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Application News

GCMS-TQ™8050 NX

Residual Pesticides Analysis in Plant-Based Meat by GC-MS/MS

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User Benefits

- Simple sample preparation procedure for high sensitivity analysis
- Ready-to-use method with optimized MRM ion transitions from Shimadzu Smart Pesticides Database

■ Introduction

Plant-based meats are artificial meats that are created using plants as the main ingredient to look and taste like real meat. Plant-based meats are healthier and tend to have less environmental impact than real meats [1]. With more awareness of healthy lifestyles and climate change, the possibility of switching to a plant-based meat diet may increase in the near future.

Plant-based meats are made of ingredients from plants, such as grains, legumes, vegetable proteins and vegetable oils. Pesticides are frequently used in farms to control weed growth and insect infestation in plants. Residual pesticides remain in the plants will cause health issues when consumed [2][3]. As such, residual pesticides are a food safety concern in plant-based meats. Hence, the detection and quantitation of pesticides in plant-based meats are crucial to ensure the food is safe for consumption.

This study describes a triple quadrupole GC-MS/MS method coupled with Shimadzu Smart Pesticides Database™ Ver.2. for screening targeted pesticides in four different types of plant-based meats. QuEChERS is used for sample extraction and clean-up.

■ Experimental

Analysis condition

GCMS-TQ8050 NX with AOC-20i/s Plus (Shimadzu Corporation, Japan) was used in this work. GCMS-TQ8050 NX is equipped with a highly efficient detector and patented ion source technology for ultra trace analysis, which is suitable for this application.

Analytical conditions, MRM transition ions and collision energies (CEs) used were obtained from the Shimadzu Smart Pesticides Database Ver.2. The Smart Pesticides Database is a database that contains a list of 530 residual pesticides (including internal standards), supplied with retention indices which allows simple method creation for MRM or SIM mode analysis of residual pesticides in food.

Table 1: GC-MS/MS analytical conditions for analysis of residual pesticides in plant-based meat.

Instruments and Column information	
GC-MS/MS	GCMS-TQ8050 NX
Auto Injector	AOC-20i/s Plus
Column	SH-I-5Sil MS (P/N 221-75954-30) 30 m x 0.25 mm ID x 0.25 µm df
GC-MS/MS parameter	
GC-MS/MS Method No. 3 from Smart Pesticides Database Ver.2 (P/N 225-30434-92)	

Standard and sample preparation

All the targeted compounds were mixed and diluted with acetonitrile to a concentration of 1 mg/L. The standard was then diluted with matrix solution (solution collected after sample preparation) to concentrations of 0.5, 1, 2, 5, 10, 20, and 50 pg/µL for matrix-match calibration curves.

The sample preparation procedure is shown in Figure 1, according to EN 15662, with some modifications. An additional step of spiking the standard is required for recovery check after adding 10 mL of acetonitrile.

Two microliters of standard and sample solutions were injected to the GC-MS/MS for subsequent analysis.

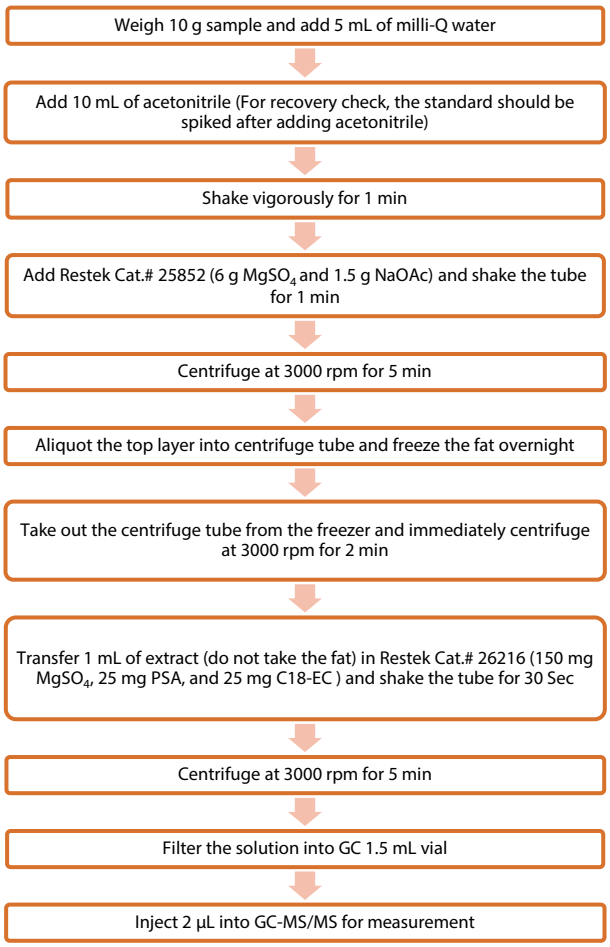

Figure 1: Workflow for sample preparation of plant-based meat

■ Results

Recovery

Four different plant-based meat samples (Sample A, B, C and D) were tested to determine whether QuEChERS sample preparation procedure was suitable. Recovery of area count of pre-spike over post-spike was calculated for each sample. Sample B could achieve area percentage recovery between 70 to 130 % for all the targeted compounds, while some pesticides in other samples were below 70% in recovery. This implies that modifications of sample preparation might be required for samples with different types of ingredients, for example, plant-based meats with much different fat content, sugar content, or additional coloring ingredients. In this experiment, the sample preparation in Figure 1, which used QuEChERS dSPE containing 150 $MgSO_4$, 25 mg PSA, and 25 mg C18-EC (meant for foodstuffs with fats and waxes [4]), is appropriate for plant-based meats which are of similar ingredients with Sample B. Accordingly, Sample B was used for the next stage of the study.

Table 2: Percentage recovery using area count for all the four samples at 5 pg/µL.

No.	Name	% Recovery using area count			
		Sample A	Sample B	Sample C	Sample D
1	Atrazine	98	98	97	109
2	Pyrimethanil	86	89	92	86
3	Terbacil	99	95	94	116
4	Tefluthrin	88	93	93	84
5	Vinclozolin	100	102	99	108
6	Transfluthrin	90	91	95	86
7	Anthraquinone	92	90	93	91
8	Chlorpyrifos	81	90	87	86
9	Triadimefon	105	100	106	107
10	Cyprodinil	84	91	91	69
11	Penconazole	99	97	99	97
12	Fipronil	125	114	128	109
13	Procymidone	99	110	105	96
14	Triflumizole	105	102	112	96
15	Paclobutrazol	114	105	114	111
16	Flutriafol	101	110	103	105
17	Fludioxonil	102	98	102	97
18	Profenofos	78	92	82	71
19	Bupirimate	97	115	107	91
20	Lenacil	97	96	92	101
21	Tebuconazole	101	99	104	97
22	Iprodione	69	78	68	82
23	Bifenthrin	72	80	77	71
24	Pyriproxyfen	82	83	85	73
25	Fenarimol	94	94	98	96
26	Permethrin-1	66	78	75	81
27	Permethrin-2	72	82	80	69
28	Cyfluthrin-1	87	102	103	84
29	Cyfluthrin-2	75	100	96	84
30	Cyfluthrin-3	79	98	94	81
31	Cyfluthrin-4	81	107	92	83
32	Cypermethrin-1	75	101	97	71
33	Cypermethrin-2	82	105	89	69
34	Cypermethrin-3	80	109	88	70
35	Flucythrinate-1	88	106	98	94
36	Cypermethrin-4	85	85	81	71
37	Etofenprox	78	79	77	71
38	Flucythrinate-2	87	108	98	89
39	Fluridone	104	107	104	115
40	Deltamethrin-1 (Tralomethrin deg.-1)	71	99	73	87
41	Deltamethrin-2 (Tralomethrin deg.-2)	76	79	70	77
42	Azoxystrobin	103	104	105	112

Note: Yellow indicates % recovery results which are outside 70-130% window.

Matrix effect

The magnitude of the matrix effect was carefully considered before setting the calibration curves. Nine pesticides were selected as representatives to investigate the matrix effect. It was found that at the same concentration, compounds in post-spike matrix solution (Sample B) had higher area count than compounds prepared in acetonitrile solvent. Sample B blank is the matrix blank solution after sample pretreatment. Figure 2 shows the overlay of some pesticides prepared in acetonitrile solvent, post-spike in Sample B and Sample B blank. This shows that higher area counts in the post-spike standard in Sample B was due to the matrix effect. The matrix effect is tabulated in Table 3. Based on the matrix effect result, it was concluded that matrix-match calibration curves were more suitable for plant-based meat analysis.

Table 3: Matrix effect of Sample B

Name	Area count of 10 pg/µL standard in acetonitrile	Area count of 10 pg/µL (post-spike in Sample B)	Matrix effect (%)
Atrazine	5358	30395	467
Pyrimethanil	16199	115598	614
Terbacil	3193	81488	2452
Tefluthrin	83801	333775	298
Vinclozolin	12265	38893	217
Fludioxonil	14138	241907	1611
Bupirimate	2977	73582	2372
Lenacil	9612	298175	3002
Tebuconazole	3439	109475	3083

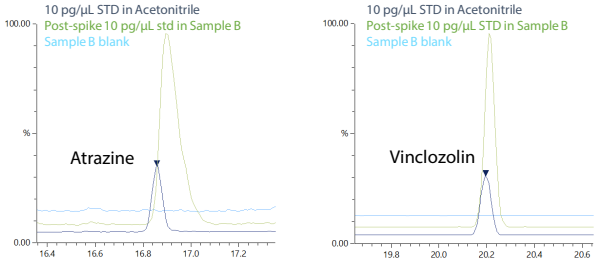

Figure 2: Overlay MRM chromatogram of pesticides at 10 pg/µL in acetonitrile solvent (dark blue), post-spike 10 pg/µL in Sample B (green) and Sample B blank (light blue).

Sensitivity, Repeatability and Linearity

Matrix-match calibration curves were plotted using Sample B matrix spiked with different concentrations of standard solution. As the pesticides displayed different sensitivity in the GC-MS/MS, the optimum calibration curve concentration range was selected for each compound. The limit of quantification (LOQ), which was set as the lowest level of a calibration curve, was determined based on the concentration at which the signal-to-noise ratio were greater than 10 and area repeatability (% RSD) were less than 15 %. About 60 % of the compounds had the lowest calibration level at 0.5 pg/µL, while 80% of compounds had at least 5-point calibration and good linearity (R^2>0.999). The calibration information for each compound is tabulated in Table 4.

Table 4: Calibration information for all the targeted compounds prepared in Sample B matrix.

No.	Name	LOQ (pg/µL)	Area %RSD (n=8)	R^2
1	Atrazine	0.5	4.5	0.99993
2	Pyrimethanil	0.5	3.0	0.99994
3	Terbacil	0.5	5.6	0.99991
4	Tefluthrin	0.5	3.0	0.99997
5	Vinclozolin	1	4.3	0.99996
6	Transfluthrin	0.5	10.4	0.99989
7	Anthraquinone	0.5	2.7	0.99996
8	Chlorpyrifos	0.5	3.8	0.99987
9	Triadimefon	0.5	7.1	0.99996
10	Cyprodinil	0.5	7.0	0.99996
11	Penconazole	0.5	3.9	0.99994
12	Fipronil	0.5	6.6	0.99993
13	Procymidone	0.5	8.5	0.99996
14	Triflumizole	0.5	4.8	0.99991
15	Paclobutrazol	2	8.7	0.99993
16	Flutriafol	0.5	5.2	0.99994
17	Fludioxonil	0.5	5.6	0.99996
18	Profenofos	0.5	9.8	0.99993
19	Bupirimate	0.5	4.7	0.99993
20	Lenacil	1	2.6	0.99999
21	Tebuconazole	0.5	3.7	0.99997
22	Iprodione	5	7.1	1.00000
23	Bifenthrin	0.5	3.1	0.99999
24	Pyriproxyfen	0.5	5.6	0.99995
25	Fenarimol	0.5	6.9	0.99996
26	Permethrin-1	0.5	2.9	0.99997
27	Permethrin-2	1	4.7	0.99997
28	Cyfluthrin-1	2	8.2	0.99994
29	Cyfluthrin-2	5	1.8	0.99999
30	Cyfluthrin-3	5	4.6	0.99999
31	Cyfluthrin-4	2	5.7	0.99984
32	Cypermethrin-1	2	9.6	0.99980
33	Cypermethrin-2	5	4.7	1.00000
34	Cypermethrin-3	10	5.6	1.00000
35	Flucythrinate-1	0.5	6.5	0.99982
36	Cypermethrin-4	10	4.2	1.00000
37	Etofenprox	0.5	6.2	0.99999
38	Flucythrinate-2	0.5	6.0	0.99980
39	Fluridone	1	2.7	0.99989
40	Deltamethrin-1 (Tralomethrin deg.-1)	10	11.5	1.00000
41	Deltamethrin-2 (Tralomethrin deg.-2)	2	9.4	0.99988
42	Azoxystrobin	0.5	10.1	0.99993

Note: Green indicates pesticide compounds with LOQ of 0.5 pg/µL

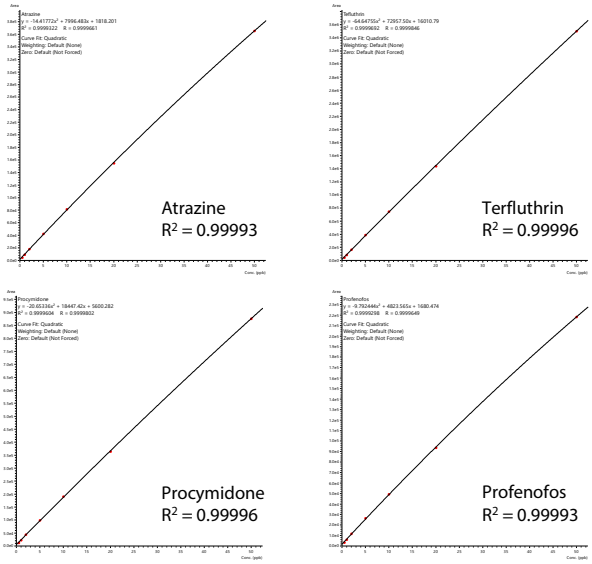

Figure 3: Calibration curves of pesticides prepared in Sample B matrix.

■ Conclusion

Method development for pesticide residue analysis in plant-based meat was carried out using GCMS-TQ8050 NX. QuEChERS dSPE, containing 150 $MgSO_4$, 25 mg PSA, 25 mg C18-EC, was used as a sample cleanup method which resulted in different recovery results for different plant-based meat samples. This suggests that modification of cleanup reagents could be required for different types of samples. The matrix effect was noticeable when comparing spiked pesticides in the matrix to those in acetonitrile; hence, matrix-match calibration curves were recommended for quantitation. More than half of the targeted compounds had LOQ of 0.5 pg/µL, while about 80% of the compounds had at least 5-point calibration curves with R^2 values greater than 0.999.

■ References

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