Application Note

Food Testing & Agriculture

Meeting European Union Maximum Residue Level Regulations for Pesticides in Tea and Honey

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Abstract

As pesticide use has increased, so has the level of concern among environmentalists, regulators, and consumers. Growing demand has increased the use of pesticides and expanded poor agricultural practices, elevating risks in the food supply and the environment. Regulating bodies around the globe have established regulations regarding the maximum residue levels (MRLs) of pesticide that can be found in or on food. In the United States, MRLs can range from 0.02 to 100 ppm depending on the matrix and pesticide in question, while the European Commission has a default value of 0.01 ppm[1]. The complexity of pesticide analysis, the low quantitation limits, and MRL ranges drive the need for a multiresidue quantification method with a reasonable linear range and low limits of detection. For this reason, tandem mass spectrometry (MS/MS) is used for screening, confirming, and quantifying low-level pesticides. It not only provides low limits of quantitation, but also allows for higher selectivity to minimize matrix interferences[2]. This Application Note evaluates multiresidue pesticide analysis for loose-leaf black tea and organic honey on the Agilent Intuvo 9000 gas chromatograph (GC) and an Agilent 7000C triple quadrupole gas chromatography/mass spectrometry (GC/MS) system. Calibration curves for targeted pesticides showed excellent linearity (97 % of compounds maintained a R^2 ≥0.990) for concentrations ranging from 5 to 500 ppb. For all compounds analyzed in honey, the limits of quantitation (LOQs) were found to be below 7 ppb where MRLs are between 10 to 50 ppb. For 94 % of the compounds analyzed in tea, the LOQs were found to be below 100 ppb where MRLs are between 20 to 200 ppb. All analyzed pesticides obtained a %RSD of repeated measurements of ≤30 % with recovery errors under 30 %.

Introduction

With the introduction of the Intuvo 9000 GC, an easy-to-use gas chromatographic platform is now available. The Intuvo features a modular flowpath and Guard Chip designed to protect downstream components from contamination and eliminate the need to trim the column. This can greatly simplify the maintenance model for many laboratories and decrease the cost per analysis. However, this only partially addresses the challenges laboratories face today. Challenges still arise in instrument configuration and method development. A complete workflow that provides a default screening method with an optimal configuration for pesticides in food is required to meet the current and future needs of food analysis laboratories worldwide.

The Agilent Pesticides Workflow Kit for Intuvo GC/TQ (G9233AA) is a comprehensive tool that guides a user through creating new or modifying existing multiresidue pesticide analyses on an Intuvo-GC/TQ system. This kit was used for this analysis.

Experimental

Sample preparation

While using multiple reaction monitoring (MRM) can reduce matrix interferences in the chromatogram, it does not remove the matrix from the sample. Injecting matrix can result in the loss of signal and tailing[3]. This means that, to analyze pesticide residues in foods, some level of sample preparation must be performed. At a minimum, the sample must be homogenized and extracted into a solvent suitable for chromatography. The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction method is commonly used for pesticide extraction, as it involves a single acetonitrile extraction and simultaneous salting out with magnesium sulfate.

Sometimes, additional cleanup is performed with dispersive solid phase extraction (dSPE)[4]. Organic honey (high sugar) and black loose-leaf tea (complex) were each extracted with their specified QuEChERS methodology in which various dSPE techniques were used for matrix cleanup (Figure 1).

Instrumentation

All analyses were run on an Intuvo 9000 GC equipped with an Agilent 7693B autosampler and a 7000C triple quadrupole GC/MS (Figure 2). The Intuvo 9000 inert flowpath 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).

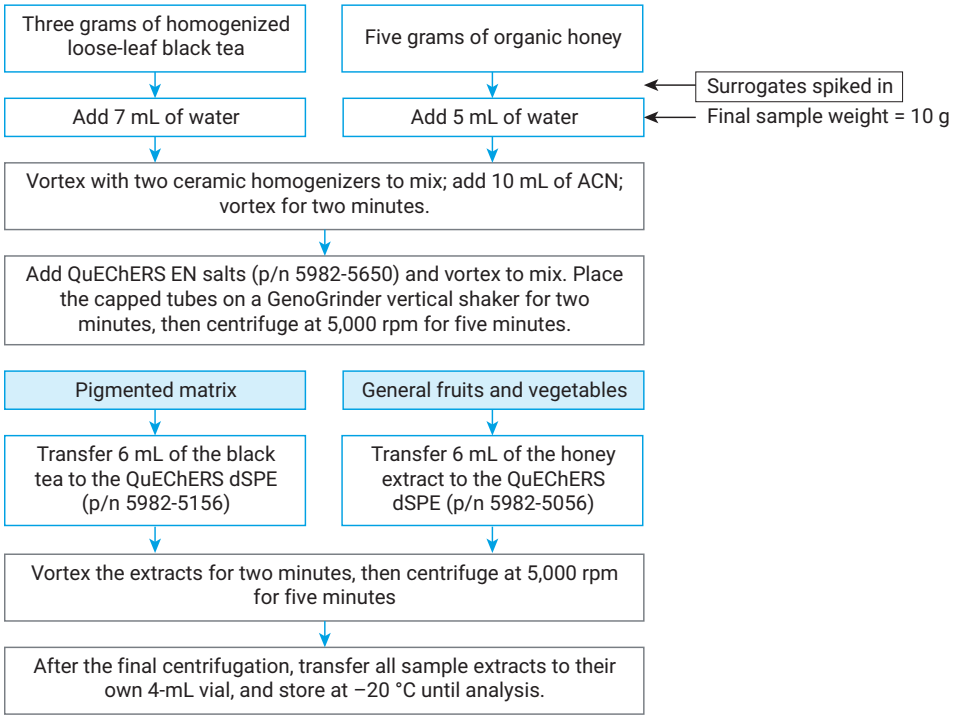

Figure 1. QuEChERS sample preparation method.

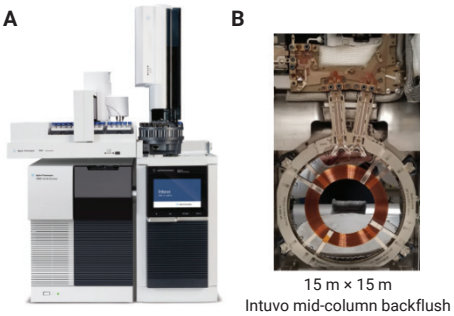

Figure 2. A) An Intuvo 9000 GC equipped with a 7693B autosampler and a 7000C triple quadrupole GC/MS and B) Intuvo column configuration for a multiresidue pesticides workflow.

Table 1 displays the GC/MS/MS method parameters. The Intuvo was also configured with a multimode inlet (MMI) equipped with a 4 mm Agilent Ultra Inert, splitless, single taper, glass wool liner (p/n 5190-2293), and an MMI Guard Chip (p/n G4587-60665).

Table 1. GC/MS/MS method parameters.

Parameter	Value
Intuvo 9000 GC	
Inert flowpath configuration	Midcolumn backflush
Syringe	10 µL (p/n G4513-80220); PTFE-tip plunger
Solvent washes	Pre-injection
	3× solvent A, acetonitrile (3 µL)
	3× solvent B, acetonitrile (3 µL)
	Post injection
	3× solvent A, acetonitrile (3 µL)
	3× solvent B, acetonitrile (3 µL)
Sample washes	0
Sample pumps	1
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 minute
Septum purge flow	3 mL/min
Gas saver	20 mL/min after 2 minutes
Intuvo guard chip	Track oven
Columns	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 minute),
	then 40 °C/min to 170 °C,
	then 10 °C/min to 310 °C (hold 3 minutes)
Midcolumn backflush	
Timing	5 minutes duration during post run
Oven temperature	310 °C
AUX EPC pressure	~30 psi
Inlet pressure	~2 psi
7000C triple quadrupole GC/MS/MS	
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 N_2 and 2.25 mL/min He
Scan type	dMRM
Electron energy	70 eV
EM gain	10
MS1 and MS2 resolution	Wide
Quant/Qual transitions	P&EP Intuvo MRM database
Right and left RT deltas	0.2 minutes
Dwell times	Optimized by dMRM
Minimum dwell time (ms)	10
Cycles per second	3.07

Results and discussion

The Intuvo 9000 GC and the 7000C triple quadrupole GC/MS system can confirm and quantitate pesticide residues at the low ppb level in loose-leaf black tea and organic honey extracts in keeping with EU MRL requirements. Tables 2 and 3 show the data for selected target compounds.

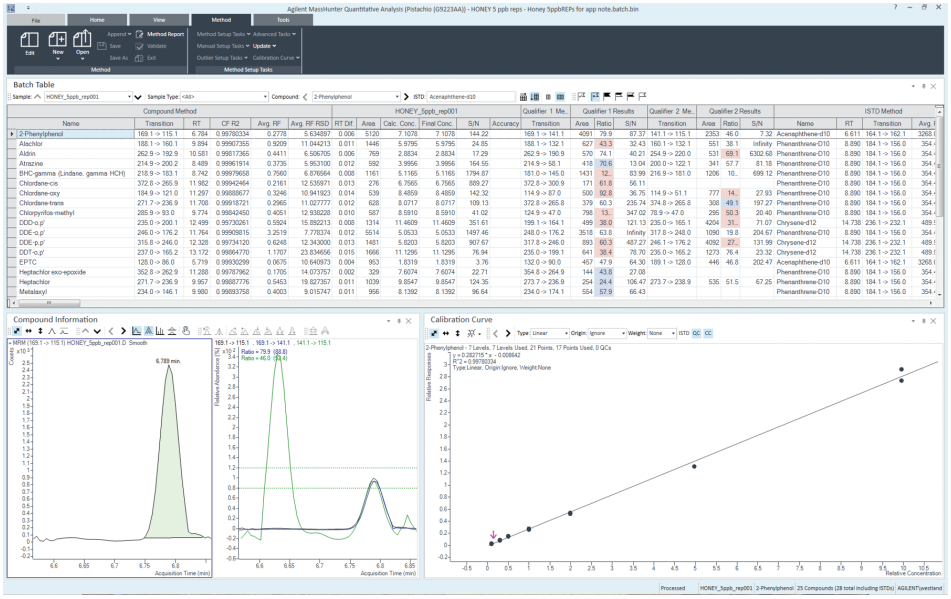

Figure 3. Display of Quant-My-Way Flavor Pistachio (G9223AA); provided with the Pesticides Workflow Kit for Intuvo/TQ.

Table 2. Data results for loose-leaf black tea.

Compound	Results for tea		
	EU MRLs (ppb)	MDL (ppb)	iLOQ (ppb)
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	200	151.34	150.81
Heptachlor	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	100	4.49	13.24
Triadimefon	50	3.80	13.97

Table 3. Data results for organic honey.

Compound	Results for honey		
	EU MRLs (ppb)	MDL (ppb)	iLOQ (ppb)
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	50	2.94	2.53
Heptachlor	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	n/a	0.21	2.92
Triadimefon	50	0.42	1.54

Conclusion

The Pesticides Workflow Kit for Intuvo/TQ (G9233AA) is a comprehensive tool that guides a user through creating new or modifying existing multiresidue pesticide analyses on an Intuvo GC/TQ system. This kit was used for this analysis to run multiresidue pesticide analyses to meet EU MRLs. Calibration curves for targeted pesticides in both loose-leaf tea and organic honey showed excellent linearity (97 % of compounds maintained a R^2 ≥0.990) for concentrations ranging from 5 to 500 ppb. For all compounds analyzed in honey, the LOQs were below 7 ppb. For 94 % of the compounds analyzed in tea, the LOQs were below 100 ppb. All analyzed pesticides obtained a %RSD of repeated measurements of ≤30 % with recovery errors under 30 %. The Intuvo 9000 GC and the 7000C triple quadrupole GC/MS can confirm and quantitate pesticide residues at the low ppb level in complex extracts, and so meet EU MRL requirements.

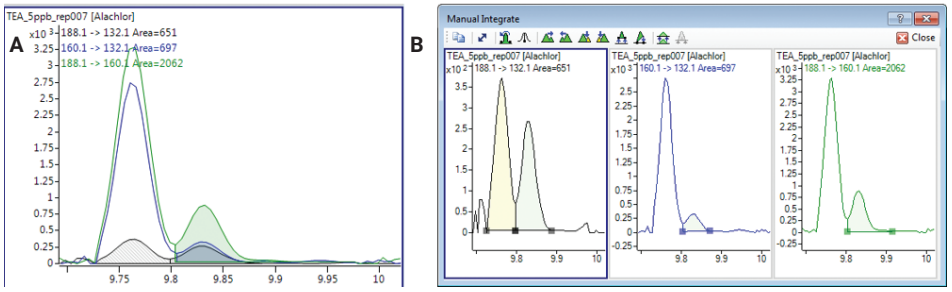

Figure 4. Alachlor at 5 ppb in Tea: A) overlay of MRM transitions, and B) view of each transition separately.

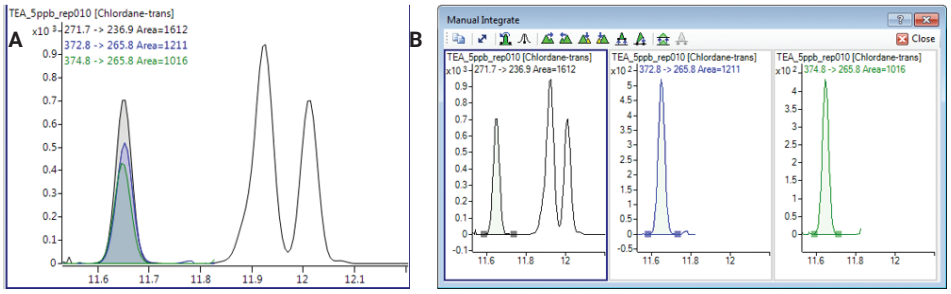

Figure 5. *trans*-Chlordane at 5 ppb in tea: A) overlay of MRM transitions, and B) view of each transition separately.

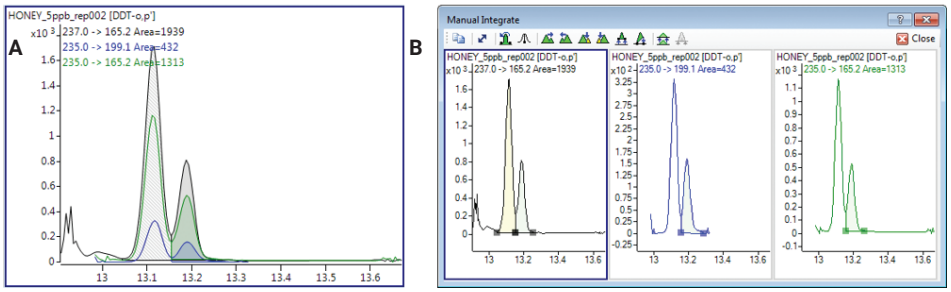

Figure 6. DDT-*o,p'* at 5 ppb in honey: A) overlay of MRM transitions, and B) view of each transition separately.

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. Churley, M. Lowering Detection Limits for Routine Analysis of Pesticides Residues in Foods Using the Agilent 7000C Triple Quadrupole GC/MS, *Agilent Technologies Application Note*, publication number 5991-4131EN, **2015**.
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