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Introduction

As pesticide use has increased so has the level of concern among environmentalists, regulators, and consumers.
Regulations regarding the maximum limit of pesticide residues that can be found in or on food (MRLs) have been established nearly worldwide, including countries in North America (United States and Canada), Europe (European Union), Asia (Japan), and Australia. In the United States, MRLs can range from 0.02ppm to 100ppm depending on the matrix and pesticide in question while the European Commission has a default value of 0.01ppm [1].

The analysis of pesticides in food can quickly become complex with increasing target compound and commodity lists and decreasing detection limit requirements. Having a robust method on an easy-to-use platform that integrates seamlessly to a large database is desired to facilitate this analysis. Coupling Agilent's 7000C Triple Quadrupole GC/MS to Agilent's newest GC platform (Intuvo 9000 Gas Chromatograph) delivers a streamlined workflow that involves the implementation an inert microfluidic retention gap (guard chip) for multiresidue pesticide analyses.

Calibration curves for various target pesticides in varied matrices showed excellent linearity for concentrations ranging from 5 ng/mL to 500 ng/mL (97% of the compounds analyzed maintained a R2 ≥ 0.990). Excellent response and peak shape consistency was obtained with the implementation of the Intuvo guard chip which protects downstream components and eliminates the need to trim the column after matrix evaluation. All analyzed pesticides obtained a %RSD of repeated measurements ≤30% with recovery errors ≤ 30%. With regular maintenance, including liner and Intuvo guard chip replacements, peak shape, response, and analyte calibration can be preserved for over 500 injections [2].

Results and Discussion

Meeting MRL Requirements

Agilent's Intuvo 9000 GC and the 7000C Series Triple Quadrupole GC/MS system can confirm and quantitate pesticide residues at the low ppb level in organic honey, jasmine rice, and loose-leaf black tea extracts that meet both the EU and the USDA MRL requirements.

Note that the commodities analyzed are more aligned with EU commodities. Generally EU MRLs are lower than those of the USDA.

Figure 4. DDT-o,p' at 5ppb in Honey: A) overlay of MRM transitions, and B) view of each transition separately.

Table 1. MRL requirements and results for Organic Honey

Compound	Results for Honey (ppb)		
	EU MRL	MDL	iLOQ
Aldrin	10	0.39	1.43
Atrazine	50	0.21	0.77
Alachlor	10	0.89	3.28
2-Phenylphenol	50	0.16	0.60
Chlordane	10	0.64	0.30
Chlorpyrifos-methyl	n/a	0.34	1.24
DDT (sum)	50	2.94	2.53
Heptachlor (sum)	10	0.45	1.65
Metalaxyl	50	0.43	1.59
Metolachlor	50	1.31	4.80
Myclobutanil	50	0.35	1.27
EPTC	20	0.04	0.15
Propyzamide	50	1.37	5.03
Propachlor	20	0.02	0.09
Simazine	10	0.29	1.06
Permethrin (sum)	n/a	0.21	2.92
Triadimefon	50	0.42	1.54

Figure 6. Lindane at 5ppb in Rice: A) overlay of MRM transitions, and B) view of each transition separately.

Table 3. MRL requirements and results for Jasmine Rice

Compound	Results for Rice (ppb)			
	EU MRL	USDA MRLs	MDL	iLOQ
Aldrin	10	20	0.29	1.07
Atrazine	50		0.31	1.16
Lindane	10	100	0.60	2.20
Alachlor	10		0.21	0.78
2-Phenylphenol	50		0.08	0.30
DDT	50	500	1.84	6.67
Endosulfan	50		0.17	1.66
Clomazone	10	20	0.51	1.88
Heptachlor	10	30	0.38	1.39
Metalaxyl	10	100	0.22	0.80
Metolachlor	50		0.56	2.07
Myclobutanil	20		0.15	0.56
EPTC	10		0.03	0.11
Propyzamide	10		0.49	1.81
Propachlor	20		0.03	0.10
Simazine	10		0.22	0.81
Permethrin	50		0.09	0.32
Triadimefon	10		0.20	0.75

Sample Preparation

Sample preparation is an essential part of successful chromatography. It extends column lifetime, reduces the need for repeated samples, and minimizes interferences that can jeopardize your separation, detection, and quantification. Many laboratories focused on pesticide residue analysis in food commodities routinely use the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [3,4]. This straightforward sample preparation allows for the analysis of hundreds of pesticides with a single extraction. However, sample prep shortcuts are becoming more common within the laboratory. Not only will this influence the data quality, but will also increase the frequency of GC/MS system maintenance.

Three diverse matrices were selected to demonstrate matrix differences. These commodities included organic honey, jasmine rice, and black loose-leaf tea. Each matrix was extracted with their specified QuEChERS methodology (Figure 1), in which specific dispersive SPE (dSPE) were used for matrix cleanup.
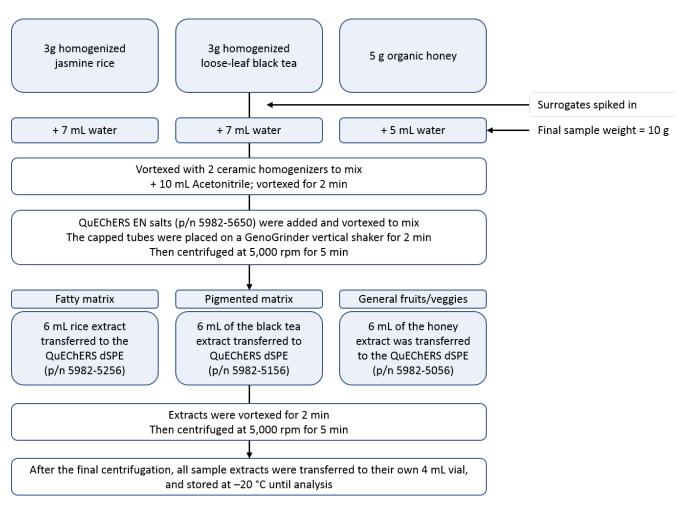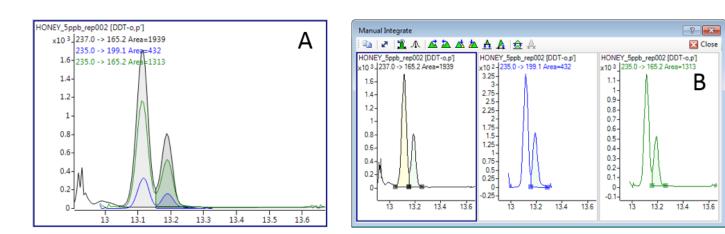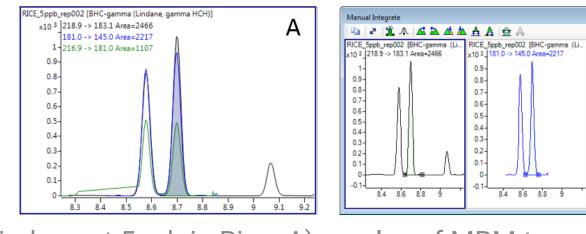

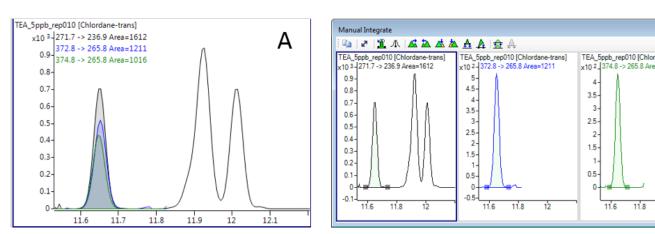Figure 1. Sample Prep Procedure

Figure 5. Trans-Chlordane at 5ppb in Tea: A) overlay of MRM transitions, and B) view of each transition separately.

Table 2. MRL requirements and results for Loose-leaf Black Tea

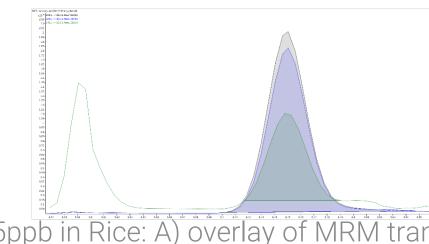| Compound	Results for Tea (ppb)		
	EU MRL	MDL	iLOQ
Aldrin	20	6.15	16.42
Atrazine	100	0.09	0.30
Alachlor	50	3.39	12.46
2-Phenylphenol	100	5.63	19.96
Chlordane	20	12.03	9.77
Chlorpyrifos-methyl	100	0.17	0.45
DDT (sum)	200	151.34	150.81
Heptachlor (sum)	20	0.16	0.34
Metalaxyl	50	1.93	7.10
Metolachlor	50	0.04	0.12
Myclobutanil	50	5.85	21.53
EPTC	50	1.17	3.74
Propyzamide	50	0.40	1.20
Propachlor	100	0.36	1.20
Simazine	50	0.10	0.30
Permethrin (sum)	100	4.49	13.24
Triadimefon	50	3.80	13.97

Figure 7. Lindane at 5ppb in Rice: A) overlay of MRM transitions, and B) view of each transition separately.

Table 4. Surrogate Recovery Data (n = 12 @ ~25 ppb)

Compound	Honey		Rice		Tea	
	%RSD	%Error	%RSD	%Error	%RSD	%Error
Aldrin	6.19	4.64	8.70	6.99	17.71	13.25
Atrazine	7.57	6.71	15.50	12.95	25.07	21.50
Lindane	4.92	4.1417	8.19	6.7100	13.89	11.74
Alachlor	3.23	2.58	16.12	13.03	25.23	18.13
2-Phenylphenol	5.26	4.78	7.78	6.19	11.16	9.37
Chlordane	5.58	4.76	13.60	11.50	23.68	19.15
Chlorpyrifos-methyl	4.40	3.12	12.68	10.59	27.24	23.84
DDT (avg of sum)	11.79	10.29	10.13	8.03	26.93	22.93
Endosulfan	11.72	10.44	12.41	10.60	25.70	22.39
Clomazone	5.65	4.52	10.32	8.50	22.16	17.33
Heptachlor (avg of sum)	6.18	5.29	10.89	8.65	26.27	23.78
Metalaxyl	3.54	3.00	16.64	14.04	25.28	17.52
Metolachlor	5.13	4.10	13.55	11.73	17.40	15.05
Myclobutanil	11.53	9.12	11.10	9.13	27.21	24.91
EPTC	8.70	7.12	10.16	9.09	12.70	10.42
Propyzamide	4.42	3.68	12.22	10.26	27.83	23.86
Propachlor	6.98	6.15	11.70	9.51	29.58	25.96
Simazine	6.77	4.82	11.78	9.81	27.06	22.48
Permethrin (avg of sum)	20.64	19.59	7.91	7.27	25.40	23.83
Triadimefon	5.10	4.14	17.52	15.60	22.39	18.19

Experimental

Instrumentation

All analyses were run on an Agilent Intuvo 9000 GC equipped with an Agilent 7693B Autosampler and an Agilent 7000C Triple Quadrupole GC/MS (Figure 2). The Intuvo 9000 inert flow path was configured with midcolumn backflush (p/n G4588-60721) with two Agilent J&W HP-5ms Ultra Inert Intuvo GC columns (15 m × 0.25 mm, 0.25 µm; p/n 19091S-431UI-INT). The Table below displays the GC/MS/MS method parameters. The Intuvo GC was also configured with a multimode inlet (MMI) equipped with a 4 mm ultra inert, splitless, single taper, glass wool liner (p/n 5190-2293), and an MMI guard chip (p/n G4587-60665).
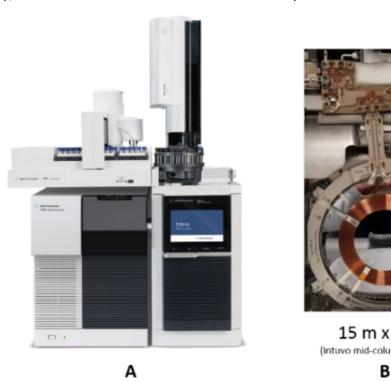

Figure 2. A) Agilent Intuvo 9000 GC equipped with an Agilent 7693B Autosampler and an Agilent 7000C Triple Quadrupole GC/MS and B) Intuvo Column Configuration for a Multiresidue Pesticides Workflow.

Agilent 9000 Intuvo GC	
Parameter	Value
Inert flow path configuration	Mid-column backflush
Syringe	10 µL (p/n G4513-80220); PTFE-tip plunger
Sandwich injection	Reversed 3-Layer Switch (L3,L1,L2)
	L1 (standard or sample) 0.5 µL
	L2 (ISTD) 0.5 µL
	L3 (matrix) 0.5 µL
Carrier gas	He
Inlet	MMI
Injection mode	Pulsed Splitless
Purge flow to split vent	30 mL/min at 1 minutes
Septum purge flow	3 mL/min
Gas saver	20 mL/min after 2 minutes
Intuvo Guard Chip	Track Oven
Columns	Agilent Intuvo HP5-MS UI (19091S-431UI-INT)
Column 1 flow	1.2 mL/min
Column 2 flow	1.4 mL/min
Oven temperature program	60 °C (hold 1 min)
	then 40 °C/min to 170 °C,
	then 10 °C/min to 310 °C (hold 3 min)
Midcolumn Backflush	
Timing	5 min duration during post-run
Oven temperature	310 °C
Aux EPC pressure	~30 psi
Inlet pressure	~2 psi

Agilent 7000C Triple Quadrupole GC/MS/MS	
Parameter	Value
Tune file	atunes.eiex.tune
Transfer line	280 °C
Source temperature	280 °C
Quad temperature	150 °C
Collison Cell Gas Flows	1.5 mL/min N2 & 2.25 mL/min He
Scan Type	dMRM
Electron Energy	70 eV
EM gain	10
MS1 & MS2 resolution	Wide
Quant/Qual transitions	P&EP Intuvo MRM Database
Right & Left RT Deltas	0.2 min
Dwell times	Optimized by dMRM
Min Dwell Time (ms)	10
Cycles Per Second	3.07

Conclusions

Agilent's Intuvo 9000 GC and the 7000C Series Triple Quadrupole GC/MS system can confirm and quantitate pesticide residues at the low ppb level in complex extracts that will meet both the EU and the USDA MRL requirements.

Calibration curves for targeted pesticides in organic honey, jasmine rice, and loose-leaf tea showed excellent linearity (97% of the compounds analyzed maintained a R2 ≥ 0.990) for concentrations ranging from 5 to 500 ppb. For all compounds analyzed in honey and rice the LOQs were found to be below 7 ppb. For 94% of the compounds analyzed in tea the LOQs were found to be below 100 ppb. All analyzed pesticides obtained a %RSD of repeated measurements ≤30% with recovery errors ≤ 30%.

Note that the commodities analyzed are more aligned with EU commodities. Generally EU MRLs are lower than those of the USDA.

References

1. Maximum Residue Limits Database. (2016, July 7). Retrieved from United States Department of Agriculture Foreign Agriculture Service: https://www.fas.usda.gov/maximum-residue-limits-mrl-database.
2. Veeneman, R. Multiresidue Pesticide Analysis with the Agilent Intuvo 9000 GC and Agilent 7000 Series Mass Spectrometer, Agilent Technologies Application Note, publication number 5991-7216EN, 2016.
3. Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. S. J. AOAC Int. 2003, 86, 412-431.
4. Lehotay, S. J.; Mastovská, K.; Lightfield, A. R. J. AOAC Int. 2005, 88, 615-629.