Technical Note: 10295

Multi-residue Pesticide Analysis in Green Tea by a Modified QuEChERS Extraction and Ion Trap GC/MS^n Analysis

David Steiniger, Guiping Lu, Jessie Butler, Eric Phillips, Yolanda Fintschenko, Thermo Fisher Scientific, Austin, TX, USA

Key Words

- ITQ 700
- Food Safety
- GC/MS^n
- Green Tea
- Pesticide Residues
- QuEChERS

Introduction

Recently formulated pesticides are quite different in their physical properties from their predecessors such as 4,4'-DDT. Most of these newer pesticides are smaller in molecular weight and were designed to break down rapidly in the environment. Therefore, to successfully identify and quantify these compounds in foods, more careful consideration must be placed on the sample preparation for extraction and the instrument parameters for analysis. This study will cover the preparation of extracts and the optimization of the analytical parameters of the splitless injection, separation, and detection.

The determination of pesticides in fruits, vegetables, grains and herbs has been simplified by a new sample preparation method, QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe), published recently as AOAC Method 2007.01.[1] The sample preparation is simplified by using a single step buffered acetonitrile (MeCN) extraction and liquid-liquid partitioning from water in the sample by salting out with sodium acetate and magnesium sulfate ($MgSO_4$).[1] QuEChERS can be used to prepare green tea samples for analysis by gas chromatography/tandem mass spectrometry (GC/MS^n) on the Thermo Scientific ITQ 700 GC-ion trap mass spectrometer.

The study was performed to determine the linear ranges, quantitation limits and detection limits for a partial list of pesticides that are commonly used on green tea crops, prepared in matrix using the QuEChERS sample preparation guidelines. A splitless injection of 22 pesticides was made in a single injection with detection in electron ionization (EI) MS/MS. Since the extracts were prepared in MeCN, a solvent exchange was made to hexane/acetone (9:1) prior to conventional splitless injection.[2] Once the calibration curve was constructed, multiple matrix spikes were analyzed at levels of 37.5, 75, 150, 225, 600, or 1200 ng/g (ppb) and low level spikes of 7.5, 15, 37.5, 75, or 300 ng/g (ppb) to verify the precision and accuracy of the analytical method. These concentrations were chosen based on the requirements of various regulatory agencies.

Experimental Conditions

The sample preparation involves careful homogenization of the sample. Extraction solvents must be buffered and the powdered reagents measured at appropriate amounts for the size of sample prepared. Some reagents cause an exothermic reaction when mixed with water, which can adversely affect the recoveries of target compounds. The recommended consumables required for sample

preparation and analysis were rigorously tested (Table 1). A list of the pesticides to be studied was created that would address all of the various functional groups and different physical properties of most pesticides. MS^n parameters were optimized with the use of variable buffer gas, the testing of the isolation efficiency, and adjustment of the Collision Induced Dissociation (CID) voltage. A surge splitless injection was made into a Thermo Scientific TRACE TR-Pesticide III 35% diphenyl/65% dimethyl polysiloxane column, (0.25 mm x 30 meter, and a film thickness of 0.25 µm with a 5 m guard column).

Item Descriptions
TRACE™ TR-Pesticide III 35% diphenyl/65% dimethyl polysiloxane column, 0.25 mm x 30 meter, 0.25 µm w/ 5 m guard column
5 mm ID x 105 mm liner (pk of 5)
10 µL syringe
Septa (pk of 50)
Liner graphite seal (pk of 10)
Ion volume, EI open
Ion volume holder
Graphite ferrule 0.1-0.25 mm (pk of 10)
Ferrule 0.4 mm ID 1/16 G/V (pk of 10)
Blank vespel ferrule for MS interface (pk of 10)
2 mL amber glass vial, silanized glass, with write-on patch (pk of 100)
Blue cap with ivory PTFE/red rubber seal (pk of 100)
Acetonitrile analytical grade (4L)
Hexane GC Resolv* (4L)
Acetone GC Resolv* (4L)
Organic bottle top dispenser
HPLC grade glacial acetic acid
50 mL Nalgene FEP centrifuge tubes (pk of 2)
Clean up tube:15 mL tube ENVIRO 900 mg $MgSO_4$, 300 mg PSA 150 mg C18 (pk of 50)
50 mL PP Tubes 6 g $MgSO_4$, 1.5 g $CH_3CHOONa$ (anhydrous) (pk of 250)
Clean up tube: 2 mL tubes 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18 (pk of 100)

Table 1: Consumables for QuEChERS sample preparation and GC/MS analysis

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Sample Extraction and Clean Up

The QuEChERS sample preparation procedure consists of the steps shown in Figure 1. There are three parts: extraction, clean up, and solvent exchange. The solvent exchange provides a final solvent that is more amenable to splitless injection and concentrates the analytes to reach lower detection limits. In addition, the solvent exchange and final clean up removed caffeine and polyphenols from the sample before injection. These compounds readily dissolve in acetonitrile, as shown in Figure 2 (red trace). However, they are not readily soluble in hexane:acetone (9:1), as shown in the black trace in Figure 2. This helps keep the analytical system clean.

Care must be taken to adequately and thoroughly homogenize the sample. A large amount of water must be added during the homogenization step when preparing the tea for extraction. This must be taken into consideration in the final calculations of spikes and standards. A total of 1200 mL of water was added to 200 g of green tea in this experiment.

An observation was made during the extraction phase of the sample preparation. If the MeCN extract was poured into the $MgSO_4$, poor spike recoveries were observed. This was due to an exothermic reaction of any water in the sample and the $MgSO_4$. Although many vendors offer the pre-measured powder reagents in a separate capped centrifuge tube, it is recommended not to add the sample to these tubes. Instead, reagents from these tubes should instead be added directly to the sample containing the acidified MeCN. Therefore, an empty 50 mL FEP extraction tube was included in the list of consumables for sample preparation. A thoroughly homogenized 15 g sample of green tea and water were weighed into the FEP extraction tube. Then 15 mL of 1% glacial acetic acid MeCN extraction solvent was poured into the tube on top of the sample. The surrogate and the pesticide solutions were spiked into this MeCN layer for the method validation (MVD) and method detection limit (MDL) samples.

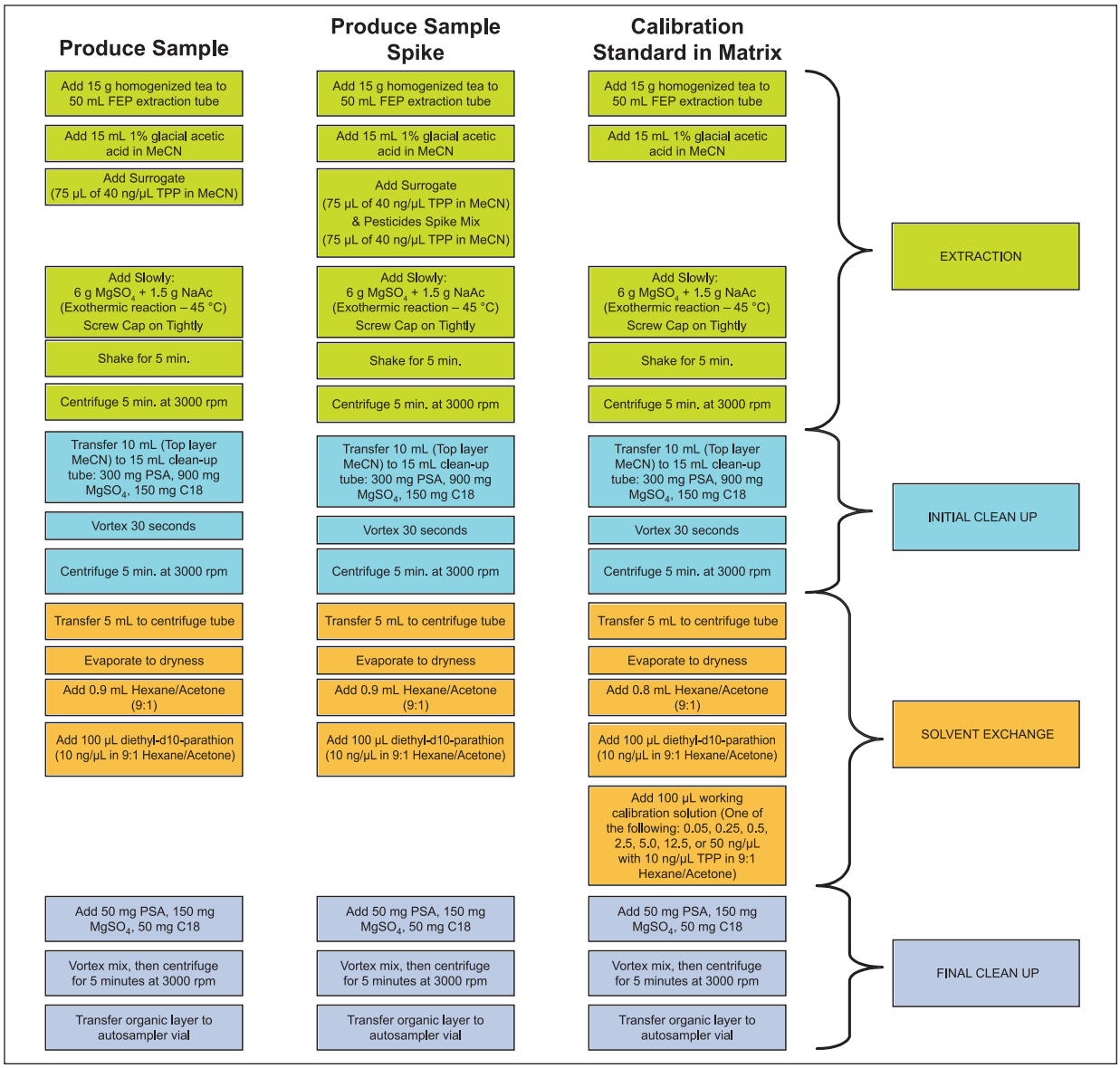

Figure 1: Flow diagram of QuEChERS sample preparation steps

The tube was capped and vortexed for 30 seconds. The cap was removed and the powder reagents were poured slowly into the MeCN layer. The cap was tightened securely on the 50 mL extraction tube, and was vortexed for 30 seconds until all of the powder reagents were mixed with the liquid layers. The tube was placed on a mechanical shaker for 5 minutes and then centrifuged for 5 minutes at 3000 rpm. Next, 11 mL of the top MeCN layer was removed and transferred to a 15 mL clean-up tube. This tube was capped and vortexed for 30 seconds and centrifuged for 5 minutes at 3000 rpm. A 5 mL aliquot of the top layer was transferred into a clean test tube for solvent exchange.

Solvent Exchange

The 5 mL aliquot of cleaned-up extract was evaporated to dryness with a gentle stream of nitrogen at 40 °C in about two hours. A film formed on top of the solvent layer and samples required mixing to break the film and continue the evaporation process. Care was taken to remove the tube immediately when dried. Approximately 1 mL of extracted compounds from the tea remained in the tubes after evaporation. A 900 µL aliquot of hexane/acetone (9:1) was added and 100 µL of the internal standard, d10-parathion, was spiked into the organic solution. The tube was capped and vortexed for 15 seconds. The 1 mL of extract was transferred to a 2 mL clean-up tube, capped tightly, and vortexed for 30 seconds. After centrifuging for 5 minutes at 3000 rpm, 200 µL of the lightly colored extract was transferred to an autosampler vial with a small glass insert for injection on the ITQ 700™. The individual calibration levels were spiked into each extract for the calibration curve in matrix before the final cleanup step (Figure 1).

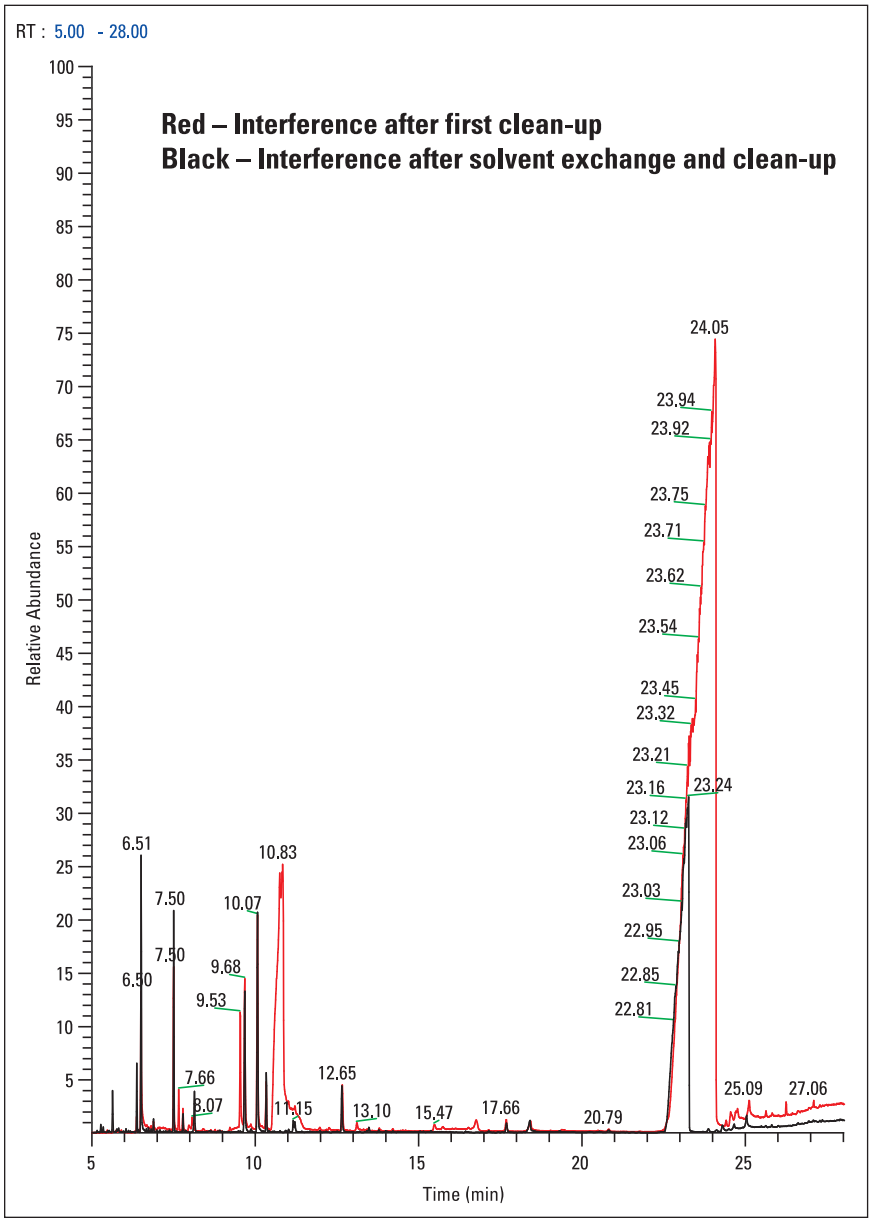

Figure 2: Comparison of a single cleanup step (red) against solvent exchange/final cleanup (black)

Injection

The ITQ 700 is paired with the Thermo Scientific FOCUS GC gas chromatograph, which is a single-channel GC with a standard split/splitless (SSL) injection port. The SSL inlet temperature was set to 250 °C. A 5 mm ID splitless liner with a volume of 1.6 mL was selected for the surged pressure injection. For the surge splitless injection, the inlet pressure was held at an elevated pressure of 250 kPa for the 0.5 minute injection (splitless) time. This technique reduces the vapor cloud of a 2 µL injection from 0.37 mL to 0.19 mL. At an elevated injection flow rate of 4.6 mL/ min, the liner was swept several times during injection. The target compounds moved through the inlet so rapidly that they had less time to interact with the inside walls of the liner. This minimized the amount of breakdown of the more fragile pesticides.

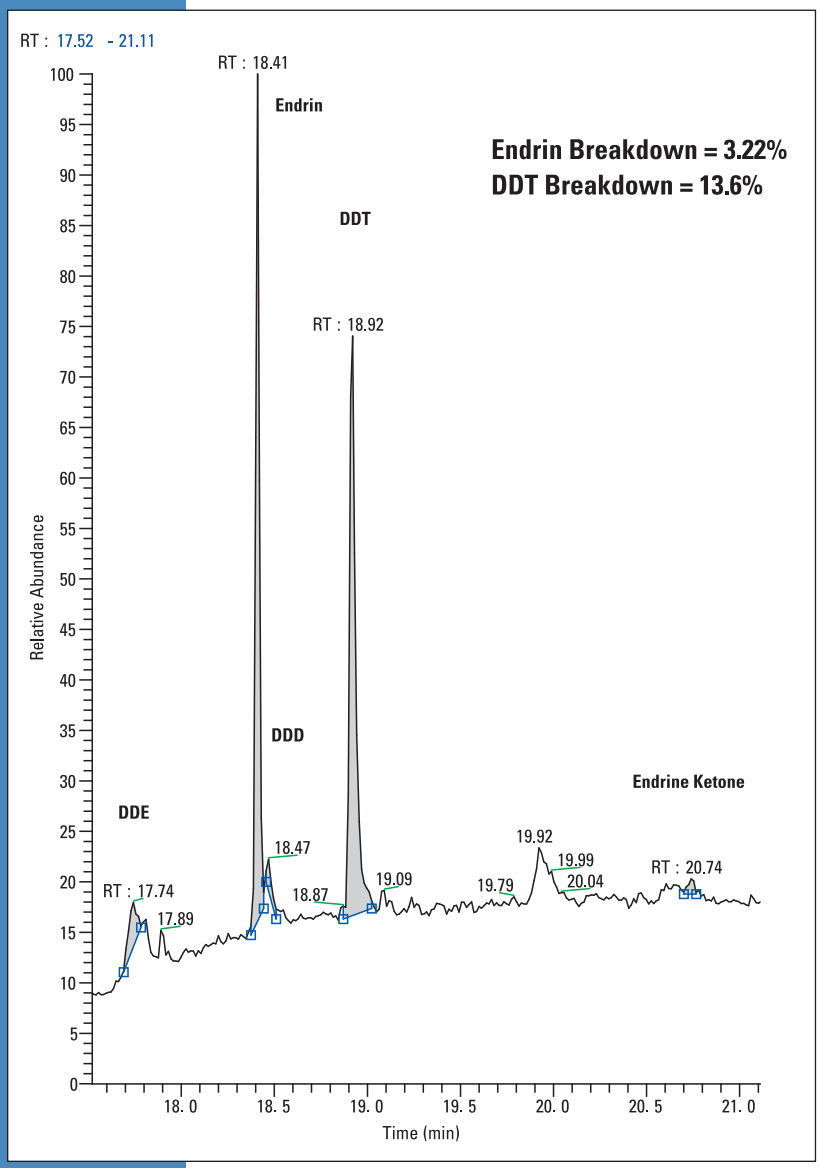

Figure 3: System performance check analysis demonstrating endrin breakdown < 5% and DDT breakdown < 15%

A Performance Solution consisting of endrin and 4,4'-DDT was analyzed as a daily check to determine system activity. The analysis of endrin, DDT, and their breakdown products as part of daily quality control can alert the analyst that the system has developed active sites and maintenance is needed. Without performing a breakdown analysis the laboratory may need to continually maintain the equipment and replace consumables, even when it may not be needed. Monitoring breakdown can decrease the cost of running the analysis and save significant amounts of time.

Endrin breakdown is determined by adding up the response for the two breakdown products: endrin aldehyde and endrin ketone and dividing by the total response for the breakdown products and endrin in percent. The breakdown products of DDT are DDE and DDD and are calculated similarly. The breakdown check results showed < 15% breakdown for both compounds on a daily basis. For routine use the liner would be changed when the breakdown of either compound reaches > 20%. The injection port liner tested showed very good results over a long period of time without the need for maintenance (Figure 3).

Separation

Chromatographic separation was achieved by using a 35% diphenyl/65% dimethyl polysiloxane column (0.25 mm x 30 meter, and a film thickness of 0.25 µm with a 5 m guard column). This column was chosen to improve the resolution of the more polar compounds. Some interactions within the stationary phase showed a loss of some pesticides at concentrations below 100 pg. The oven was programmed as follows: Initial Temp: 40 °C, 1.5 min, 25 °C/min to 150 °C, 0.0 min, 5 °C/min to 200 °C, 7.5 min, 25 °C/min to 290 °C with a final hold time of 12 min and a constant column flow rate of 1 mL/min. The entire set of instrument parameters is listed in Table 2.

Detection

The detection of the pesticides was performed using the ITQ 700 ion trap mass spectrometer with optional MS^n mode and a variable damping gas option. The MS/MS scan mode offers significantly enhanced selectivity over scanning modes such as full scan and selected ion monitoring (SIM). The ITQ 700 operated in the MS/MS mode generates unique product ion spectra by collision induced fragmentation of each of the detected pesticides. Because of the highly effective elimination of matrix interfering ions, more accurate results are produced at the lower levels. The MS^n parameters for the ITQ 700 are listed in Table 3. Figures 4 and 5 show a comparison between a Full Scan TIC and MS/MS extracted ion profile.

AS 3000 II Autosampler	
Sample Volume	2 µL
Plunger Strokes	5
Viscous Sample	No
Sampling Depth in Vial	Bottom
Injection Depth	Standard
Pre-inject Dwell Time	0
Post-inject Dwell Time	0
Pre-inject Solvent Wash Vial Position	A + B
Pre-inject Solvent Wash Cycles	3
Sample Rinses	3
Post-inject Solvent	A
Post-inject Solvent Cycles	3

FOCUS™ GC	
Column	TRACE TR-Pesticide III 35% diphenyl/65% dimethyl polysiloxane, 0.2 5 mm x 30 m x 0.25 µm w/ 5 m guard column
Column Constant Flow	1 mL/min
Oven Program	40 °C, 1.5 min, 25 °C/min; 150 °C, 0.0 min, 5 °C/min; 200 °C, 7.5 min, 25 °C/min; 290 °C, 12 min
S/SL Temperature	250 °C
S/SL Mode	Splitless with Surge Pressure
Surge Pressure	250 kPa
Inject Time	0.5 min
Split Flow	50 mL/min
Transferline Temperature	290 °C

ITQ Mass Spectrometer	
Damping Gas Flow	2
Source Temperature	250 °C
Ion Volume	EI
Emission Current	250 µA
Detector Gain	3 (1421 V)
Lens 1	-25V
Lens 3	-25V
Gate Lens On	-100
Gate Lens Off	100
Electron Lens On	15V
Electron Lens Off	85
Electron Energy	-70 eV
Trap Offset	-10
Waveforms	Off

Table 2: Selected instrument parameters for the ITQ 700 GC-ion trap MS

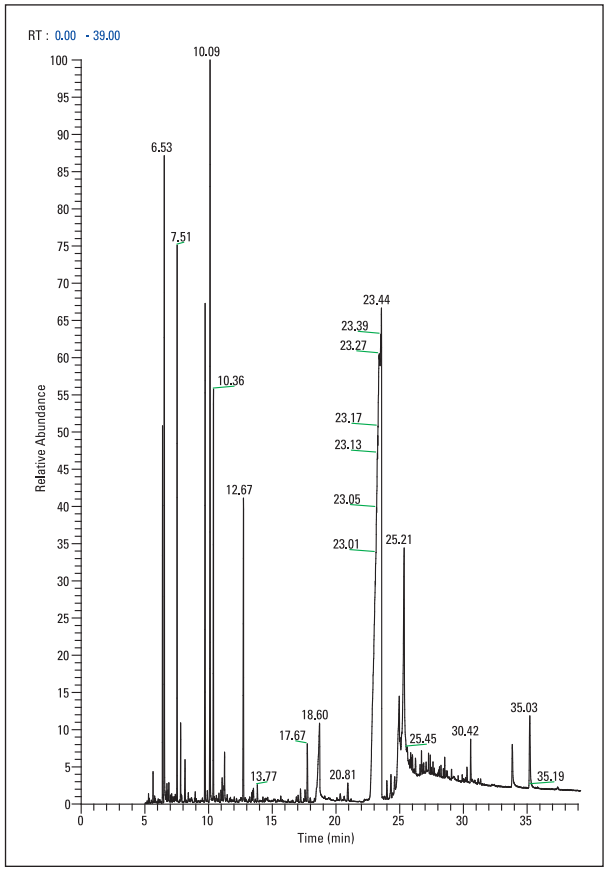

Figure 4: Full scan chromatogram of 600 ng/g pesticide spike in tea matrix

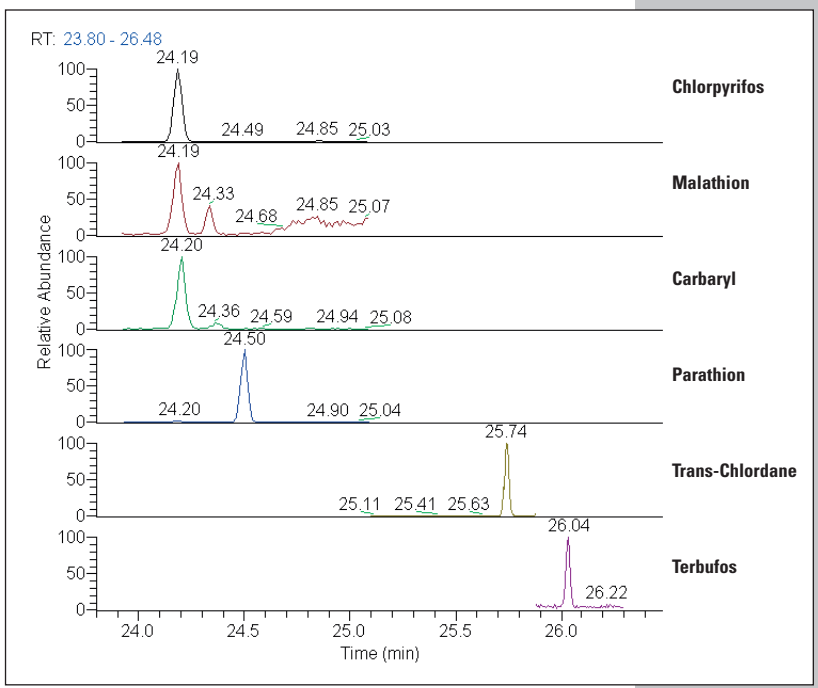

Figure 5: MS/MS scan of 600 ng/g pesticides in tea matrix, highlighting the elution range of 24 to 26 minutes

Compound	RT (min)	Precursor *(m/z)*	Width (amu)	Collision Energy (V)	Max. Excitation Energy (q)	Range *(m/z)*	Product Ion *(m/z)*	Qualifiers *(m/z)*
Dichlorvos	8.48	185	1	3	0.225	53-195	93	131, 109, 170, 63
Molinate	13.05	126	2	3	0.3	45-136	98	83, 55, 82, 81
Trifluralin	13.34	264	2	3	0.225	150-274	206	188, 160, 171, 177
Ethoprophos	14.57	158	2	2	0.225	84-168	114	130, 94, 140
Di-allate	15.45	234	3	3	0.225	140-244	192	150,193
Phorate	15.73	231	2	3	0.225	165-241	203	175, 185
Propyzamide (Pronamide)	16.86	173	2	3	0.225	135-183	145	146
Atrazine	17.42	200	6	4	0.225	84-210	122	132, 94, 134, 158
Diazanon	17.51	179	1	4	0.225	86-189	137	164, 138, 161, 96
Gamma BHC (Lindane)	17.92	219	4	3	0.225	171-229	181	183, 182, 184
Aldrin	22.15	263	1	5	0.225	217-273	229	228, 227, 230, 249
Metribuzin	23.69	198	2	4	0.225	93-208	151	103, 110, 153, 128
Dursban (Chlorpyrifos)	24.16	314	5	3	0.225	248-324	286	258, 287, 288, 285
Malathion	24.16	173	3	4	0.225	125-183	136	145, 137, 138, 135
Sevin (Carbaryl)	24.16	144	1	3	0.3	105-154	116	115
d-10 Parathion	24.34	301	2	3	0.225	105-311	269	147, 115, 148, 271
Parathion	24.49	291	4	3	0.225	99-301	142	263, 137, 109, 114
trans-Chlordane	25.73	375	4	4	0.225	256-385	301	266, 337, 303, 339
Terbufos	26.02	199	7	3	0.225	133-209	171	172, 153, 143, 173
cis-Chlordane	26.08	373	5	4	0.225	254-383	301	337, 299, 264, 335
Bifenthrin	28.29	181	7	4	0.225	143-191	166	165, 167, 178, 153
cis-Permethrin	30.99	183	3	4	0.225	143-193	168	165, 155, 153, 181
trans-Permethrin	31.19	183	3	4	0.225	143-193	168	165, 155, 153, 181

Table 3: MS/MS parameters for pesticides in tea

Results and Discussion

Linearity

The calibration curve was spiked into the tea matrix. Levels ranged from 1 ng/g to 1200 ng/g, depending on the compound and its MRL in green tea. The linearity for most compounds was $R^2 > 0.995$. The results of the linearity are shown in Table 4. Figures 6 and 7 are two examples of calibration curves.

Limits of Detection and Quantitation

The actual LOD and LOQ were determined by preparing matrix spikes at a level near or below the MRL. Concentrations of 7.5, 15, 37.5, 75, or 300 ng/g were analyzed in seven matrix samples and the LOD and LOQ calculated from these results by multiplying the standard deviation by 3.143 and 10 respectively. The results are shown in Table 5. These results exhibit that this method is able to meet or exceed the MRL requirements for most of the compounds, even at the most stringent level.

Method Validation Results

The method validation calculations were performed on five matrix samples spiked at a concentration of 37.5, 75, 150, 225, 600, or 1200 ng/g. Samples had an average of 104% recovery with an average % RSD of 10.8%. MVD results for selected concentrations are shown in Table 6.

Conclusions

The ITQ 700 GC-ion trap MS was thoroughly evaluated and showed excellent accuracy at low concentrations for a large number of pesticide residues analyzed in green tea. Using the instrument's MS^n functionality allows the user to identify, confirm, and quantify in one analytical run. The injector demonstrated low endrin and DDT breakdown (< 15%) on a daily basis, proving that the system can analyze active compounds without the need for continual, expensive, and time-consuming maintenance. Calibration curves for most pesticides studied met a linear least squares calibration with a correlation coefficient of $R^2 > 0.995$. The Method Validation Study generated an average % RSD of 10.8% for five replicate analyses at 37.5, 75, 150, 225, 600, or 1200 ng/g and a calculated average LOD of 14 ng/g in tea based on 7 replicate analyses of 7.5, 15, 37.5, 75, or 300 ng/g These results demonstrate that the ITQ 700 can comply with international regulations for the control of pesticides in tea.

Compound	(R^2)	Compound	(R^2)	Compound	(R^2)
Dichlorvos	0.9990	Gamma BHC (Lindane)	0.9949	*cis*-Chlordane	0.9944
Molinate	0.9996	Aldrin	0.9879	Bifenthrin	1.0000
Trifluralin	0.9994	Metribuzin	0.9966	*cis*-Permethrin	0.9993
Ethoprophos	0.9997	Dursban (Chlorpyrifos)	0.9998	*trans*-Permethrin	0.9998
Di-allate	0.9996	Malathion	0.9998	**Average**	**0.9989**
Phorate	0.9979	Sevin (Carbaryl)	0.9997		
Propyzamide (Pronamide)	0.9985	Parathion	0.9993		
Atrazine	0.9993	*trans*-Chlordane	0.9958		
Diazanon	0.9917	Terbufos	0.9993		

Table 4: Calibration curve results, demonstrating excellent linearity with average R^2 of 0.9978

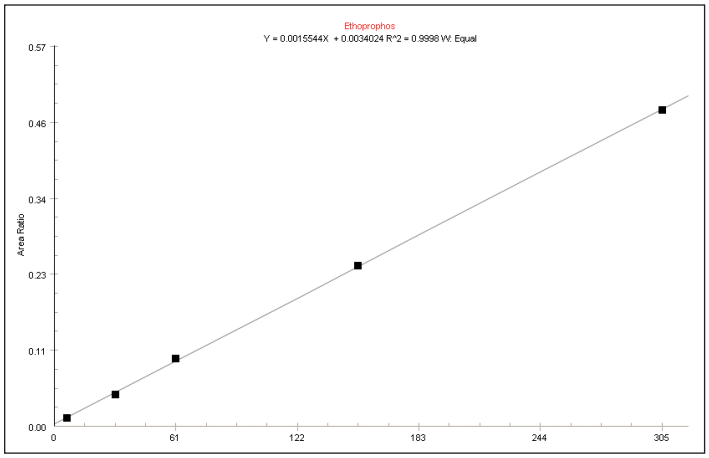

Figure 6: Calibration curve for Ethoprophos in tea

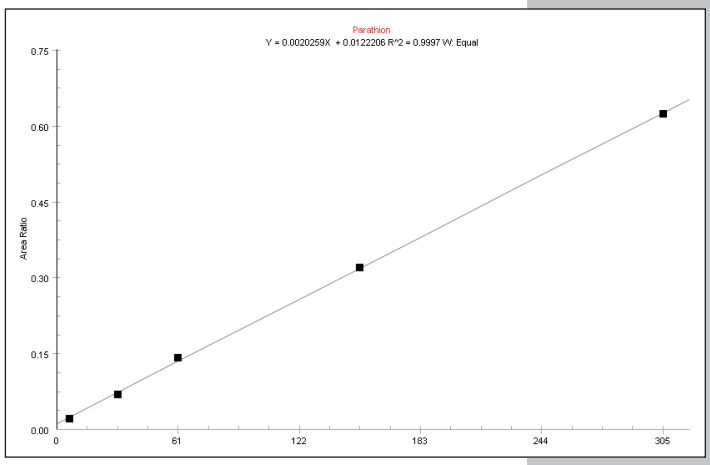

Figure 7: Calibration curve for Parathion in tea matrix

Component	Avg Conc (ng/g)	Std. Dev.	% RSD	LOD (ng/g)	LOQ (ng/g)	Japan[2] MRL (ng/g)	EU[3] MRL (ng/g)	EU[3] LOD (ng/g)	WHO[1] MRL (ng/g)
Dichlorvos	14	1.57	11.3	5	16	100	100	100	
Molinate	9	0.75	8.7	2	7	20			
Trifluralin	7	0.44	6.0	1	4	50			
Ethoprophos	6	0.89	14.0	3	9	5			
Di-allate	43	4.03	9.3	13	40	100	100	100	
Phorate	9	0.59	6.6	2	6	100	100	100	
Propyzamide (Pronamide)	11	1.04	9.6	3	10	50	50	50	
Atrazine	6	0.46	7.6	1	5	100	100	100	
Diazanon	13	0.60	4.7	2	6	100	50	50	
Gamma BHC (Lindane)	18	0.94	5.3	3	9	50	50	50	
Aldrin	7	1.70	25.2	5	17	ND	20	20	
Metribuzin	36	6.04	16.9	19	60	100			
Dursban (Chlorpyrifos)	19	2.80	14.7	9	28	10,000	100	100	2,000
Malathion	336	34.83	10.4	109	348	500	500		
Sevin (Carbaryl)	27	4.48	16.9	14	45	1000			
Parathion	46	7.82	17.2	25	78	300	100	100	
trans-Chlordane	8	1.21	14.8	4	12	20			
Terbufos	43	4.38	10.3	14	44	5			
cis-Chlordane	7	0.72	9.7	2	7	20			
Bifenthrin	32	6.09	19.0	19	61	25,000	5,000	100	
cis-Permethrin	78	7.71	9.9	24	77	20,000	100	100	20,000
trans-Permethrin	78	9.41	9.4	30	94	20,000	100	100	20,000
Average			**11.7**						

1. CODEX alimentarius (www.codexalimentarius.net/mrls/pesticides/jsp/pest-q-e.jsp)

2. Japanese Food Chemical Research Foundation (www.m5.ws001.squarestart.ne.jp/foundation/search.html)

3. Informal coordination of MRLs established in Directives 76/895/EEC, 86/362/EEC, 86/363/EEC, and 90/642/EEC (5058/VI/98)

Table 5: Comparison of LODs and LOQs to selected MRLs from international agencies and reporting bodies

Component	Avg Conc	Theo Conc	% RSD	% Recovery
Dichlorvos	158	150	4.5	105
Molinate	64	75	6.8	85
Trifluralin	66	75	4.2	89
Ethoprophos	64	75	3.4	86
Di-allate	59	75	4.1	79
Phorate	60	75	4.3	80
Propyzamide (Pronamide)	133	150	7.9	88
Atrazine	61	75	5.5	82
Diazanon	49	37.5	8.4	130
Gamma BHC (Lindane)	81	75	5.0	108
Aldrin	36	37.5	13.7	96
Metribuzin	67	75	11.7	89
Dursban (Chlorpyrifos)	175	225	6.3	78
Malathion	730	600	13.3	122
Sevin (Carbaryl)	72	75	9.9	96
Parathion	75	75	9.4	100
trans-Chlordane	43	37.5	16.0	115
Terbufos	76	75	9.8	101
cis-Chlordane	39	37.5	9.9	105
Bifenthrin	65	600	11.6	109
cis-Permethrin	1236	1200	15.1	103
trans-Permethrin	1239	1200	11.6	103
Average			**10.8**	**104**

Table 6: Results of method validation study, showing good precision and high recoveries

References

1. AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate, S. Lehotay, *Journal of AOAC International*. Vol. 90, No. 2, (2007) 485-520.
2. Rapid Method for the Determination of 180 Pesticide Residues in Foods by Gas Chromatography/Mass Spectrometry and Flame Photometric Detection, M. Okihashi, *Journal Pesticide Science*. 304 (4), (2005) 368-377.
3. Commission Decision of August 12, 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results, *Official Journal of European Communities*. 17.8.2002.
4. MRLs for tea as listed at *http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp*.

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