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Screening for 34 pesticides in hops using GC-MS/MS

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Introduction

From a consumer safety point-of-view, quantitation of the pesticide residues hops has begun to attract wide interest. There are several problems associated with analysis of pesticide residues in hops. First and foremost, there are very few regulatory guidelines established to define which pesticides to include or what the detection limits should be. In fact the US government’s 40 CFR Part 180 states individual tolerances must be established for miscellaneous commodities intentionally not included in any group including hops. Secondly the matrix is very complex with significant interferences. Finally, sample load is growing exponentially, so the chosen method must be quick and easy to perform. Trace level pesticide analysis in complex food matrices have been done for many years with similar challenges, thus many of the analytical protocols emerging for the hops matrix are based on these well-established techniques. Triple-quadrupole GC-MS/MS operated in MRM mode provides significant sensitivity and selectivity, but method development can be expensive and time consuming. This poster describes streamlined method development process for analysis of 34 pesticide residues in hops using a QuEChERS sample preparation method, followed by GC-MS/MS detection and quantitation. The pesticides are from 5 classes of compounds including organonitrogen, organophosphorus, organochlorines, carbamates, and synthetic pyrethroids.

Experimental

Compound List

For this study 34 pesticides were selected for analysis based on the types of pesticides that are commonly used in hop production. The list includes several different compound classes (Table 1).

Organonitrogen Compounds	Synthetic Pyrethroid Compounds	Organophosphorus Compounds
Bupirimate	Bifenthrin	Chlorpyrifos
Etofenprox	Permethrin	Diazinon
Etridiazole (Terrazole)	Cyfluthrin	Malathion
Fenarimol	Deltamethrin	Mevinphos (Phosdrin)
Flutriafol	Flucythrinate	Phosalone
MGK-264	Lambda-cyhalothrin	Pirimiphos methyl
Myclobutanil	Tefluthrin	**Carbamates and others**
Paclobutrazol	Transfluthrin	Metalaxyl
Penconazole	**Organochlorines compounds**	2-Phenylphenol
Tebuconazole (Folicur)	Dichlorvos (DDVP)	Vinclozolin
Terbuthylazine	Endosulfan sulfate	
Triadimefon	gamma-BHC (Lindane)	
Triadimenol (Baytan)	p,p’-DDT	

Table 1: Selected Pesticide Compound Classes Included Organonitrogens, Synthetic Pyrethroids, Organochlorines, Organophosphates, and Carbmates

Method Development

The most difficult part of any triple quadrupole method development process, is determination and optimization of the Multiple Reaction Monitoring (MRM) transitions and collision energies (CE). For this study, the Shimadzu Smart Pesticide Database was used as the foundation for creating the MRM analysis method. The Smart Pesticide Database includes up to six fully optimized MRM transitions and CEs for 479 pesticides and Retention Indices (RI) for accurately predicting compound retention times. The transitions and CEs in the database were optimized using the Shimadzu GCMS-TQ8040 triple quadrupole GC-MS/MS. Figure 1 shows a portion of the Smart Pesticide Database and the method, compound, and transition information.

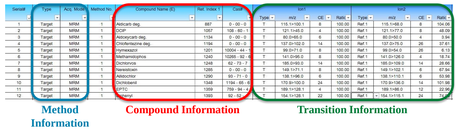


Figure 1: Example of Information Found in the Smart Pesticide Database Used to Create an MRM Analysis Method

A few of the target pesticides were not included in the Smart Pesticide Database. For these compounds, the MRM Optimization Tool was used to automatically determine the optimized MRM transitions and collision energies (CE). Once determined, the new transitions are added to the Smart Pesticide Database. Figure 2 shows the graphic results from the MRM Optimization Tool, with 6 transitions for two of the pesticides.

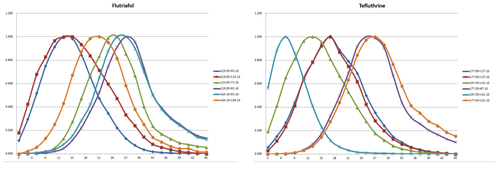


Figure 2: Optimized Transitions for Two Pesticides Using the MRM Optimization Tool

After adding the optimized transitions for the new pesticides to the existing Smart Pesticide Database, the MRM analysis method was created automatically. The program uses pesticide RIs in the database to accurately predict retention times for the target compounds. The Smart MRM function automatically adjusts Loop, Event, and Dwell times to optimize sensitivity for all compounds in the list simultaneously. Flexible MS events can create optimized methods with 400+ compounds. Used together, the Smart Pesticide Database and MRM Optimization Tool shortened the method development time from hours to just a few minutes.

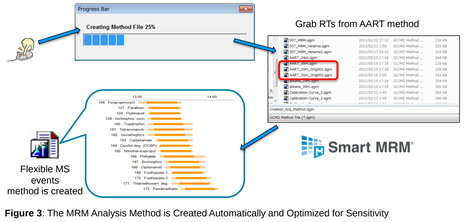


Figure 3: The MRM Analysis Method is Created Automatically and Optimized for Sensitivity

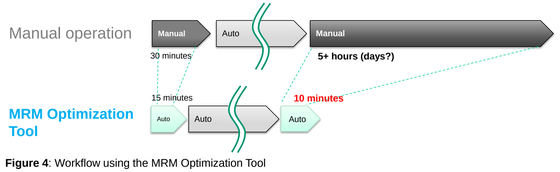


Figure 4: Workflow using the MRM Optimization Tool

Information used to create the analysis method is shown in Table 2. It includes a compound table, retention indices and retention times, one transition with optimized CE for quantitation, and two reference transitions. Area ratios are also empirically determined, and can be used as part of the laboratory QAQC program.

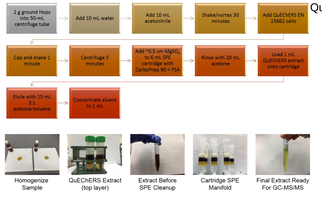
Serial#	Compound Name	Ret. Index I	Ret. Time	Ion1				Ion2				Ion3			
				Type	m/z	CE	Ratio	Type	m/z	CE	Ratio	Type	m/z	CE	Ratio
1	Dichlorvos	1252	4.345	T	109.00>79.00	7	100.00	Ref.1	185.00>93.10	13	44.15	Ref.2	219.95>185.00	5	10.19
2	Mevinphos	1427	5.642	T	127.05>109.00	11	100.00	Ref.1	192.05>127.00	13	47.84	Ref.2	127.05>95.00	15	35.24
3	Etridiazole	1439	5.891	T	210.95>183.00	11	100.00	Ref.1	182.90>140.00	15	96.56	Ref.2	210.95>140.00	23	91.67
4	2-Phenylphenol	1533	6.483	T	169.10>141.10	13	100.00	Ref.1	169.10>115.10	25	91.99	Ref.2	170.10>141.10	23	86.39
5	Lindane	1779	8.060	T	180.95>145.00	15	100.00	Ref.1	218.90>183.00	7	86.47	Ref.2	218.90>145.00	19	63.83
6	Terbuthylazine	1782	8.694	T	229.10>173.10	7	100.00	Ref.1	214.10>71.10	19	78.34	Ref.2	214.10>132.10	9	59.36
7	Diazinone	1790	8.766	T	304.10>179.20	13	100.00	Ref.1	248.05>152.10	7	61.75	Ref.2	248.05>137.10	17	61.34
8	Tefluthrine	1816	9.002	T	177.05>127.10	17	100.00	Ref.1	177.05>137.10	17	33.83	Ref.2	197.05>141.10	13	31.28
9	Vinclozoline	1894	9.730	T	212.00>172.00	15	100.00	Ref.1	212.00>145.00	23	80.05	Ref.2	285.00>212.00	15	71.13
10	Transfluthrin	1903	9.815	T	163.05>127.10	7	100.00	Ref.1	163.05>91.10	23	82.75	Ref.2	163.05>143.00	17	75.80
11	Metalaxyl	1915	9.926	T	234.10>146.10	19	100.00	Ref.1	234.10>174.10	11	75.22	Ref.2	249.15>190.20	9	64.50
12	Pirimiphos methyl	1940	10.167	T	290.10>125.10	23	100.00	Ref.1	290.10>233.10	13	53.89	Ref.2	276.05>125.00	17	54.23
13	Malathion	1964	10.377	T	127.05>99.10	7	100.00	Ref.1	173.10>99.10	13	66.84	Ref.2	173.10>127.10	7	64.75
14	Chlorpyrifos	1980	10.529	T	313.95>257.80	19	100.00	Ref.1	313.95>259.90	19	74.59	Ref.2	285.95>257.90	9	47.29
15	Triadimefon	2003	10.738	T	208.05>111.10	25	100.00	Ref.1	208.05>127.10	15	89.54	Ref.2	210.05>183.10	9	43.88
16	MGK-264	2030	10.980	T	164.10>93.10	13	100.00	Ref.1	164.10>98.10	13	68.56	Ref.2	164.10>80.10	25	55.15
17	Penconazole	2063	11.281	T	248.10>157.10	25	100.00	Ref.1	248.10>192.10	15	50.04	Ref.2	248.10>192.10	15	45.77
18	Triadimenol	2092	11.541	T	168.15>70.00	9	100.00	Ref.1	128.00>65.10	23	38.42	Ref.2	112.05>somo	11	27.60
19	Paclobutrazol	2132	11.899	T	236.05>125.10	11	100.00	Ref.1	236.05>167.10	9	37.10	Ref.2	238.05>127.10	11	32.42
20	Flutriafol	2155	12.104	T	123.05>95.10	13	100.00	Ref.1	219.05>123.10	15	83.60	Ref.2	123.05>75.10	25	53.38
21	Myclobutanil	2200	12.502	T	179.05>125.00	15	100.00	Ref.1	179.05>152.00	9	35.34	Ref.2	179.05>90.10	29	36.10
22	Bupirimate	2204	12.535	T	273.10>108.10	15	100.00	Ref.1	273.10>193.10	7	67.72	Ref.2	193.15>81.10	25	74.80
23	Endosulfan sulfate	2360	13.865	T	271.80>236.80	21	100.00	Ref.1	271.80>204.90	17	22.20	Ref.2	271.80>141.00	31	22.31
24	p,p’-DDT	2367	13.919	T	235.00>165.20	29	100.00	Ref.1	237.00>165.20	23	64.85	Ref.2	235.00>199.10	17	13.84
25	Tebuconazole	2399	14.184	T	250.10>125.10	19	100.00	Ref.1	250.10>70.10	9	40.63	Ref.2	252.10>127.10	23	35.30
26	Bifenthrin	2471	14.767	T	181.15>166.10	13	100.00	Ref.1	181.15>165.10	27	90.00	Ref.2	166.10>164.20	29	4.99
27	Phosalone	2536	15.432	T	182.05>115.00	15	100.00	Ref.1	182.05>75.10	27	53.37	Ref.2	182.05>138.00	9	30.67
28	lambda-Cyhalothrin	2597	15.748	T	197.05>141.10	11	100.00	Ref.1	208.10>181.10	7	97.01	Ref.2	197.05>161.10	7	54.32
29	Fenarimol	2631	16.001	T	251.00>139.00	15	100.00	Ref.1	251.00>111.10	29	42.14	Ref.2	330.05>139.10	9	34.45
30	Permethrin	2706	16.562	T	183.00>153.10	15	100.00	Ref.1	183.00>168.10	13	107.11	Ref.2	163.05>127.10	7	109.13
31	Cyfluthrin	2793	17.202	T	226.05>206.10	13	100.00	Ref.1	199.10>170.10	25	70.95	Ref.2	206.05>151.10	19	64.85
32	Etofenprox	2870	17.812	T	163.15>135.10	11	100.00	Ref.1	163.15>107.10	17	89.29	Ref.2	376.20>163.20	11	5.78
33	Flucythrinate	2876	17.860	T	199.10>157.10	9	100.00	Ref.1	199.10>107.10	23	94.17	Ref.2	225.10>133.10	19	18.37
34	Deltamethrin	3061	19.030	T	252.90>93.10	19	100.00	Ref.1	181.10>152.10	23	87.40	Ref.2	252.90>172.00	7	56.01

Table 2 Results of MRM Optimization Used to Create the MRM Method

Gas Chromatograph	GC-2010 Plus
Injection Port	250 °C 1 µL splitless injection, 1 minute sampling time
Column	SH-Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm film Helium carrier gas Constant Linear Velocity mode, 40.0 cm/second
Oven Program	85 °C (hold 1 minute) 25 °C/minute to 160 °C 10 °C/minute to 240 °C 10 °C/minute to 290 °C (hold 3 minutes)
Transfer Line	300 °C
Mass Spectrometer	**GCMS-TQ8040**
Acquisition Mode	MRM
Ion Source	230 °C Electron ionization mode, 70 eV
Collision Gas	Argon, 200 kPa
MRM Loop Time	Optimized with Smart MRM

Table 3 Optimized Instrument Conditions for Analysis of Pesticides in Hop Samples using the Shimadzu GCMS-TQ8040

Sample Preparation - QuEChERS



QuEChERS Extraction Steps Followed by Cartridge SPE Cleanup

Calibration

A 5-point calibration curve was generated for all 34 target pesticides, covering the range from 1 to 200 parts-per-billion (ppb) (Figure 5). Figure 6 shows the overlaid MRM chromatograms from three transitions for two of the pesticides in the 1-ppb calibration standard.

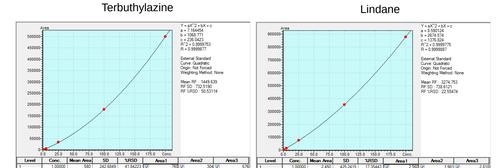


Figure 5 Exponential Calibration Curves for Two Pesticides, 1 to 200 ppb

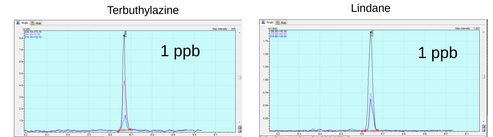


Figure 6 Example of Overlaid MRM Chromatograms For Two Pesticides in the 1-ppb Calibration Standard

Sample Repeatability

Two different hops samples were processed using the QuEChERS procedure. The extracts were spiked with the pesticide mix at 25 ppb and analyzed in triplicate using the optimized MRM method. Chromatograms in Figure 7 illustrate how the MRM technique can be used to select the target compound from a complex matrix background, and produce reliable, reproducible results at low concentrations.

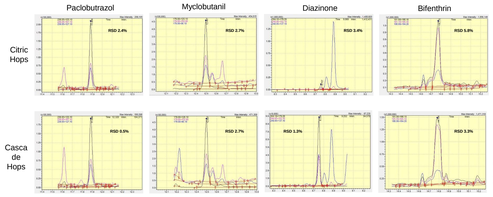


Figure 7 MRM Chromatograms of Two Hops Samples Spiked at 25 ppb and Analyzed in Triplicate

Summary and Conclusion

The data presented illustrate how a triple quadrupole GC-MS/MS operated in the MRM mode, can be used to analyze for trace-level pesticide residues in complex plant matrices such as hops. The matrix was extracted using a QuEChERS kit, and interferences removed using an SPE cartridge. The resulting extracts were analyzed in triplicate using MRM transitions provided in the Smart Pesticide Database or individually optimized using the MRM Optimization Tool, with repeatability of 6% or better. The MRM method was fully optimized in just a few minutes, target compounds were selectively identified against the co-eluting matrix interferences, and quantitated at the parts-per-billion range.

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