



Trace-level quantitation of pesticide residues in red chili powder using LC-(HESI)-MS/MS

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Goal

The objective of this work was to develop a method for the trace-level quantitation of pesticides and their metabolite residues in chili powder, using liquid chromatography-triple quadrupole mass spectrometry. The optimized method performance was verified in accordance with the EU SANTE guidelines and assessed for compliance with the Food Safety and Standards Authority of India (FSSAI) and EU MRLs in chili powder.

Introduction

Spices are widely used for flavoring foods in both commercial catering and households, but potential contaminants that can cause food safety and quality issues receive little attention. This is particularly the case in the myriad of small volume spice trade networks in India and in Asian countries. Food testing of spices generally focuses on microbial impurities or mycotoxins and less on pesticides, perhaps because the difficulties and hence the cost of analyzing a large number of pesticides in a complex matrix are high. Few pesticides are registered for chili crop management to control diseases and pest attacks.¹ Recently, the Rapid Alert System for Food and Feed (RASFF) issued an alert due to flonicamid and formentate residues found in chili powder.² However, the FSSAI does not have MRLs for flonicamid and formentate but the EU has set 0.1 and 0.05 mg/kg, respectively. The lowest MRL set in chili powder is

0.005 mg/kg for fipronil and fipronil sulfone (sum of both).^{3,4} Consequently, a robust and sensitive analytical method is required to check that spices on the market are compliant with these new MRLs for LC-amenable compounds.

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method has been widely adopted for the extraction of pesticide residues from a wide range of food samples including spices.⁵ In the case of spices, the instrument method plays a critical role to deliver accurate, precise, and rugged results in compliance with regulatory requirements.

Therefore, the aim of this work was the development, optimization, and validation of a QuEChERS-based multi-residue method for the analysis of pesticide residues in chili powder using LC-MS/MS with the Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer. The data acquisition and processing were carried out using Thermo Scientific™ TraceFinder™ software. The optimized method was verified according to the SANTE/11813/ 2017 guidelines⁶ and evaluated for compliance with the EU and FSSAI MRL requirements.

Experimental

Chemicals and apparatus

- Acetonitrile, Optima™ LC/MS Grade, Fisher Scientific™
- Methanol, Optima™ LC/MS Grade, Fisher Scientific™
- Water, Optima™ LC/MS Grade, Fisher Scientific™
- Formic acid (85%), Fisher Scientific™
- Acetic acid (100%), Fisher Scientific™
- Ammonium formate, LC/MS Grade, Fisher Scientific™
- Anhydrous magnesium sulfate, Fisher Scientific™
- Sodium acetate, Fisher Scientific™
- Reference standards procured from Restek™
- Other equipment such as a weighing balance, vortex mixer (model, Thermo Scientific), centrifuge, micropipettes, water purification system, ultrasonic bath were used in sample preparation.

LC-MS/MS analysis

The Thermo Scientific™ Vanquish™ Flex UHPLC system was coupled to a TSQ Quantis triple quadrupole mass spectrometer (fitted with a heated electrospray ionization (HESI) source). Chromatographic conditions and LC-MS/MS parameters are given in Table 1, while details of

collision energy (CE), retention time (RT), precursor and product ion transitions are given in Table 2.

Table 1. LC-MS/MS instrument conditions

Liquid chromatography method				
Instrumentation:	Vanquish Flex UHPLC			
Column	Thermo Scientific™ Hypersil GOLD™ (100 mm × 2.1 mm × 1.9 μm) (P/N 25002-102130)			
Sample compartment temp.:	10 °C			
Column oven temp.:	25 °C			
Mobile phase:	A: 2 mM ammonium formate + 0.1% formic acid in water: acetonitrile(90:10, v/v) B: 2 mM ammonium formate + 0.1% formic acid in water: acetonitrile (10:90, v/v)			
Autosampler:	Vanquish (216 vials capacity)			
Total run time:	18.0 min			
Gradient program:	Time	Flow Rate	%B	Curve
	0.000	0.400	1	5
	1.500	0.400	1	5
	5.000	0.400	50	5
	8.500	0.400	95	5
	13.500	0.400	95	5
	14.000	0.400	1	5
	18.000	0.400	1	5
Mass spectrometry method				
Instrumentation:	TSQ Quantis triple quadrupole tandem mass spectrometer			
Method type:	Time-based selective-reaction monitoring (t-SRM)			
Ion source type:	HESI			
Polarity:	Positive/Negative switching			
Spray voltage:	Static Positive: 3500 V Negative: 2500 V			
Sheath gas:	50 Arb			
Aux gas:	10 Arb			
Sweep gas:	1 Arb			
Ion transfer tube temp.:	325 °C			
Vaporizer temp.:	350 °C			

Sample preparation

The chili powder samples were collected from the local market and mixed well before selecting the test portion for extraction. The particle size was approximately 200–500 μm .⁷ The QuEChERS method was used for extraction.⁵

Sample extraction:

- Weigh 2 g chili powder into a 50 mL extraction tube.
- For recovery experiment, spike samples before the addition of water and extraction solvent.
- Add 15 mL of HPLC grade water (containing 1% acetic acid) and leave the sample for 10 min soaking.
- Add 15 mL acetonitrile to the above tube.
- Mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Add 6 g anhydrous MgSO_4 and 1.5 g sodium acetate to the tube and again mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge at 5000 rpm for 5 min at ambient conditions.
- Take an aliquot (1 mL) of the acetonitrile supernatant layer.
- Add 50 mg PSA + 7 mg GCB + 150 mg MgSO_4 .
- Centrifuge at 5000 rpm for 5 min at ambient conditions.
- Take 0.25 mL supernatant and dilute with 0.75 mL of water.
- Inject 5 μL into the LC-MS/MS.

Data acquisition and processing

The data acquisition was performed by using the instrument conditions in Table 1. The data acquisition and processing methods were carried out using Thermo Scientific™ TraceFinder™ software version 4.1. For each analyte two precursor to product ion transitions were acquired in t-SRM mode. For data processing, the user-

defined criteria included an ion ratio ($\pm 30\%$), retention time (± 0.1 min), linearity (> 0.99 with residuals ± 20), recovery (70–120%) and precision ($\pm 20\%$) in accordance with the SANTE guidelines.⁶

Results and discussion

Sample preparation

Chili powder is a dry powder and complex matrix containing carbohydrates ($< 9\%$) and proteins ($< 2\%$) with high amounts of alkaloids and colored pigments such as β -carotene, which are very difficult to remove without incurring losses of pesticides. Chili has a pH in the range 6.2–6.7 and less moisture content ($< 5\%$). So, the pH of the sample was reduced by using 1% acetic acid in water, which is essential for liquid-liquid partitioning with acetonitrile. To improve the stability of base-sensitive and organophosphorus compounds during extraction, GCB was added to reduce the concentration of pigments, while PSA was added to remove acidic matrix co-extractives. The dSPE cleanup followed by dilution of the extract provided an acceptable recovery by a reduction in matrix co-extractives without substantial losses of the target pesticides. The cleanup and dilution approach increased the instrument uptime by increasing the intervals between cleaning of the sampling cone. The final extract was diluted (30x) as per the defined protocol (e.g., 0.01 mg/kg corresponds to 0.00033 $\mu\text{g/mL}$), but this low concentration was easily detected by the TSQ Quantis LC-MS/MS system.

LC-MS/MS analysis

The optimized LC-MS/MS method conditions showed excellent sensitivity for 127 compounds. The total ion chromatogram (TIC) is shown in Figure 1. The optimized liquid chromatographic method offered excellent separation for the target analytes (spinetoram and spinosad D, Figure 2). In this method, the dwell time was automatically optimized for the target list of analytes (two transitions per analyte), which offered ≥ 12 points per peak (Figure 3).

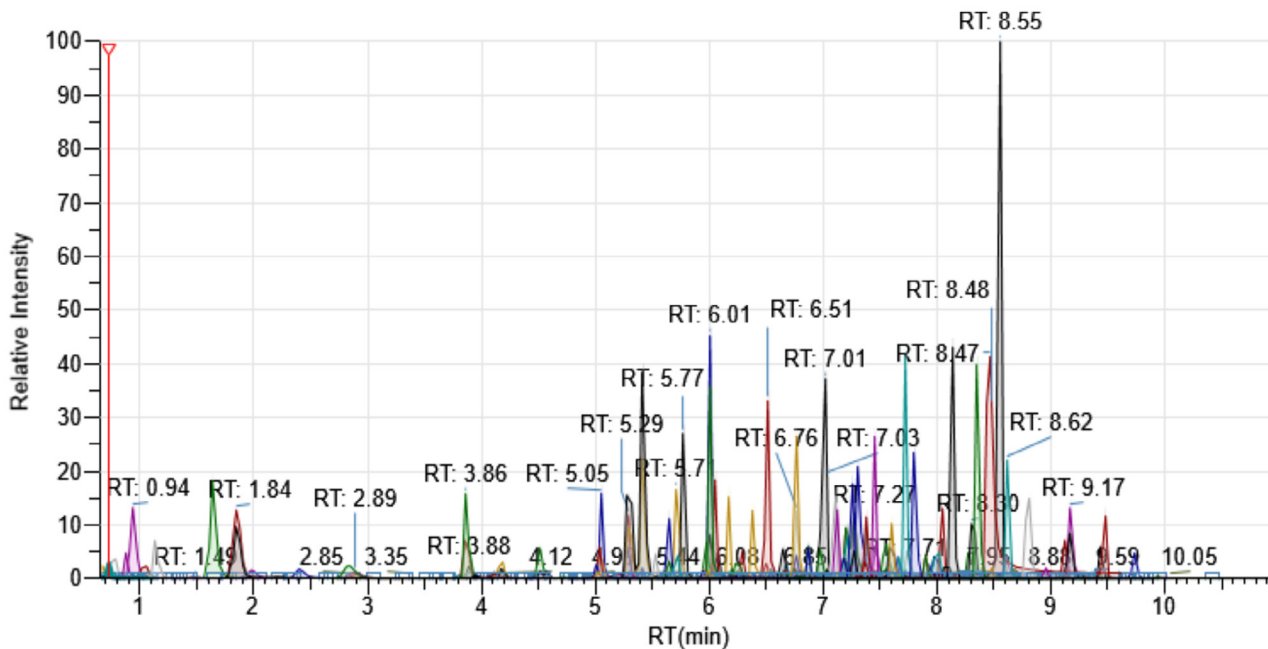


Figure 1. Total ion chromatograms with overlay extraction ions (127 compounds)

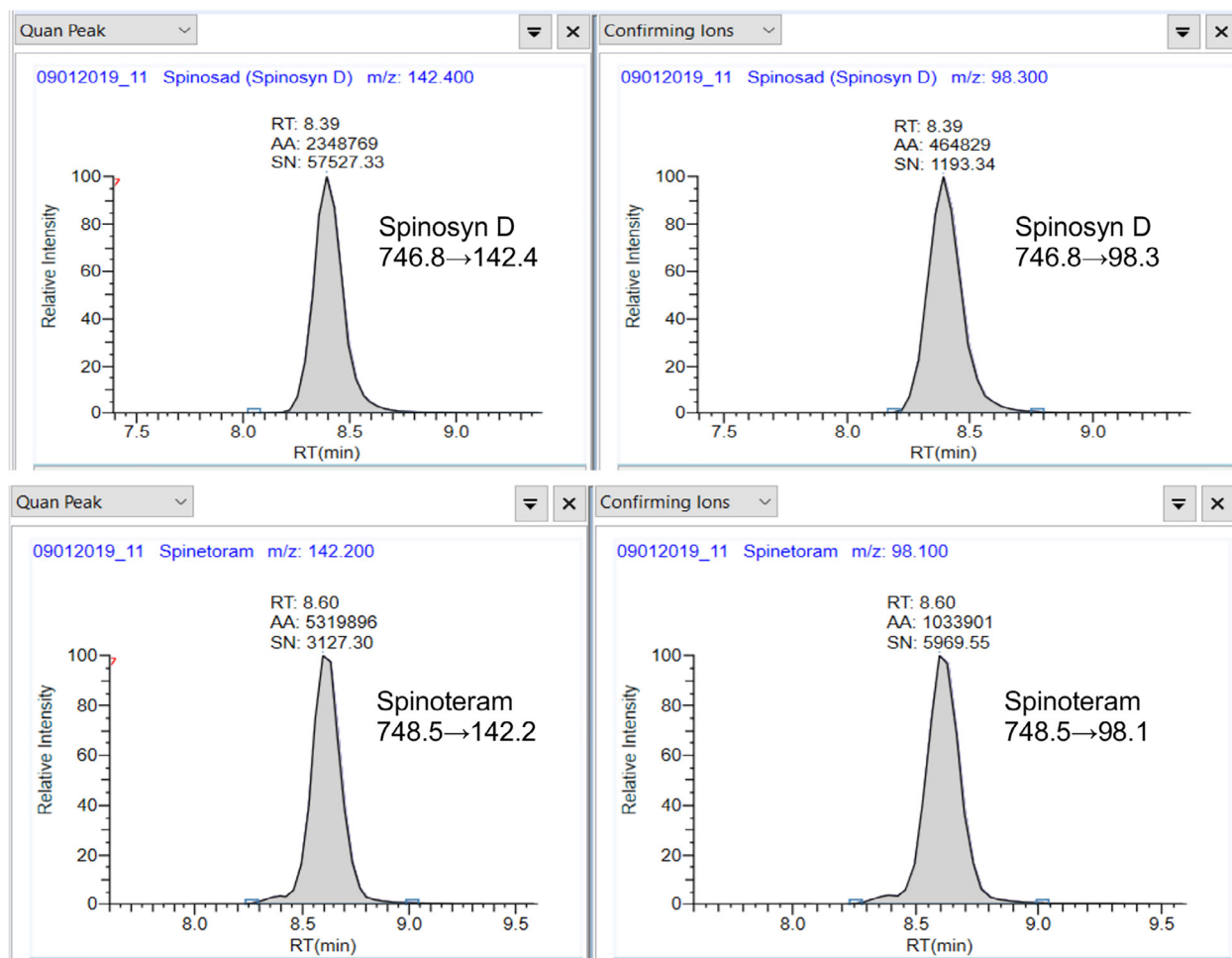
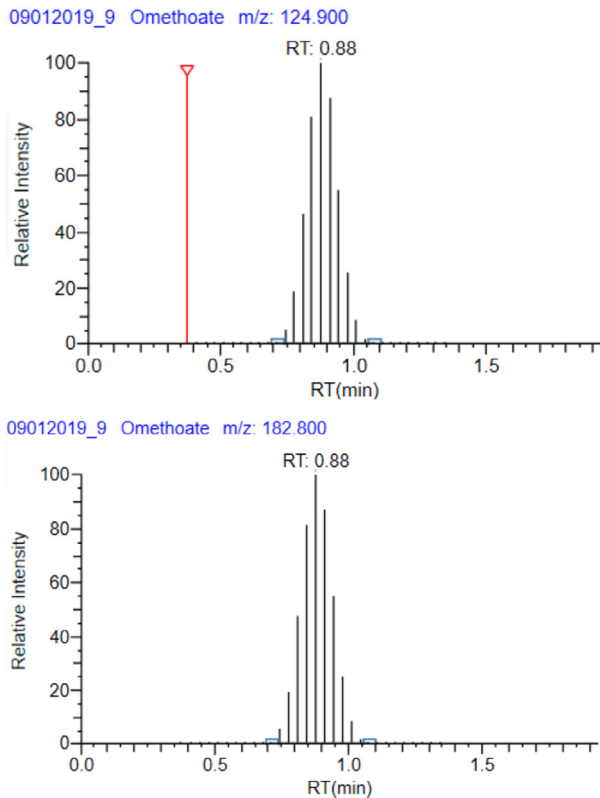


Figure 2. Chromatographic separation of isobaric compounds, i.e. spinosad D and spinoteram



Identification and quantitation

User-defined parameters for data processing including two transitions per analytes, retention time, correlation coefficient, and residuals were set in the data processing method of TraceFinder software (Master method).

Based on these parameters, the data was processed automatically with flagging. These flags indicate through color codes whether results pass or fail against acceptance criteria set in the processing method. The results that passed user-defined criteria (SANTE guidelines) are shown in green (Figure 4). An identification of aminocarb in chili powder was demonstrated with two transitions, 209→137 (quantitative) and 209→152 (confirmatory) at the same retention time (0.94 min, ±0.1) with an ion ratio of 75.83% (64.23–119.28%) observed in chili powder in comparison with neat standard. This approach meets the requirement of the SANTE guidelines for identification and quantitation.

Figure 3. Impact of optimized dwell time on the data points per peak

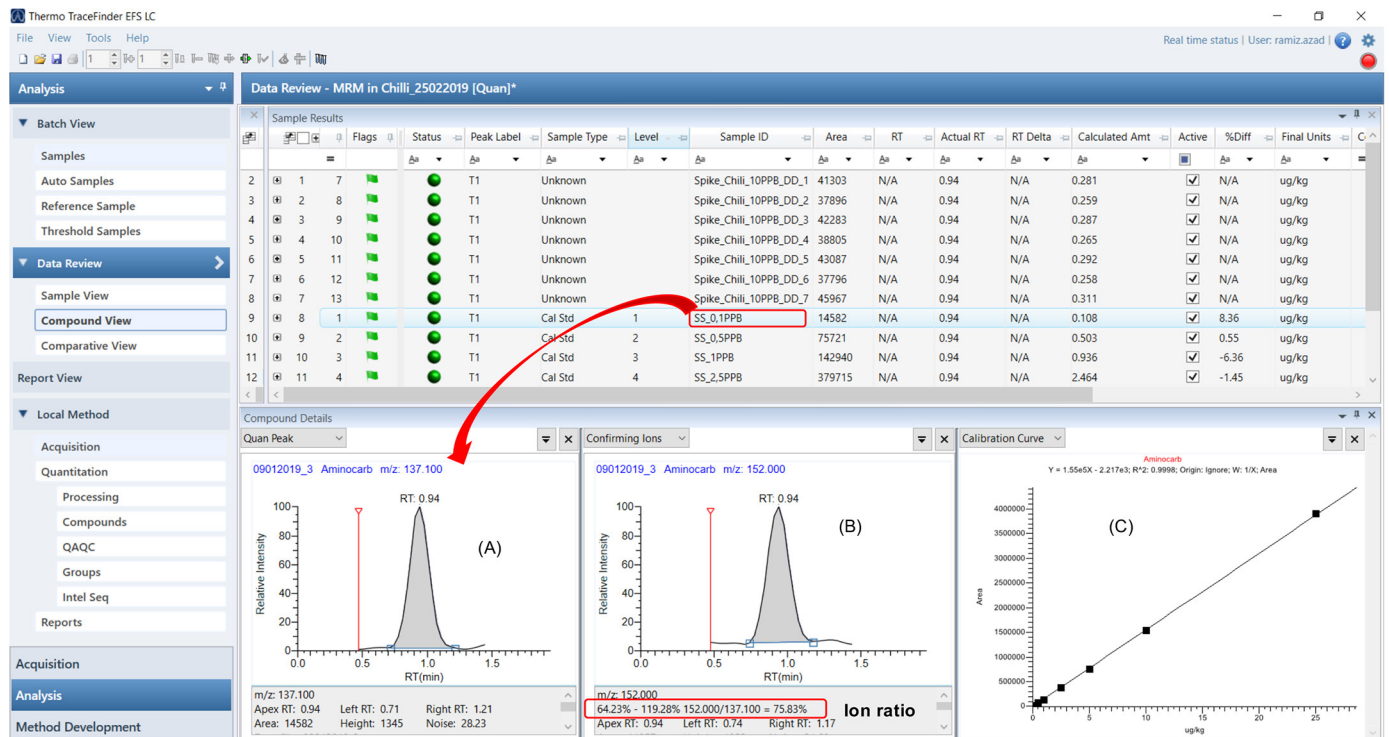


Figure 4. Extracted ion chromatogram (XIC) for quantifier ion of aminocarb (A), identification based on qualifier/ confirmatory ions with confirmed with ion ratio (B), and calibration curve (C)

Method performance

The MS response was linear over the range of 0.0001–0.025 mg/L. This range offered excellent correlation coefficients (>0.99) with $<15\%$ residuals for all the target analytes in the solvent, which was further used for quantitation. For the quantitative approach, the sensitivity achieved at such low levels (0.1 ng/mL) provided $S/N >10:1$ supporting with the confirmatory ion (Figure 4). But as per the extraction protocol, the sample gets diluted (30x). Hence, the limit of quantitation (LOQ) values observed in chili powder matrix were 0.005 mg/kg with acceptable recoveries (70–120%) and precision ($<20\%$) for target analytes ($>96\%$) except bitertanol, clethodim, diniconazole, difenoconazole, fluazinam, hexythiazox, monolinuron, and spirodiclofen (LOQ = 0.01 mg/kg). The recovery experiment was carried out at 0.005 mg/kg (LOQ) and 0.01 mg/kg (LOQ $\times 2$ = reporting limit) to demonstrate the method accuracy and precision. The recoveries were observed in the range of 76–116% with $<15\%$ RSD

(Table 2, Appendix), which were within acceptance criteria (recovery 70–120% and precision $<20\%$).⁶ The XICs of a few compounds at LOQ level spiked in chili matrix are shown in the supplementary information in Appendix II. The optimized method was tested for repeatability of results obtained from a continuous sequence of 50 injections. The repeatability was $<15\%$ for area and ± 0.05 min retention time (Figures 5 and 6).

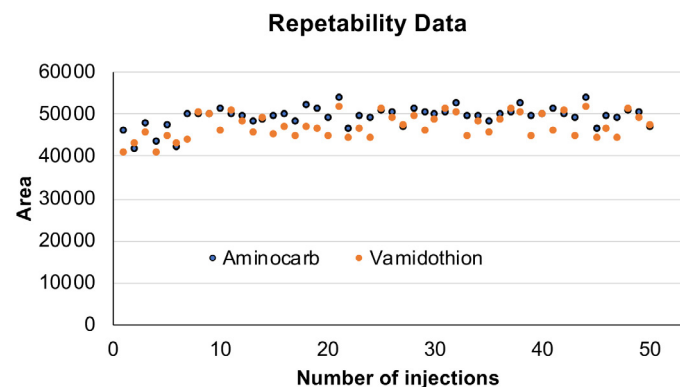


Figure 5. Area repeatability for aminocarb and vamidothion (n=50)



Figure 6. Retention time repeatability for aminocarb and thiabendazole (n=50)

Conclusion

The developed method provides a robust analytical solution for the trace-level (sub ppb) quantitation of more than 120 pesticides in chili powder. The simplicity of the method based on extraction using a QuEChERS method and detection using LC-HESI-MS/MS is suitable for routine analysis in a high-throughput commercial food testing laboratory. The dSPE cleanup followed by the dilution approach minimized the need for cleaning of the sample cone to increase system up time. By following this approach, at least 70 injections (standards, samples, blank) could be completed in a day (24 h cycle). The method validation data at the reporting limit (RL) meets the recovery and precision requirement as per SANTE guidelines. Also, this method complies with the EU and FSSAI MRL requirements by achieving excellent lower limits of quantitation.

References

1. Insecticides / Pesticides Registered under section 9(3) of the Insecticides Act, 1968 for use in the Country:(As on 15/05/2019) http://ppqs.gov.in/sites/default/files/list_of_pesticide_registered_us_93.pdf.
2. European Commission RASFF Portal, Notification details - 2019.1223, Formetanate (1.3 mg/kg - ppm) and flonicamid (1.5 mg/kg - ppm) in chilli peppers from Italy https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationDetail&NOTIF_REFERENCE=2019.1223
3. FSSAI Manual for food safety, 17th Edition-2017 (THE FOOD SAFETY AND STANDARDS ACT, 2006)
4. EU Pesticides Database. <http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=product.resultat&language=EN&selectedID=237>
5. Lehotay, S.J.; Maštovská, K.; Lightfield, A. R. Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. *J. AOAC Int.* **2005**, *88*, 615–630; (Note: Basis of the AOAC Method 2007.01).
6. SANTE guidelines https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf.
7. Oh, S.H. Chungbuk National University, Chungbuk, Republic of Korea Kang, Y.R., Chungbuk, Physical Properties of Red Pepper Powder at Different Particle Sizes [2013], National Academy of Agricultural Science, Gyeonggi, Republic of Korea <http://agris.fao.org/agris-search/search.do?recordID=KR2015003122>

Appendix I: list of pesticides

Table 2 (part 1). List of pesticides with MRM transitions used

Sr. No.	Name of pesticide	RT (min)	ESI (+/-)	Q1	Q3	CE	Q3	CE	0.005 mg/kg (n=6)		0.01 mg/kg (n=6)		Ion Ratio Absolute	Ion ratio (±30)
									% Rec	% RSD	% Rec	% RSD		
1	3-Hydroxycarbofuran	3.89	+	238.1	163	19	181	15	120	7.2	106	6.9	53.7	-0.72
2	Acephate	4.17	+	184.1	49	35	143	11	99	0.2	85	6.3	95.9	5.60
3	Acetamiprid	4.51	+	223	99	53	126	29	94	13.7	88	14.1	10.6	-10.66
4	Aldicarb sulfone	1.46	+	240.1	86.2	28	148.2	19	104	15.1	96	13.5	86.6	1.84
5	Ametryn	5.75	+	228.1	96	35	186.1	25	108	3.9	85	9.1	14.7	-15.86
6	Aminocarb	0.94	+	209.1	137.1	33	152	19	114	1.6	92	4.6	87.2	-5.39
7	Azoxystrobin	6.96	+	404.1	344.1	33	372.1	19	74	7.9	100	2.9	4.3	-18.95
8	Benalaxyl	7.72	+	326.2	148.1	29	294.1	15	102	3.7	85	6.4	20.6	-19.61
9	Benzoximate	8.1	+	364	105	31	199	11	104	7.8	92	6.9	15.5	15.07
10	Bifenazate	7.64	+	301.1	170.1	27	198.1	13	92	4.7	76	9.6	40.6	6.99
11	Bitertanol	7.35	+	338.2	70	29	269.2	13	ND	ND	109	7.3	86.4	-6.07
2	Bupirimate	7.13	+	317	108	35	159.1	33	105	15.4	76	7.1	34.9	-0.26
13	Buprofezin	8.28	+	306.2	116.2	23	201.1	17	108	9.5	114	11.4	30.8	8.94
14	Butafenacil	7.6	+	492.1	331	27	349	19	113	8.7	119	9.1	33.6	0.27
15	Carbendazim	1.64	+	192.2	132.1	41	160.2	25	92	12.5	86	19.1	8.5	-2.51
16	Carbetamide	5.02	+	237.1	118.1	17	192	13	107	12.9	88	9.1	71.1	10.64
17	Carbofuran	5.98	+	222.1	123	29	165.1	17	108	15.8	78	8.6	3.8	2.11
18	Carboxin	5.98	+	236.1	87	33	143	21	106	4.5	109	7.3	8.6	19.33
19	Chlorantraniliprole	6.47	+	484	285.9	17	452.9	21	115	9.3	106	8.8	79.3	3.33
20	Chlorotoluron	5.97	+	213.1	46.2	35	72.2	31	99	3.7	98	4.4	13.3	-13.01
21	Chloroxuron	6.99	+	291.1	72.4	47	218.1	33	102	5.6	81	11.7	22.9	10.07
22	Clethodim	8.27	+	360.1	164	29	268.1	17	ND	ND	75	2.5	31.3	2.69
23	Clothianidin	3.86	+	250	132	21	169	19	118	12.3	80	5.3	72.3	-16.73
24	Cycluron	6.24	+	199.1	89	15	69	21	103	7.8	83	16.2	112.8	7.79

*ND = not detected

*Ion ratio absolute value provided by software and calculated by considering reference value of standard

Table 2 (part 2). List of pesticides with MRM transitions used

Sr. No.	Name of pesticide	RT (min)	ESI (+/-)	Q1	Q3	CE	Q3	CE	0.005 mg/kg (n=6)		0.01 mg/kg (n=6)		Ion Ratio Absolute	Ion ratio (±30)
									% Rec	% RSD	% Rec	% RSD		
25	Cyproconazole	6.79	+	292	70	31	125	29	103	13.8	94	10.8	70.5	1.08
26	Cyromazine	0.66	+	167.1	85.1	26	125.1	24	108	11.2	106	5.8	53.5	-4.21
27	Desmedipham	6.73	+	318.1	154	35	182	19	93	8.7	94	15.5	9.2	27.98
28	Diclotophos	2.78	+	238.1	112.1	17	193	13	119	1.5	103	5.0	71.1	-8.73
29	Difenoconazole	8.01	+	406.1/ 408.2	251.1	41	253.1	31	ND	ND	102	2.8	40.4	-18.05
30	Dimethoate	4.15	+	230	125	29	199	13	99	4.5	89	6.3	61.0	6.21
31	Dimethomorph Isomer 1	6.38	+	388.1	165.1	45	301	29	94	7.1	79	15.2	51.7	7.02
32	Dimethomorph Isomer 2	6.61	+	388.1	165.1	45	301	29	103	19.7	76	14.4	48.5	-1.00
33	Dimoxystrobin	7.41	+	327.1	116	29	205	23	84	12.9	74	5.0	6.0	-17.49
34	Diniconazole	7.61	+	326.2	70.2	50	159	43	ND	ND	103	18.9	20.6	-28.53
35	Dinotefuran	1.07	+	203.1	129.2	17	157.2	11	86	15.7	96	8.7	68.8	-12.13
36	Dioxacarb	4.11	+	224.1	123	21	167	11	83	15.7	94	8.0	75.0	2.75
37	Emamectin-benzoate b1a	8.54	+	886.5	82.1	65	158.1	49	88	14.6	87	12.2	5.7	14.91
38	Epoxiconazole	7.1	+	330	101.1	65	121.1	49	81	12.3	98	12.8	35.5	-3.69
39	Eprinomectin	8.65	+	914.6	154.2	49	186.2	25	81	11.1	92	19.9	35.4	20.15
40	Etaconazole Isomer 1	7.17	+	328.1	159	31	205	23	102	5.7	106	8.2	5.6	3.46
41	Etaconazole Isomer 2	7.27	+	328.1	159	31	205	23	81	15.9	78	16.2	5.5	-8.17
42	Ethirimol	4.2	+	210.2	98.1	39	140.1	31	98	14.6	86	14.0	80.5	0.89
43	Etoxazole	8.9	+	360.1	57.2	51	141	59	100	11.6	74	19.5	5.5	1.64
44	Fenamidone	6.96	+	312.1	92	35	236.1	21	93	10.7	87	18.8	34.0	1.47
45	Fenazaquin	9.69	+	307.1	147	25	161.1	27	97	15.4	71	5.7	79.5	-19.72
46	Fenbuconazole	7.31	+	337	70	39	124.9	55	78	17.9	74	4.5	79.9	13.84
47	Fenpropimorph	7.23	+	304	117	65	147	39	78	15.7	80	5.9	84.9	19.87
48	Fenpyroximate	8.92	+	422	135.1	53	366.1	23	104	8.2	85	3.6	0.8	8.86
49	Fenuron	3.8	+	165.1	46	29	72.1	45	88	15.9	92	13.9	69.9	-12.51
50	Fipronil	7.7	-	435	250	35	330	20	74	16.6	74	14.1	22.5	-24.44
51	Fluazinam	8.63	-	462.7	397.8	24	415.7	26	ND	ND	72	13.5	50.1	1.21
52	Flubendiamide	7.63	+	683.1	274.1	41	408	9	100	19.8	75	16.4	28.7	-6.62
53	Fluometuron	5.95	+	233.1	46	37	72.1	35	112	8.5	102	8.8	13.1	-16.73
54	Fluoxastrobin	7.46	+	459.2	188	47	427.2	23	120	6.1	117	11.4	24.3	-21.97
55	Flusilazole	7.34	+	316.1	165.1	37	247.1	21	73	14.2	90	9.7	64.3	-13.78
56	Forchlorfenuron	6.05	+	248	93.1	49	129.1	25	73	7.5	76	11.7	18.4	13.74
57	Furalaxyl	6.76	+	302.1	95	39	242.1	21	110	10.5	104	10.8	81.0	-5.45
58	Furathiocarb	8.43	+	383.1	195.1	25	252.1	17	116	13.0	110	5.1	38.3	-15.36
59	Hexaconazole	7.44	+	314.1	70	55	159	33	73	6.7	119	6.5	91.3	6.44
60	Hexythiazox	8.67	+	353.1	168	37	228	19	ND	ND	83	14.0	52.3	4.20
61	Hydramethylnon	9.17	+	495.2	151.1	65	323.2	41	83	7.1	71	6.9	27.4	7.06
62	Imazalil	6.28	+	297	159	29	201	25	87	18.6	75	9.2	13.9	-2.00
63	Imidacloprid	4.09	+	256	175.1	25	209.1	21	106	16.4	97	2.5	74.7	13.99
64	Ipconazole	7.68	+	334.2	70	37	125	47	119	13.0	89	16.5	92.8	12.04
65	Isoproturon	6.14	+	207.2	46.1	35	72.1	29	87	8.5	85	13.6	11.6	4.94
66	Ivermectin	9.78	+	892.6	307.3	31	569.5	19	107	11.5	101	10.1	92.8	9.78
67	Mandipropamid	7.08	+	412.1	328.1	19	356.1	15	97	15.5	89	7.9	23.4	7.31
68	Mefenacet	7.06	+	299	120.1	35	148.1	21	85	7.3	73	7.2	81.9	9.71
69	Metalaxyl	6.02	+	280.1	192.2	25	220.2	19	104	5.6	95	9.9	39.7	-13.11
70	Methabenzthiazuron	5.98	+	222.1	150.3	45	165.2	21	99	4.3	80	2.7	20.0	21.31
71	Methamidophos	0.63	+	142	94	19	125	19	88	18.0	82	19.2	35.8	11.54
72	Methoprotryne	5.72	+	272.2	198	31	240.2	27	74	10.3	73	11.4	50.9	-5.00

*ND = not detected

*Ion ratio absolute value provided by software and calculated by considering reference value of standard

Table 2 (part 3). List of pesticides with MRM transitions used

Sr. No.	Name of pesticide	RT (min)	ESI (+/-)	Q1	Q3	CE	Q3	CE	0.005 mg/kg (n=6)		0.01 mg/kg (n=6)		Ion Ratio Absolute	Ion ratio (±30)
									% Rec	% RSD	% Rec	% RSD		
73	Methoxyfenozide	7.25	+	369.1	149.1	21	313.2	11	72	9.2	77	7.7	49.2	-13.15
74	Metribuzin	5.28	+	215.1	84.1	31	187.1	25	88	8.5	106	4.7	4.5	13.15
75	Mevinphos Isomer 1	3.91	+	225.1	127.1	21	193.2	11	109	15.3	97	11.3	25.7	-9.89
76	Mevinphos Isomer 2	4.55	+	225.1	127.1	21	193.2	11	90	17.1	84	8.9	28.6	-4.73
77	Mexacarbate	1.88	+	223.2	151	31	159.1	21	106	2.6	95	5.2	59.2	-6.91
78	(Monceren) Pencycuron	7.95	+	329.1	125	31	218.1	23	83	1.8	73	3.2	3.3	11.49
79	Monocrotophos	1.94	+	224.1	98	17	127.1	21	82	12.8	79	4.0	38.3	22.28
80	Monolinuron	6.07	+	215.1	99	47	126.1	23	ND	ND	74	18.8	32.9	11.01
81	Myclobutanil	6.99	+	289	70	41	125	39	109	15.4	117	2.4	93.6	20.61
82	Nitenpyram	2.22	+	271	126	35	225.2	17	97	12.6	97	10.3	58.1	-14.21
83	Omethoate	0.85	+	214	124.9	31	182.8	17	77	10.0	96	6.4	65.2	-1.86
84	Oxadixyl	5.26	+	279.1	132.1	43	219.1	15	92	9.1	87	5.5	33.2	18.47
85	Penconazole	7.57	+	284.1	70	37	159	35	100	15.2	75	6.5	41.8	-15.33
86	Phenmedipham	6.72	+	301.2	107.9	44	168	12	101	16.2	71	9.1	36.5	0.44
87	Picoxystrobin	7.62	+	368	145	29	205	13	108	6.4	99	7.1	67.7	-8.28
88	Piperonyl butoxide	8.37	+	356.2	119.1	47	177.2	13	97	4.6	110	6.3	7.5	-14.22
89	Pirimicarb	3.89	+	239.2	72.1	33	182.1	21	98	4.3	89	4.1	62.6	-5.18
90	Prochloraz	7.56	+	376	70	43	308	15	72	11.2	91	12.9	9.3	5.06
91	Prometon	5.32	+	226.1	86	39	142	33	87	7.4	82	8.1	82.2	17.06
92	Prometryne	6.41	+	242.2	158.1	33	200.1	25	80	12	73	6.2	59.5	0.91
93	Propiconazole	7.65	+	342.1	69	39	159	31	94	14.5	77	17.5	9.6	8.09
94	Pyracarbolid	5.78	+	218.1	97	37	125	25	73	8.7	72	5.9	8.3	-1.53
95	Pyraclostrobin	7.83	+	388	163	31	194	17	91	19.9	85	14.7	137.9	5.46
96	Pyridaben	9.23	+	365	147	33	309	19	74	11.7	73	8.4	75.1	15.50
97	Pyrimethanil	6.24	+	200	82	37	107	33	102	16.2	82	13.3	36.9	-0.19
98	Pyriproxyfen	8.57	+	322	96	21	185	31	76	9.6	74	10.9	18.3	-0.43
99	Quinoxifen	8.91	+	308.1	162.1	63	197.1	45	92	8.6	70	8.3	78.0	-9.86
100	Secbumeton	5.38	+	226.2	100	37	170.1	25	94	3.5	83	2.9	20.4	-13.40
101	Siduron	6.61	+	233.3	94	31	137.2	23	72	12.1	74	2.9	72.9	0.65
102	Simetryn	5.04	+	214	124	29	144	29	115	12.2	79	11.5	42.0	-11.16
103	Spinetoram	8.44	+	748.5	98.1	65	142.2	43	111	14.1	97	11.9	21.6	4.18
104	Spinosad (Spinosyn A)	7.85	+	732.5	98.1	65	142.2	39	77	12.1	84	15.6	15.5	-10.79
105	Spinosad (Spinosyn D)	8.24	+	746.8	98.3	65	142.4	41	105	13.4	97	9.1	32.3	28.50
106	Spirodiclofen	9.91	+	411.3	71.3	31	313.3	17	ND	ND	94	1.2	41.9	8.93
107	Spiromesifen	9.21	+	371.2	255.2	31	273.2	11	108	11.8	82	7.2	11.5	-17.41
108	Spirotetramat	6.8	+	374.2	302.2	27	330.2	23	104	10.4	115	2.7	145.2	-10.21
109	Spiroxamine 1	7.3	+	298.2	100.1	43	144.2	29	92	12.9	77	6.0	25.9	0.23
110	Spiroxamine 2	7.3	+	298.2	100.1	43	144.2	29	91	3.4	75	9.2	28.6	-8.69
111	Tebufenozide	7.59	+	353.2	133	23	297.2	11	95	6.5	107	16.8	73.9	-1.44
112	Tebufenpyrad	8.28	+	334	117	47	145	37	85	7.8	93	4.1	30.9	-25.80
113	Tebuthiuron	5.05	+	229.1	116.1	37	172.4	25	92	11.6	89	10.6	21.0	3.74
114	Terbumeton	5.38	+	226.1	100	41	170.1	23	87	3.2	82	3.8	8.7	5.31
115	Terbutryn	6.51	+	242.1	68.1	61	186.1	25	91	6.8	72	5.7	27.6	6.84
116	Tetraconazole	7.18	+	372.1	70	47	159	35	75	11.8	76	12.2	24.3	10.37
117	Thiabendazole	2.41	+	202.1	131.2	45	175.1	35	78	4.6	71	3.1	61.9	-10.93
118	Thiacloprid	5.03	+	253	99	59	126	29	77	10.0	92	14.0	5.6	-24.70
119	Thiamethoxam	2.86	+	292	181	31	211	17	83	19.2	96	11.2	16.4	-5.08
120	Thiophanate-methyl	5.5	+	343	151.1	31	311	17	98	16.5	96	12.1	5.7	26.42

*ND = not detected

*Ion ratio absolute value provided by software and calculated by considering reference value of standard

Table 2 (part 4). List of pesticides with MRM transitions used

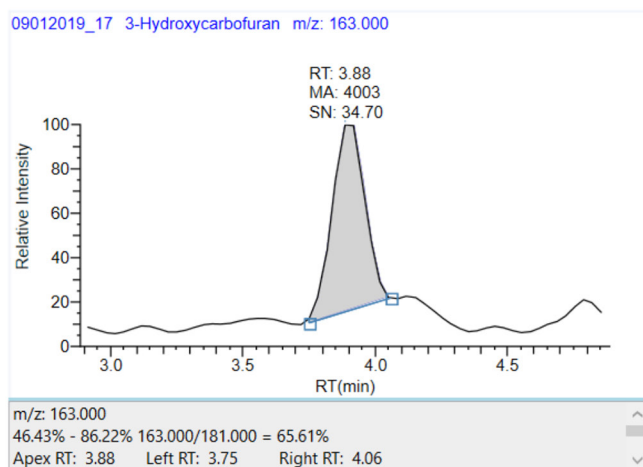
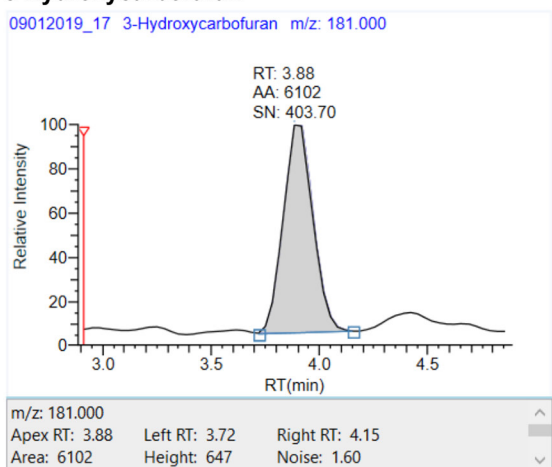
Sr. No.	Name of pesticide	RT (min)	ESI (+/-)	Q1	Q3	CE	Q3	CE	0.005 mg/kg (n=6)		0.01 mg/kg (n=6)		Ion Ratio Absolute	Ion ratio (±30)
									% Rec	% RSD	% Rec	% RSD		
121	Triadimefon	7.06	+	294	197.1	21	225	19	119	4.8	82	15.6	36.8	-8.26
122	Triadimenol	6.65	+	296.1	70	33	227.1	17	102	18.4	86	9.8	2.0	-4.63
123	Tricyclazole	5.03	+	190	136	39	163	33	97	19.7	72	14.1	70.8	13.32
124	Trifloxystrobin	8.2	+	409	186	21	206	19	84	16.0	81	7.8	29.4	13.41
125	Triflumizole	7.85	+	346.1	73	27	278.1	17	76	5.7	77	5.2	7.8	-26.68
126	Vamidothion	3.86	+	288	118	37	146	17	102	3.7	89	4.0	8.6	10.06
127	Zoxamide	7.93	+	336.1	159	55	187	29	111	13.1	77	4.4	14.9	-0.47

*ND = not detected

*Ion ratio absolute value provided by software and calculated by considering reference value of standard

Appendix II: supplemental information

3-Hydroxycarbofuran



Vamidothion

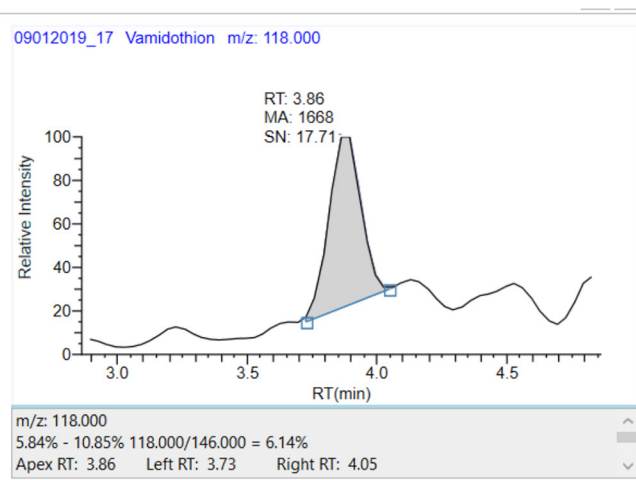
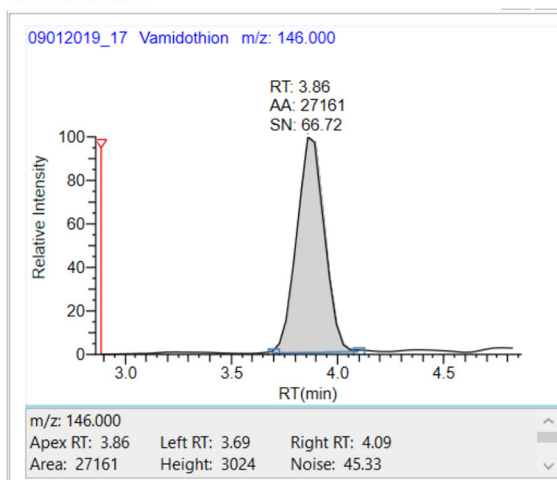


Figure 7 (part 1). Supplemental information for 3-hydroxycarbofuran and vamidothion

Prometon

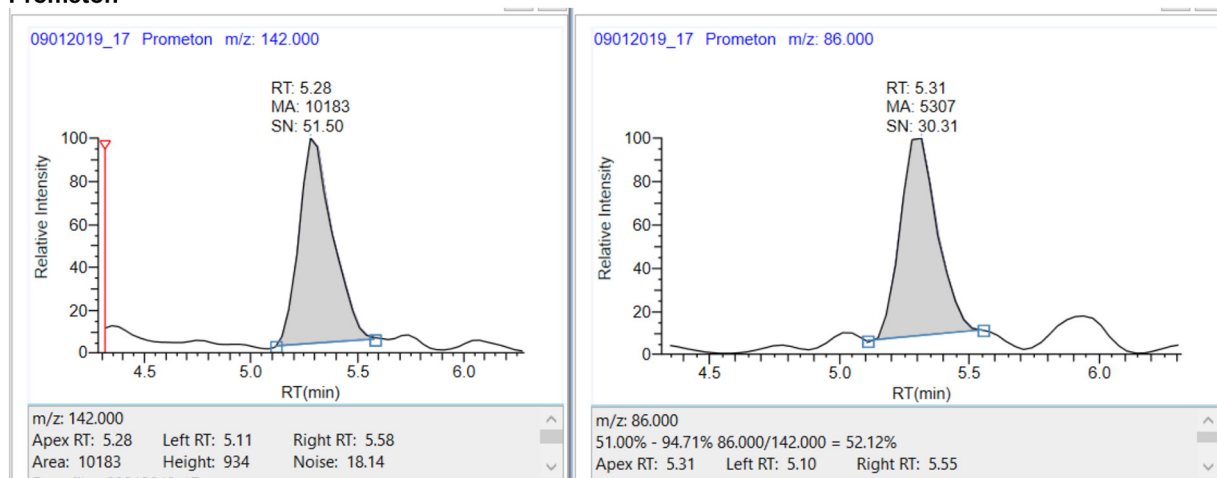


Figure 7 (part 2). Supplemental information for prometon

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