Low Part-per-Billion Level Pesticides Screening in Traditional Chinese Medicine Using the Agilent 7000A GC/MS/MS

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Application Brief

The control of pesticide residue levels is an increasing global concern. Several international organizations and governments have established maximum residue limits (MRLs) for a growing list of pesticides within an ever-widening scope of commodities. The primary task for pesticide residue monitoring laboratories is to develop analytical methodologies to screen for a large number of analytes at trace levels within a limited time frame. Gas chromatography coupled with mass spectrometry (GC/MS) has been adopted as the standard instrumentation for this purpose. As regulations in Japan and the European Union require lower MRLs for pesticide residues, the latest challenge has been to reach part-per-billion level concentrations for hundreds of pesticides in complex matrices, which in turn has required greater sensitivity and efficiency in pesticide screening. The Triple Quadrupole mass spectrometer, when used in Multiple Reaction Monitoring (MRM) mode, can dramatically remove matrix interferences and significantly increase the effective signal-to-noise ratio (S/N). This paper describes the use of gas chromatography coupled with triple quadrupole mass spectrometry (GC/MS/MS) to screen pesticides in Traditional Chinese Medicine (TCM) at levels as low as 1 ppb. The target pesticides and associated MRM conditions are listed in Table 1.

Highlights

- Consistent ion ratios over a wide linearity range and peak area repeatability at ultra-low concentrations in matrix make pesticide screening more reliable.
- The Agilent collision cell allows for excellent repeatability at 2 ppb level in matrix using just 5 ms dwell times. A single time segment can accommodate more MRM transitions for greater productivity.
- Reduction of matrix interference using MRM mode substantially decreases the detection limits that are required in pesticide screening.

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Table 1. Target Pesticides List and MRM Conditions

Segment	Compound name	R.T.	Quant	Collision energy	Qual1	Collision energy	Qual2	Collision energy
1	Dichlorvos	6.00	185 → 93	15	185 → 109	15	109 → 79	5
1	Methamidophos	6.10	141 → 95	5	95 → 80	5		
1	Acephate	7.82	136 → 94	10	142 → 96	5		
1	Dimethoate	8.53	125 → 79	5	125 → 93	15		
1	Omethoate	9.53	156 → 110	5	156 → 79	20		
2	Dimethipin	11.12	118 → 90	5	118 → 73	5		
2	Cyanophos	11.41	243 → 109	10	243 → 116	5		
3	Phosfamidon-E	12.41	264 → 127	15	127 → 109	10		
3	Parathion-methyl	12.58	263 → 109	10				
3	Phosfamidon-Z	13.13	264 → 127	15	127 → 109	10		
3	Quinoclamine	13.23	207 → 172	15	207 → 179	12	172 → 128	10
3	Chlorpyriphos	13.52	314 → 258	15	314 → 286	5	197 → 169	15
3	Cyanazin	13.52	225 → 189	15	240 → 225	5	198 → 91	10
3	Parathion	13.54	291 → 109	10	291 → 81	10		
3	Fosthiazate 1-2	13.85	195 → 103	5	195 → 139	5		
4	Fipronil	14.33	367 → 225	25	367 → 224	20		
4	Quinalphos	14.36	146 → 118	10	157 → 129	15		
4	Endosulfan-alpha	14.83	241 → 206	15	229 → 194	10	195 → 159	10
4	Chlorfenapyr	15.77	247 → 227	15	247 → 197	20	408 → 59	10
4	Endosulfan-beta	15.90	241 → 206	15	229 → 194	10	195 → 159	10
5	Triazophos	16.38	161 → 134	5	161 → 106	10	257 → 162	5
5	Bromopropylate	17.64	341 → 183	20	341 → 185	20		
5	Azinphos-methyl	18.31	160 → 77	20	160 → 132	5	132 → 77	15
6	Cafenstrole	19.92	100 → 72	5	188 → 119	25	188 → 82	20
6	Cyfluthrin 1-4	20.03	163 → 127	5	163 → 91	15	206 → 151	25
7	Flucythrinate 1	20.46	199 → 157	5	199 → 107	30	157 → 107	15
7	Flucythrinate 2	20.64	199 → 157	5	199 → 107	30	157 → 107	15
8	Fenvalerate 1	21.14	167 → 125	10	225 → 119	15		
8	Fenvalerate 2	21.33	167 → 125	10	225 → 119	15		
8	Difenoconazole 1	21.53	323 → 265	15	265 → 202	20		
8	Difenoconazole 2	21.60	323 → 265	15	265 → 202	20		
8	Delthamethrin	21.85	181 → 152	30	253 → 172	5	253 → 93	20

Experimental

Quantitation of trace level compounds is complicated by matrix, resulting in qualifier ion ratios out of range, or target ions buried in the complex background. With single quadrupole mass spectrometry, selected ion monitoring (SIM) is often used to improve the detection limit and quantitative reproducibility. In SIM mode, the MS monitors only a few ions for each target compound within the retention time (RT) range that the target elutes from the column. By monitoring only a few specific ions, the signal-to-noise ratio (S/N) improves dramatically. SIM may not work well for trace levels in matrix as the interferences in SIM are the same as scan. Triple quadrupole mass spectrometry, through further fragmentation in a hexapole collision cell of a selected precursor ion, allows for drastic reduction or elimination of matrix interference. This process, referred to as Multiple Reaction Monitoring or MRM, is based on acquisition of highly selective precursor to product ion transitions that are

not likely to result from fragmentation of matrix components. Precursor selectivity is the same as in SIM but there is a high probability that at least one of the resultant product ions will be a unique dissociation product of the precursor and not the interference.

The primary benefit is the improvement of S/N at ultra-low analyte levels with consistent qualifier ion ratios over a wide concentration range, even in the most complex matrices. Figure 1 illustrates the Agilent GC/MS/MS workstation-Quantitative view. The numbers inside the red frame are the qualifier ion ratios of cyanophos over the range from 0.1 ppb to approximately 1,000 ppb in TCM matrix, showing very good accuracy in matrix. The numbers inside the green frame indicate the accuracy of the calibration curve. The enlarged view of the low end of the calibration curve is shown. Table 2 is the repeatability of the peak area of 1 ppb pesticides in TCM matrix with six parallel injections.

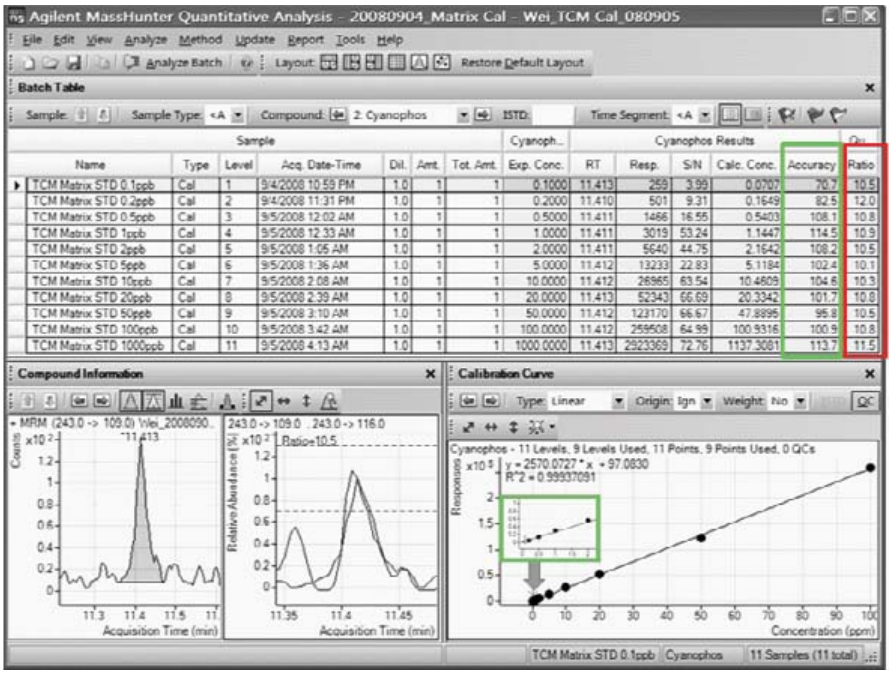

Figure 1. Calibration curve and qualifier ion ratio of cyanophos in TCM matrix.

Table 2. Peak Area Repeatability of Spiked 1 ppb Pesticides in TCM Matrix

	RSD of peak area (%)(n = 6)
Cyanophos	4.83
Bromopropylate	5.12

The Agilent hexapole collision cell, with its linear acceleration design, is optimized for high-speed performance without ion ghosting or cross-talk. High-speed MRM capability at 500 MRM/sec maximizes the number of allowable transitions as well as minimizes the dwell time for each transition in one time segment so that more compounds can be screened per run. The dwell time experiment described in Table 3 was performed using TCM matrix spiked with 2 ppb bromopropylate. As dwell time is decreased, the RSD for replicate run peak areas remains at < 5.0% until 5 ms is reached. The results at 2 ms are less precise; however, the results are acceptable for 2 ppb analysis in complex matrix.

Table 3. Dwell Time Experiment Results: 2 ppb Bromopropylate in TCM Matrix

	100 ms	50 ms	10 ms	5 ms	2 ms
Area 1	5849	5910	6265	6704	6747
Area 2	5712	6167	6189	6728	6279
Area 3	5895	5941	5966	6131	6523
Area 4	5921	6471	6551	6243	6397
Area 5	5999	6299	6119	6504	4831
Area 6	5999	6524	6415	6796	4737
Sum	35375	37312	37505	39106	35514
Avg	5895.83333	6218.667	6250.833	6517.667	5919
Std	107.618617	260.1528	209.5638	276.2496	893.2359
RSD	1.83	4.18	3.35	4.24	15.1

Figure 2 is the total ion chromatogram of the TCM extract in MRM mode by GC/MS/MS with injection volume of 1 µL. Eleven pesticides are identified in ppb level as shown in Table 4.

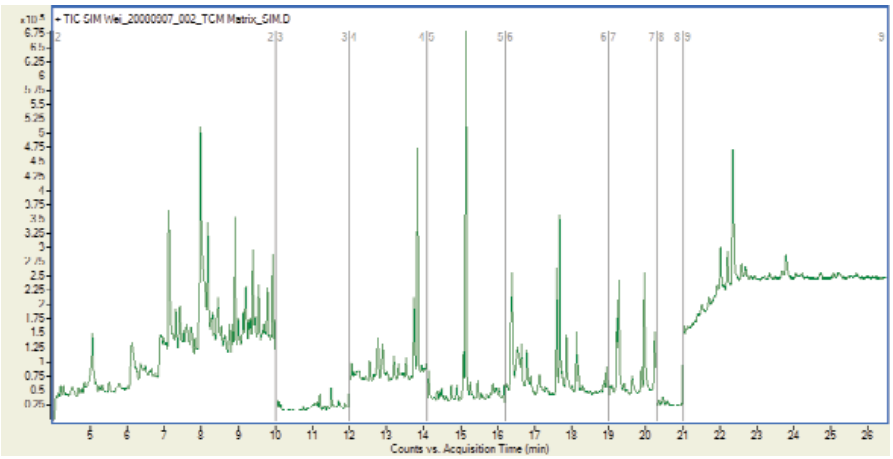

Figure 2. Total ion chromatogram of the TCM extract in MRM mode by GC/MS/MS.

Table 4. Screening Result of TCM Matrix Blank by GC/MS/MS

RT	Name	Quant transition	Matrix areas (not spiked)	Cal result
5.995	Dichlorvos	185.0 → 93.0	68	—
6.100	Methamidophos	141.0 → 95.0	0	—
7.760	Acephate	136.0 → 94.0	0	—
8.526	Dimethoate	125.0 → 79.0	0	—
9.478	Omethoate	156.0 → 110.0	0	—
11.100	Dimethipin	118.0 → 90.0	0	—
11.414	Cyanophos	243.0 → 109.0	0	—
12.390	Phosfamidon-E	264.0 → 127.0	0	—
12.560	Parathion-methyl	263.0 → 109.0	1227	6.21*
13.200	Quinoclamine	207.0 → 172.0	38	—
13.500	Cyanazin	225.0 → 189.0	16	—
13.510	Chlorpyrifos	314.0 → 258.0	1224	1.99*
13.520	Parathion	291.0 → 109.0	3950	5.62*
13.840	Fosthiazate	195.0 → 103.0	0	—
14.320	Fipronil	367.0 → 255.0	0	—
14.340	Quinalphos	146.0 → 118.0	3970	1.11*
14.820	Endosulfan-a	241.0 → 206.0	156	1.89*
15.750	Chlorfenapyr	247.0 → 227.0	0	—
15.880	Endosulfan-b	241.0 → 206.0	208	1.61*
16.360	Triazophos	161.0 → 134.0	531	1.63*
17.620	Bromopropylate	341.0 → 183.0	63	—
18.290	Azinphos-methyl	160.0 → 77.0	0	—
19.900	Cafenstrole	100.0 → 72.0	1260	3.37*
20.026	Cyfluthrin	163.0 → 127.0	0	—
20.439	Flucytrinate-1	199.0 → 157.0	0	—
20.624	Flucytrinate-2	199.0 → 157.0	0	—
21.122	Fenvarelate-1	167.0 → 125.0	4188	1.79*
21.326	Fenvarelate-2	167.0 → 125.0	13070	1.79*
21.520	Difenoconazole-1	323.0 → 265.0	0	—
21.574	Difenoconazole-2	323.0 → 265.0	0	—
21.840	Delthamethrin	181.0 → 152.0	2078	3.53*

*Potential positive sample

Summary

Pesticide residue monitoring is required to develop a methodology to accomplish the screening for hundreds of target compounds in a very limited time and ultra low concentration levels. Gas chromatography coupled with triple quadrupole mass spectrometry can perform very selective MRM, which can dramatically remove chemical noise from matrix interferences and improve the S/N, as well as detection limit. This application develops a method to screen for ppb level pesticide residue in TCM. GC/MS/MS can really help to improve the capability of pesticide residues screening in ultra low level.

References

1. Wei Luan, and Zhixiu Xu, "Screening for 430 Pesticide Residues in Traditional Chinese Medicine Using GC/MS: From Sample Preparation to Report Generation in One Hour," Agilent Technologies publication 5989-9341EN

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