Determination of Multi-Pesticide Residues in Dried Tea Samples using an Optimized Extraction/Cleanup Regime and the Agilent 7000 Series Triple Quadrupole GC/MS System

Application Note

Food Safety

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Abstract

A novel sample preparation regime has been developed for the extraction and cleanup of dried tea samples. The sample preparation regime, based on the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method effectively extracts pesticides from the tea matrix while at the same time minimizes the extraction of caffeine and other co-extractives which can cause degenerative effects on chromatographic peak shape, analyte retention time shifts and loss of sensitivity. The tea extracts were analyzed by GC/MS/MS using MRM mode on an Agilent 7890 GC with an Agilent 7000B Triple Quadrupole GC/MS system.

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Introduction

Tea (*Camellia sinensis*) is one of the most popular beverages in the world and has been consumed for centuries. Green tea in particular is well known for its anticarcinogenic and anti-ageing properties. Every year, millions of tons of tea are grown and exported from tea producing countries with China and India being the world's largest exporters. The intensive use of agro-chemicals, for example, insecticides (to control insect pests such as mites, leaf eating beetles, caterpillars, and so forth) as well as acaricides and fungicides on tea plantations has given rise to concerns over consumers' exposure to pesticide residues and hence the potential health risks. Therefore, it is necessary to provide effective residue control methods. Dried tea leaf samples provide a challenging, complex matrix which contains many different classes of chemical compounds. Table 1 shows the typical constituents of dried tea leaves.

Table 1. Main Constituents of the Dried Tea Leaves of Camellia Sinensis

Component	Content (% dry weight)
Polyphenols	25–35
Flavonoids	80% of total polyphenols
Saccharides	25
Polysaccharides	14–22
Proteins	15
Minerals	5
Free amino acids	4
Chlorophyll	0.5
Caffeine	2.5–5.5

The relatively high caffeine content in tea leaves can cause chromatographic problems such as retention time shifts and suppression of response in the mass spectrometer if it is not reduced significantly during the sample preparation procedure.

This application note describes a novel extraction and sample cleanup regime which removes the vast majority of the caffeine and other co-extractives from the final sample extract and, coupled with the use of post-column, post-run backflush to remove high-boiling matrix components that would remain on the column between runs, facilitates more robust chromatographic performance and enhanced detection levels of pesticide residues in tea samples using GC/MS/MS.

Experimental

Sample Preparation

Samples of homogenized, dried tea leaves (2 g) were weighed into 50 mL plastic centrifuge tubes and left to hydrate for 30 minutes after the addition of distilled water (10 mL) and agitation for 30 seconds. Acetonitrile (10 mL) was then added and the tube agitated vigorously for 1 minute. Magnesium sulphate (4 g) and sodium chloride (1 g) was then added (Agilent Bond Elut p/n 5982-5550) along with tri-phenyl phosphate (TPP) internal standard (ISTD). After further vigorous agitation for 1 minute, the sample tube was centrifuged for 5 minutes at 10,000 rpm.

A 1-mL amount of the (upper) acetonitrile layer was then transferred to a 15-mL plastic centrifuge tube. *n*-Hexane (1 mL) and 20% w/w aqueous sodium chloride solution (5 mL) was then added. The tube was agitated vigorously for 1 minute and then centrifuged for 1 minute at 10,000 rpm.

An aliquot of the (upper) *n*-hexane layer was transferred to a 2-mL auto-sampler vial for injection into the GC/MS/MS system. Figure 1 shows a summary of the overall sample extraction and cleanup regime.

Using this rapid and simple sample preparation method, an analyst can prepare more than six samples in 1 hour with the minimum use of organic solvents.

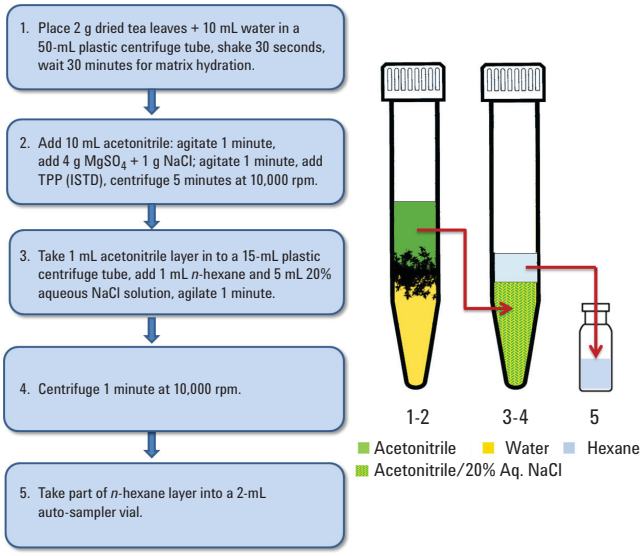


Figure 1. Flow diagram of sample extraction and clean-up regime.

Sample Analysis

The GC method was retention time locked to trifluralin at 6.219 minutes and employed post-run, post-column back flush. The use of backflush ensures that any high-boiling matrix material remaining on the column at the end of each run is quickly and efficiently removed (through the split vent) prior to the next injection in a sequence. Backflushing [1] has been proven to provide:

- Consistent analyte retention times and responses
- Robust chromatography and consistent analyte chromatographic peak shapes
- Prevention of high boiling matrix from contaminating the MS ion source
- Extended column life-time and reduced cycle times by removing the need for high-temperature bake-out between runs

Full GC analysis conditions are given in Table 2.

Table 2. GC Conditions for the Analysis of Pesticides in Tea Extracts

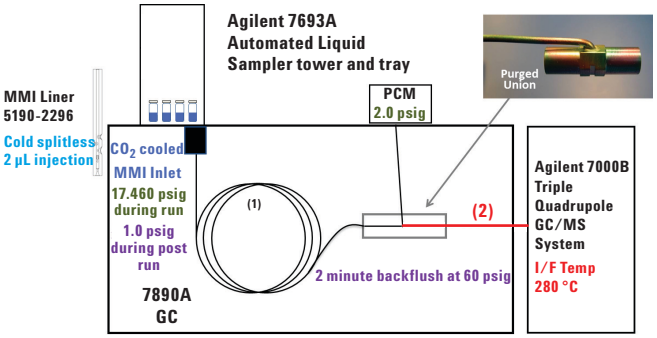
Column (1)	15 m × 0.25 mm, 0.25 µm HP-5MSUI (19091S-431UI)
Column (2)	0.50 m × 0.15 mm, 0.15 µm DB-5MSUI (cut from 165-6626)
Capillary flow device	Pressure controlled tee (PCT) with pneumatics control module (PCM)
Auto-sampler	Agilent 7693A Automated Liquid Sampler
Injection	2 µL cold splitless using CO_2 cooled Multimode Inlet (MMI)
Splitless period	1 minute
Injection port liner	2 mm id dimpled deactivated liner (5190-2296)
Inlet temperature program	50 °C (0.1 minute), 600 °C/min to 300 °C
Purge flow to split vent	50 mL/min at 1.0 minute
RTL compound	Trifluralin, locked to 6.219 minutes
Carrier gas	Helium
Inlet pressure	17.460 psig constant pressure mode (during run)
PCM pressure	2.0 psig constant pressure mode (during run)
Oven program	50 °C (1.0 min), 50 °C/min to 150 °C, 6 °C/min to 200 °C, 16 °C/min to 280 °C (4.07 minutes)
Post run time	2.0 minutes
Post run temperature	280 °C
Post run pressures	Inlet 1.0 psig, PCM 60.0 psig
MS transfer line temperature	280 °C

The Agilent 7000B Triple Quadrupole LC/MS System was operated in MS/MS electron ionization (EI) mode and analytes identified/quantified by using multiple reaction monitoring (MRM) mode using 2–8 transitions for each target analyte. MS conditions are given in Table 3.

Table 3. MS Conditions for the Analysis of Pesticides in Tea Extracts

Ionization mode	Electron ionization
Electron energy	–70 eV
Tune	EI autotune
EM gain	10
MS1 resolution	1.2 amu full width at half maximum
MS2 resolution	1.2 amu full width at half maximum
Transitions	See reference [2]
Collision energies	See reference [2]
Dwell times	2–28 ms depending on the number of transitions per time window to achieve 5 cycles/s
Collision cell gas flows	Nitrogen at 1.5 mL/min, helium at 2.25 mL/min
MS temperature zones	Ion source 280 °C, Q1 150 °C, Q2 150 °C

Figure 2 shows a schematic diagram of the GC/MS/MS system hardware configuration.



(1) Constant pressure, Column 15 m × 25 mm, 25 µm HP-5MSUI
(2) Constant pressure, Restrictor 0.50 m × 0.15 mm, 0.15 µm DB-5MSUI

Figure 2. Schematic diagram of the GC/MS/MS hardware configuration.

Results and Discussion

Chromatography

The efficiency of the cleanup procedure for removing caffeine and other co-extractives in the crude QuEChERS extract and the extract after liquid–liquid extraction is shown in Figure 3 by the full scan MS chromatograms.

Figure 4 shows the stability of GC/MS/MS analyte responses. After the first five injections of tea extracts (required to stabilize the responses of analytes when a new liner is used, so called priming the GC system with the matrix), the responses of the eight example pesticides spiked into a tea sample at 0.1 mg/kg are very reproducible over the course of 150 injections in a sequence. This demonstrates the effectiveness of backflush for removing high boiling matrix from the capillary column between sample injections.

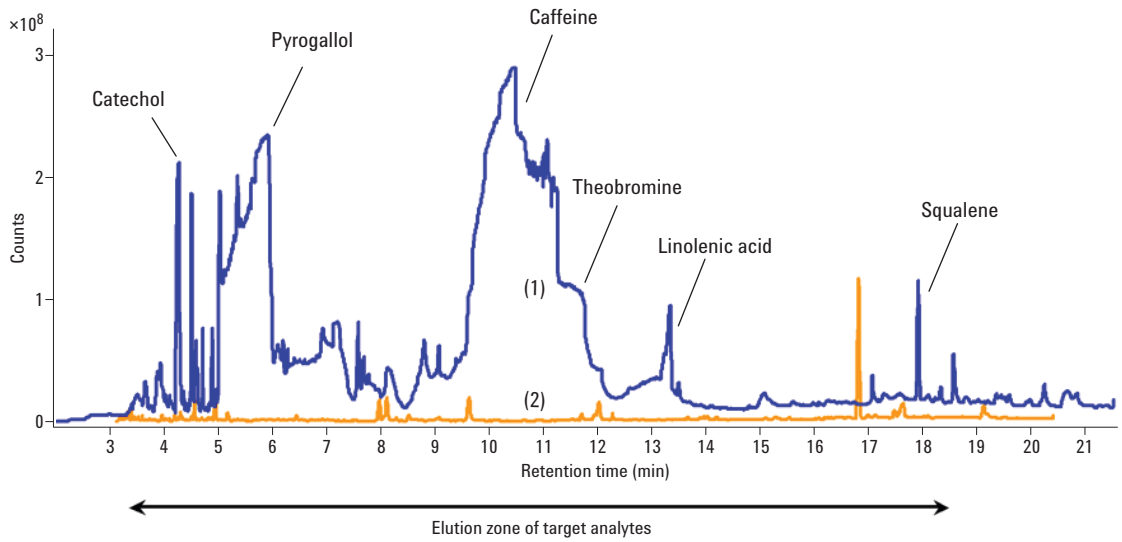


Figure 3. Full scan MS chromatograms showing very high abundance of caffeine and other co-extractives remaining in the acetonitrile extract after the QuEChERS extraction (1 blue trace) compared to their significant reduction when subsequent liquid–liquid extraction is employed (2 yellow trace).

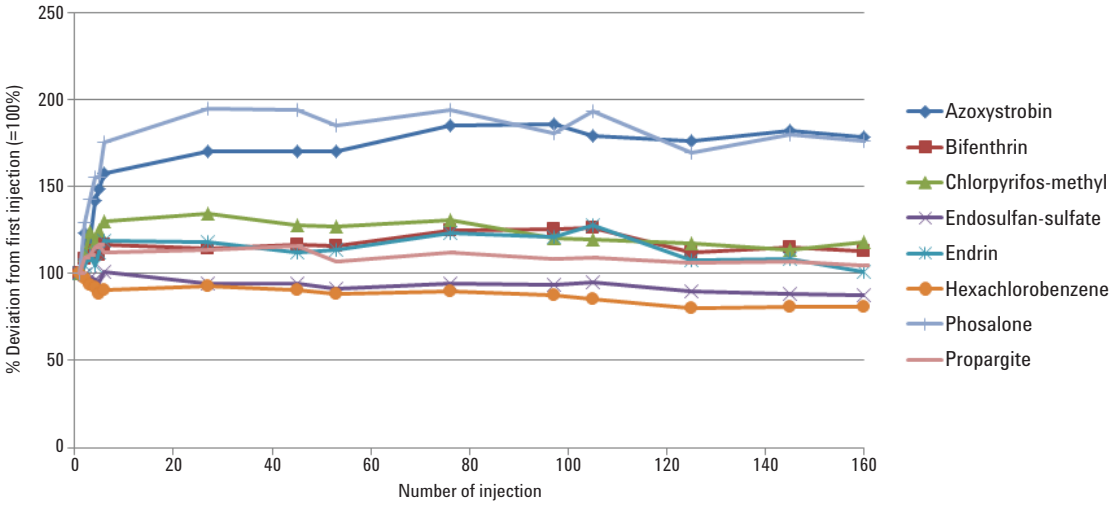


Figure 4. Long-term stability of GC/MS/MS system responses for the injection of tea extracts. Each point corresponds to the injection of a matrix-matched standard at 0.1 mg/kg concentration in a sequence.

Instrument Calibration

Tea matrix-matched calibration standards of pesticides were prepared in hexane at concentrations of 0.2, 0.5, 1, 2, 5, 10, 20, 30, 50, 100, 200, and 300 ng/mL which correspond to 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1, 0.15, 0.25, 0.5, 1, and 1.5 mg/kg in the sample. Each matrix-matched standard also contained TPP ISTD at a concentration of 20 ng/mL (corresponding to 0.1 mg/kg).

Figure 5 shows an example of MRM chromatograms for the 0.1 mg/kg (20 ng/mL) tea matrix-matched calibration standard.

Figure 6 shows an example of SRM chromatograms for an early, middle and late-eluting analyte (dichlobenil, triazophos, and azoxystrobin, respectively) from the 0.05 mg/kg (10 ng/mL) matrix-matched calibration standard.

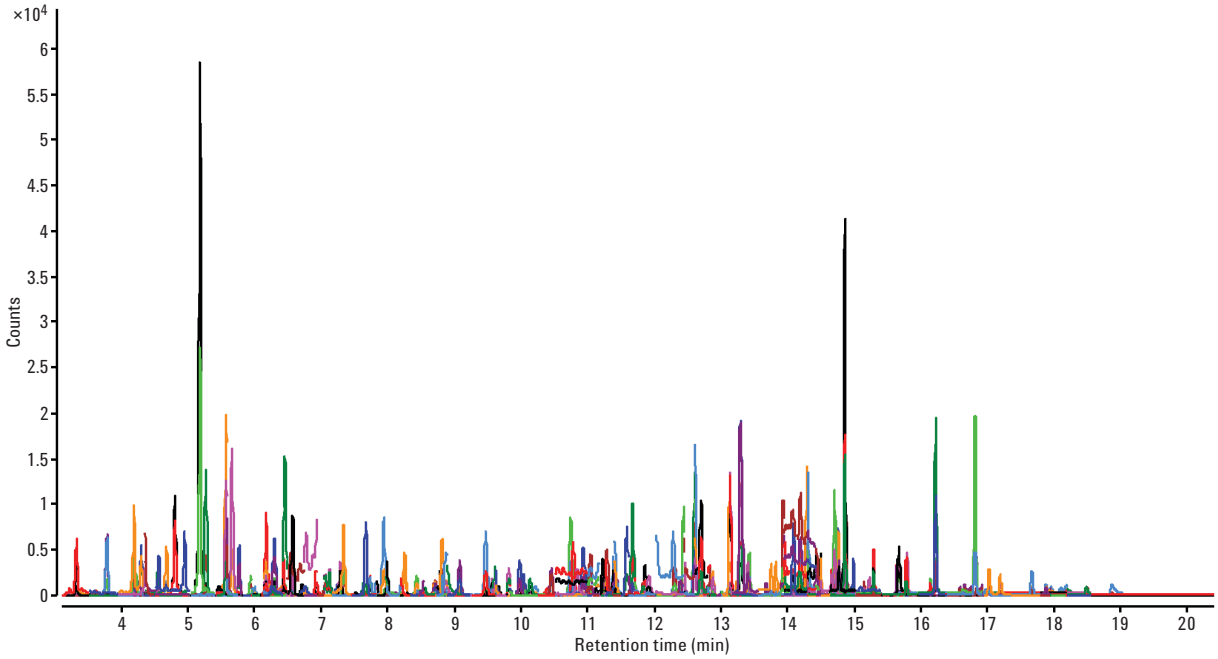


Figure 5. An overlay of MRM chromatograms of pesticides in the matrix-matched standard (0.1 mg/kg) acquired using the optimized MS/MS method.

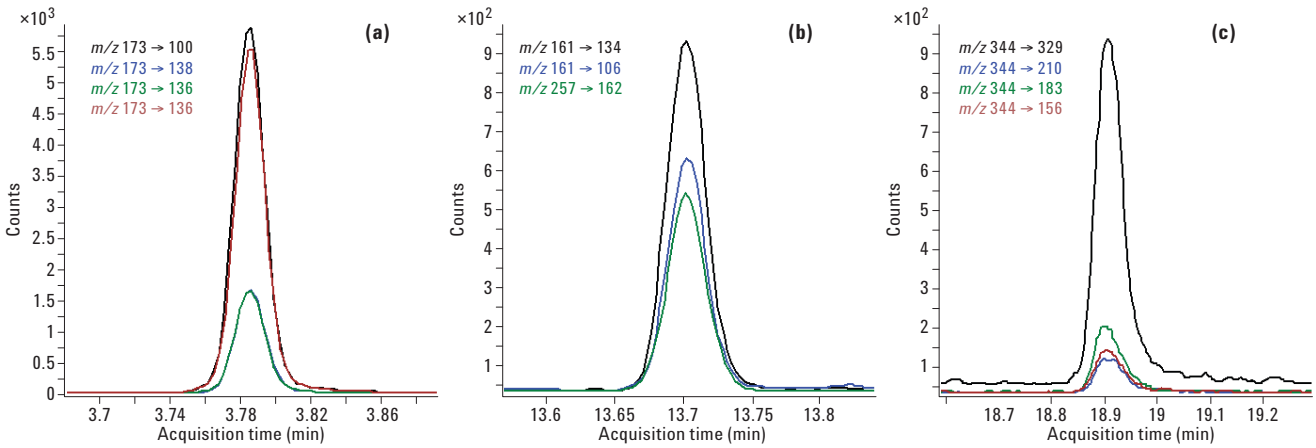


Figure 6. SRM chromatograms for (a) dichlobenil, (b) triazophos, and (c) azoxystrobin in a 10 ng/mL tea matrix-matched calibration standard, equivalent to 0.05 mg/kg in the sample.

Method Performance

The sample preparation method and optimized GC/MS/MS analysis conditions detailed in the Experimental section of this application note were evaluated in a validation study involving the analysis of six replicates of green and black tea samples, each spiked with pesticides at concentrations of 0.01, 0.1, and 1 mg/kg. Figure 7 shows the mean recoveries and relative standard deviations (RSDs) obtained from the analysis of the extracts of both matrices. The data indicate the excellent recoveries and reproducibility of quantitative data down to the 0.01 mg/kg level in both tea sample types.

Sample Analysis

The validated method was used to analyze 37 samples in a pilot study to further evaluate its performance and applicability. The analyzed samples included green and black teas, some of them aromatized. The test results are presented in Figure 8.

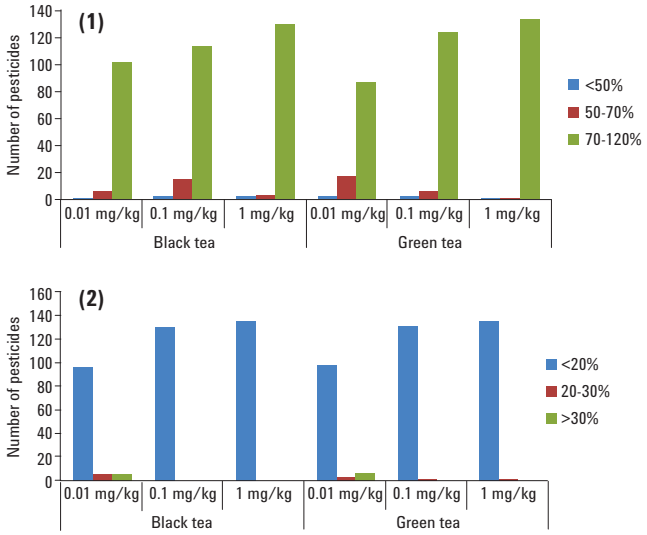


Figure 7. Distribution of (1) overall recoveries and (2) RSDs for the pesticide residues in green and black tea at spiking levels of 0.01, 0.1 and 1 mg/kg.

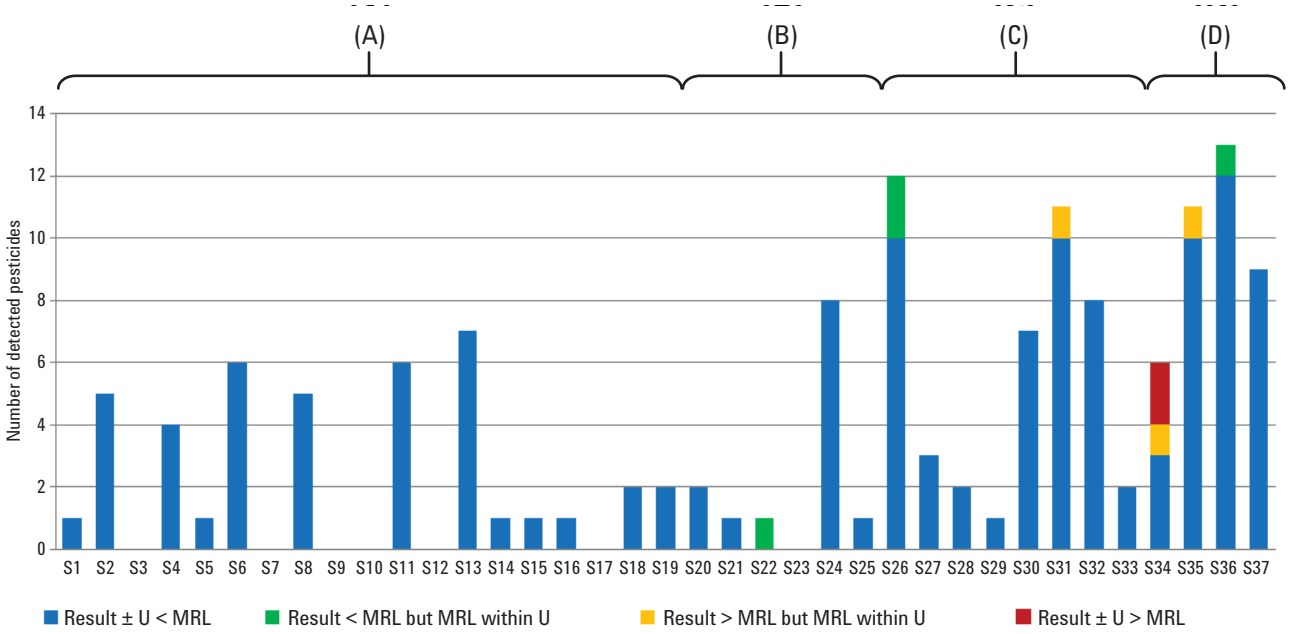


Figure 8. Number of detected pesticide residues in tea samples. (A) Black tea; (B) Black tea, aromatized; (C) Green tea; (D) Green tea, aromatized. For interpretation a default value of 50% as expanded measurement uncertainty (U) was applied (according to SANCO/12495/2011, Appendix C [4]).

In total, 81% of the samples were tested positive (≥ 0.01 mg/kg) containing at least one pesticide residue. Cypermethrin (68%), endosulfans (41%), propargite (38%), bifenthrin (38%), cyhalothrin-lambda (24%), and buprofezin (24%) were the most frequently found pesticides. Some samples contained residues around the EU MRLs and in one sample, two residues (buprofezin and triazophos), exceeded the EU MRLs. Green tea resulted in more positive hits than black tea. The higher occurrence of pesticide residues was observed for aromatized green teas, in which case essential oils and flower petals might introduce pesticides to those teas. This indicates that to reduce consumers' dietary exposure, tea represents one of the food commodities worthy of frequent monitoring for pesticide residues.

Conclusions

The determination of pesticide residues in dried tea is very challenging. Dried tea is a complex food matrix and multi-pesticide residue analysis challenges both sample preparation and analysis by gas chromatography/mass spectrometry.

A novel extraction and rapid cleanup method has been developed for the analysis of pesticide residues in dried tea samples. The extraction and cleanup, based on the QuEChERS method, includes extraction with acetonitrile followed by liquid–liquid extraction. This sample preparation regime significantly reduces the content of caffeine (a natural constituent of dried tea leaves) and other semivolatile co-extractives in the final extract. Reduction of caffeine content in the final extract improves retention time reproducibility and detection of analytes that would elute with, or just after, the caffeine peak.

Post-run, post-column back flush was implemented using capillary flow technology in order to reduce chromatographic cycle times, remove high-boiling matrix components between analyses and reduce contamination of the mass spectrometer ion source.

For most target analytes, method performance characteristics were in line with the SANCO/12495/2011 document [3], that is, recoveries were within the acceptable range of 70–120% and repeatabilities were ≤ 20% at all three spiking levels (0.01, 0.1 and 1 mg/kg). For a few of the analytes, lower recoveries between 50 and 70% were obtained (for example, azoxystrobin and hexachlorobenzene). Since the consistency of the results (≤ 20% RSD) for these pesticides was achieved, the results could be corrected for the known recovery factors in the analyses. In terms of sensitivity, the majority of the analytes could be quantified at 0.001–0.005 mg/kg (corresponding to 0.2–1 ng/mL), thus unbiased identification and reliable quantification of target analytes at EU MRLs, which are in most cases well above these levels, was possible.

Overall, the optimized sample preparation and GC/MS/MS method gave performance characteristics that are suitable to provide reliable control of pesticide residues in tea at the MRLs set in EU Legislation (Regulation (EC) No. 396/2005) [4].

References

[1] C. Sandy, "Improving GC-MS Method Robustness and Cycle Times using Capillary Flow Technology and Back flushing", Agilent Technologies publication 5990-3367EN (2009).

[2] T. Cajka, C. Sandy, V. Bachanova, L. Drabova, K. Kalachova, J. Pulkrabova, J. Hajslova: Streamlining sample preparation and gas chromatography–tandem mass spectrometry analysis of multiple pesticide residues in tea. Analytica Chimica Acta, 742 (2012) 51-60. (doi:10.1016/j.aca.2012.06.051)

[3] Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

[4] Document No. SANCO/12495/2011: Method validation and quality control procedures for pesticides residues analysis in food and feed.

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