epoxide	192	Napropamide	226	Phenthoate	260	Pyrimethanil
159	Hexachlorobenzene	193	Nitralin	227	Phorate	261	Pyriproxyfen
160	Hexaconazole	194	Nitrofen	228	Phosalone	262	Quinalphos
161	Hexazinone*	195	Norflurazon	229	Phosmet	263	Quinoxyfen
162	Iodofenphos	196	Nuarimol	230	Phosphamidon-1	264	Quintozene
163	Iprobenfos	197	o,p'-DDD	231	Phosphamidon-2	265	Resmethrin-1
164	Iprodione	198	o,p'-DDE	232	Picoxystrobin	266	Resmethrin-2 (Bioresmethrin)
165	Isazofos	199	o,p'-DDT	233	Piperonyl butoxide	267	Sebuthylazin-desethyl
166	Isodrin	200	Ofurace	234	Pirimicarb	268	Sebuthylazine
167	Isopropalin	201	Oxadiazon	235	Pirimiphos ethyl	269	Simetryn
168	Isoprothiolane	202	Oxadixyl	236	Pirimiphos-methyl	270	Spiroxamine-1
169	Kresoxim-methyl	203	oxy-Chlordane	237	Pretilachlor	271	Spiroxamine-2
170	lambda-Cyhalothrin	204	Oxyfluorfen	238	Prochloraz	272	Sulfotep

Table 4. List of GC-MS/MS compounds

273	Sulprofos	283	Terbuthylazine*	293	THPI (Tetrahydrophthalimide)	303	Tributyl phosphate
274	tau-Fluvalinate-1	284	Terbuthylazine (des-ethyl)	294	Tolclofos-methyl	304	Trifloxystrobin
275	tau-Fluvalinate-2	285	Terbutryn	295	Tolylfluanid	305	Triflumizole
276	Tebuconazole	286	Tetrachlorvinphos	296	trans-Chlordane	306	Trifluralin
277	Tebufenpyrad	287	Tetradifon	297	Transfluthrin	307	Triticonazole
278	Tecnazene	288	Tetramethrin-1	298	trans-Nonachlor	308	Vinclozolin
279	Tefluthrin	289	Tetramethrin-2	299	trans-Permethrine	309	Zoxamide
280	Terbacil	290	Thiazopyr	300	Triadimefon		
281	Terbufos	291	Thiobencarb	301	Tri-allate		
282	Terbumeton	292	Thiometon	302	Triazophos		

4. Conclusion

The method developed and validated for quantifying 513 compounds via LC-MS/MS and 308 compounds using GC-MS/MS in cucumber samples represents a substantial milestone. Confronting the inherent challenges posed by high water content, the modified QuEChERS extraction technique facilitated precise and efficient sample preparation. The use of Shimadzu's LC/MS/MS Method Package for Residual Pesticides Ver.3 and Smart Pesticides Database Ver.2 for GC-MS/MS allowed for the creation of a rapid acquisition method, significantly reducing the method development time. Additionally, the Edit flag settings of LabSolutions insight software proved instrumental in identifying any outliers in the processed data. The Shimadzu LCMS-8060NX and GCMS-TQ8040 NX platforms showcased exceptional sensitivity and reproducibility, aligning seamlessly with SANTE guidelines, thereby affirming the method's reliability. This solidifies its status as an invaluable tool for testing laboratories engaged in comprehensive multi-residue analysis of cucumber samples.

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6. Reference

1. EN 15662: Determination of pesticide residues using GC-MS and/or LC-MS (/MS) following acetonitrile extraction/partitioning and cleanup by dispersive SPE – QuEChERS method 2007 Brussels, Belgium, European Committee for Standardization.

2. Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed SANTE 11312/2021.

Note: This study can be reproduced on the LCMS-8050/LCMS-8060 with slight change in dilution factor/injection volume.

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