GC-MS/MS Analysis of Pesticide Residue in Green Tea Extracted by QuEChERS with Acetonitrile as Final Solvent

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Overview

Purpose

This poster describes the analysis of several challenging pesticides from green tea samples using GC-MS/MS and acetonitrile as final extraction solvent. The compounds analysed are representatives of various classes of pesticides, such as carboxamids, OC, OP, pyrethriods, aromatic, phenylamides. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) is a well known approach used for the extraction and clean-up of pesticide residue in various matrices. Typically, the final extract ends up with the pesticides in acetonitrile. Direct injection of acetonitrile extracts is problematic in GC-MS compared to LC-MS because of poor focusing of chromatographic peaks due to the high polarity of acetonitrile, limitations on injection volumes due to the high expansion coefficient of acetonitrile and contamination of the system by matrix co-extractives [1]. Here we present a simple and robust analytical method which employs low volume splitless injections of acetonitrile sample extracts and the selectivity of the Thermo Scientific™ TSQ™ 8000 triple quadrupole GC-MS/MS instrument. With this approach, pesticide target reporting limits of <0.01 mg/kg can be easily achieved. This also overcomes the problems associated with the thermal expansion of acetonitrile and reduces the amount of matrix injected.

Methods

Green tea samples have been extracted using a typical QuEChERS protocol, and the final extracts were spiked with a mixture of 19 pesticides at levels corresponding to 0.005 to 0.5 mg/kg. The analysis was done by GC-MS/MS using a timed-SRM detection method on the TSQ 8000 instrument, employing two SRM transitions for each pesticide compound in a typical MRM method setup. Data processing and reporting is performed by using the Thermo Scientific™ TraceFinder™ software with one SRM transition used for quantitation and the second one for ion ratio confirmation of the positively identified pesticide compounds.

Results

The described method can be confidently used for the routine analysis of pesticides in complex matrices, such as teas with challenging heavy matrix impact for the control of the regulated maximum pesticide residue levels. Excellent sensitivity, linearity and reproducibility were obtained for all target compounds spiked in the green tea samples.

Introduction

QuEChERS involves an initial step when a few grams of the sample are extracted with acetonitrile followed by a clean-up step (with dispersive-SPE) used to remove, to a certain extent, unwanted matrix compound (such as pigments, sugars, organic acids). With QuEChERS, the final extract ends up with the pesticides in acetonitrile, which, being polar solvent, can be problematic in GC-MS. Poor focusing of chromatographic peaks and high expansion coefficient are issues that need to be addressed when acetonitrile is used as a solvent for GC-MS analysis. To overcome this, an additional step can be added to the QuEChERS method where acetonitrile is replaced with solvents that are more amenable to splitless injections in GC-MS.

The aim of this study was to assess the chromatography, repeatability, robustness and linearity of these compounds when using acetonitrile as extraction solvent and splitless injections.

Methods

Sample Preparation

Organically grown green tea leaves (Pure Tea Ltd., Radstock, UK) were used for the experiments described below. For the QuEChERS, 2 g of green tea was weighted and hydrated for 30 min in 10 mL deionized water. Acetonitrile (10 mL) was added followed by 4g $MgSO_4$ and 1g NaCl. After a centrifugation step (10k rpm for 5 min), 6 mL of the supernatant were transferred to a dSPE tube containing 1200 mg $MgSO_4$, 400 mg PSA, 400 mg C_{18} and 400 mg GCB. This mixture was vortexed and centrifuged and 1 mL of the upper layer was spiked with the pesticides of interest at various levels ranging from 1.0 – 100 pg/µL (corresponding to 0.005 – 0.5 mg/kg) and used for the GC-MS analysis.

TSQ 8000 GC-MS/MS Method setup

All experiments were performed using the Thermo Scientific TSQ 8000 Pesticide Analyzer (P/N TSQ8000EI-PA230) which comprises of sample handling (Thermo Scientific™ TriPlus™ RSH liquid autosampler), sample introduction and chromatographic separation (TRACE™ 1300 Series GC equipped with a SL/SSL injector), and the TSQ 8000 triple quadrupole mass analyser.

The TSQ 8000 MS was operated in SRM mode using two transitions per compound. SRM transitions are readily available from a Thermo Scientific Pesticide Compound Database (CDB) containing >600 with retention times and pre-optimized SRMs.

TriPlus RSH Autosampler

Injection vol. and type:	1.0 µL, fast liquid band injection
Washing cycles:	5 x 7 µL, solvent acetonitrile

TRACE 1310 Gas Chromatograph

Injector Split/Splitless:	splitless mode
Liner:	SSL single taper (P/N: 453A2342)
Inj. temp.:	250 °C
Flow:	const flow, 1.5 mL/min, helium
Analytical column	30 m, ID 0.25 mm, 0.25 um TraceGOLD TG-5SILMS (P/N 26096-1420)
Pre-column deactivated	5 m x 0.25 mm, empty
Column oven	temp. programmed
Start	100 °C, for 2.0 min
Ramp 1	50 °C/min to 150 °C
Ramp 2	6°C/min to 200°C
Ramp 3	16°C/min to 320°C, 6 min hold
Transfer line	280 °C

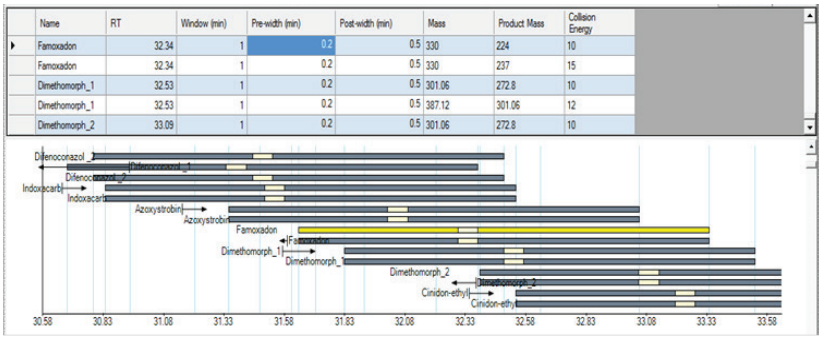
TSQ 8000 Mass Spectrometer in EI mode

Source Temp.: 300 ° C
Ionization: EI+, 70 eV
Emission Current: 50 µA
Resolution: Q1 & Q3 @ 0.7 Da
Collision Gas: Argon
MRM Detection timed SRM mode, see Figure 1

Data Acquisition/Processing

Each compound SRM transition was only monitored for a narrow time window around the established retention time (timed-SRM). This led to a fully optimized instrument duty cycle for maximum analytical performance being handled automatically by the system (Figure 1). The data processing and reporting was achieved by using TraceFinder quantitation and reporting software suite [2].

FIGURE 1. Principle of the Timed-SRM acquisition setup of the TSQ 8000 GC-MS. The white center parts show the peak width, and the gray area shows the full SRM acquisition window.



Name	RT	Window (min)	Pre-width (min)	Post-width (min)	Mass	Product Mass	Collision Energy
Famoxadon	32.34	1	0.2	0.5	330	224	10
Famoxadon	32.34	1	0.2	0.5	330	237	15
Dimethomorph_1	32.53	1	0.2	0.5	301.06	272.8	10
Dimethomorph_1	32.53	1	0.2	0.5	387.12	301.06	12
Dimethomorph_2	33.09	1	0.2	0.5	301.06	272.8	10

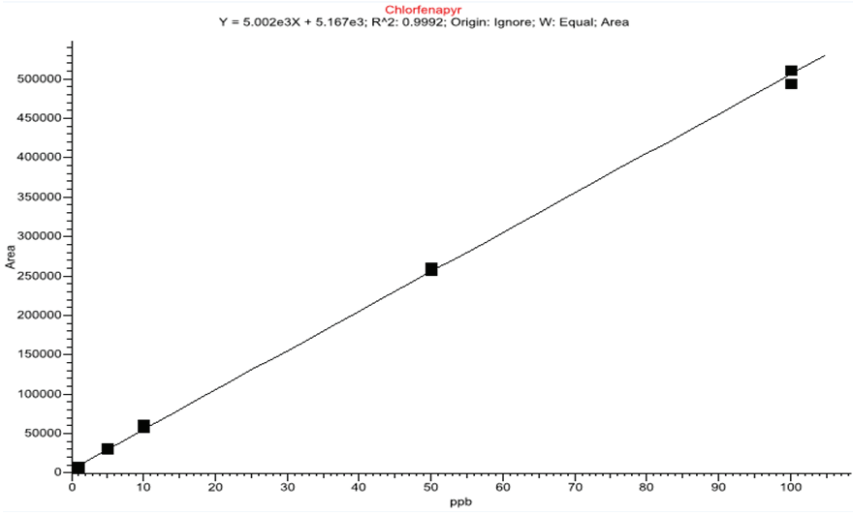
Results

This method describes the methodology used for the multi-residue pesticides analysis in green tea using acetonitrile as final extraction solvent and splitless injections of low sample volume. The performance of the TSQ 8000 GC-MS/MS system was evaluated by assessing the sensitivity, linearity and reproducibility of the targeted compounds in green tea samples.

Calibration and Linearity

The calibration solution have been prepared from green tea extracts spiked in the range of 1.0 pg/µL to 100 pg/µL (corresponding to 0.005 to 0.5 mg/kg level for each of the pesticides in the samples). Two repeat injections per calibration point were performed. The standard matrix blank consisted of green tea extracted as of the standard procedure. The pesticide blank level was tested before applying as blank standard matrix. Excellent linearity with correlation coefficients R^2 exceeding 0.996 (residual error for each calibration point <10% RSD had been achieved for all pesticides (see an example for Chlorfenapyr in Figure 2).

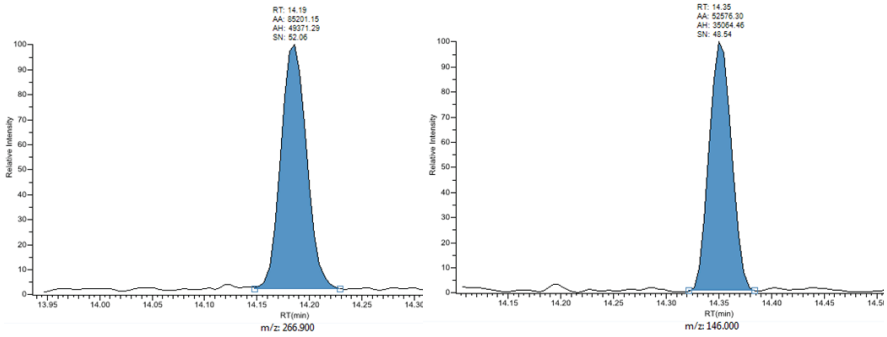
FIGURE 2. Quantitative calibration for Chlorfenapyr, range 1 ppb to 100 pg/µL, 2 injections/calibration point. No internal standard correction.



Sensitivity

All 19 pesticides were easily detected in the lowest calibration matrix-matched standard with excellent chromatography (Figure 3).

FIGURE 3. Pesticide peaks at 5 ppb (0.005 mg/kg) in green tea matrix for Profenofos (337 > 267, CE 12V) and Oxyfluorfen (252 > 146, CE 30V)

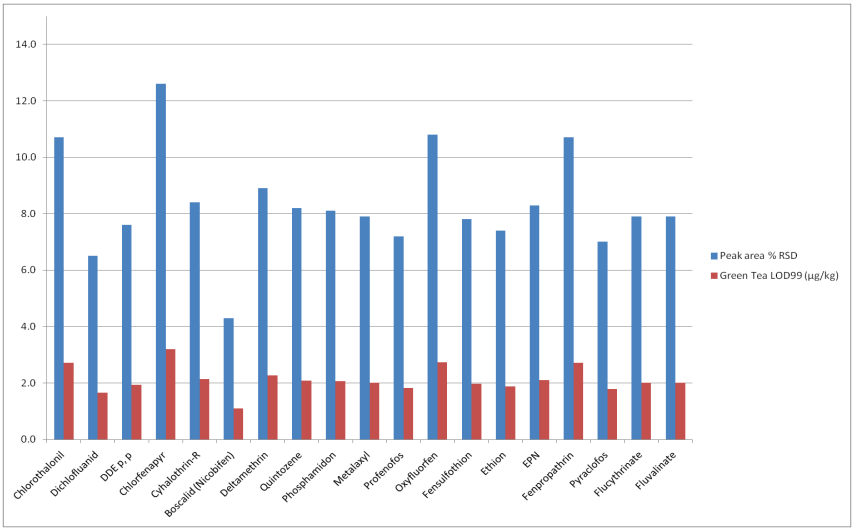


The instrument LOD was assessed by repeatedly (n = 20) injecting the 10 ppb (0.01 mg/kg) calibration standard taking into account the student's-*t* critical values for the corresponding degrees of freedom (99% confidence), the concentration of each native compound, and %RSD. The results of this test show excellent LODs for the pesticides analyzed with values between 1 ppb (200 fg on column) (Boscalid) - 3 ppb (600 fg on column) (Chlorfenapyr) (Figure 4).

Repeatability

Peak area repeatability was assessed using n = 20 replicate injections of the green tea extracts spiked at 10 ppb level (2 pg on column). The results of this experiment shows excellent coefficients of variation values (%RSD) with minimum values of 4.3% for Boscalid, maximum of 12.6 % for Chlorfenapyr and an overall average value of 8.3% (Figure 4).

FIGURE 4. Limits of Detection (LOD) and peak area repeatability (%RSD) of n=20 consecutive injections of green tea spiked at 10 ppb (0.01 mg/kg) level.



Conclusion

The QuEChERS-GC/MS/MS multi-residue method described here allows for rapid and accurate monitoring of GC amenable pesticides in green tea extracts using acetonitrile as final solvent without the need of an additional solvent exchange step.

Low volume splitless injection of the green tea sample extracts overcomes the problems associated with the thermal expansion of acetonitrile and reduces the amount of matrix injected.

The sensitivity and selectivity of the TSQ 8000 GC-MS/MS reached significantly below the regulated levels in green tea samples.

Excellent linearity, chromatography, sensitivity and peak area repeatability were reported.

Taken together, the TSQ 8000 GC-MS/MS system delivers very reliable results, reducing significantly the manual quality control reducing a typical bottleneck in trace analysis laboratories and increasing the productivity for the final sample report processing.

References

1. Rapid Analysis of Pesticides in Difficult Matrices Using GC-MS/MS, Thermo Fisher Scientific Application Note 51880, 2010.
2. Pesticide Method Reference 2nd Edition, Thermo Fisher Scientific, p/n 120390.

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