Enhanced ion sampling efficiency allows sample dilution in order to minimize matrix effects for multi-residue pesticide analysis in black tea

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Introduction

Challenges in multi-residue pesticide analysis include the diversity of pesticide compound classes found in food analysis, matrix complexity, low concentration and poor ionization efficiency of some pesticides. In this work we evaluate the use of dilution as a means of minimizing matrix effects and achieving rugged and high performance quantitation at trace levels in black tea using UHPLC-triple quadrupole mass spectrometry and dynamic MRM with fast polarity switching. Black tea was selected as test matrix due to its high complexity. Increased ion sampling efficiency achieved using dual ion funnel technology allowed a 20-fold sample dilution while still achieving the maximum residue level (MRL) stipulated by the European Union.

Experimental

Sample preparation

The extraction procedure was based on the QuEChERS method. The final extract contained 0.2 g of black tea sample per mL of acetonitrile (ACN). Stock solutions of the pesticides mixture at different levels were prepared in ACN. These levels were spiked always in the same amount of blank matrix to prepare the calibration curves in black tea in the range of 0.5 ppt to 50 ppb.

ME [%] calculations were performed comparing area response in spiked matrix and pure solvents. Both samples were in the same composition solution (ACN).

ME [%] = [(avg. area response in spiked matrix/ avg. area response in solvents) – 1] x 100

ME [%] > 0 indicates ionization enhancement; < 0 indicates ionization suppression and ± 20% is considered acceptable.

ME [%] calculations were performed after no dilution and 1:20 dilution with ACN.

Analyzed pesticides:

65 pesticides of different chemical classes including organophosphates, organothiophosphates, tetrazines, carbamates, oxime carbamates, carbanilates, tetronic acid insecticides and acid herbicides were selected for the evaluation covering the full polarity range and including pesticides classified as difficult to analyze due to their low proton affinities, thermal lability or susceptibility to matrix effects

Experimental

Agilent 1290 Infinity UHPLC:

Column: Agilent Rapid Resolution High Definition (RRHD)Zorbax Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm

Mobile phase: A= 5 mM ammonium formate in water + 0.01% HCOOH, B= 5 mM in methanol + 0.01% HCOOH; Injection volume: 5 µL. Gradient analysis at 0.6 mL/min, stop time at 15 min. Post time: 2 min.

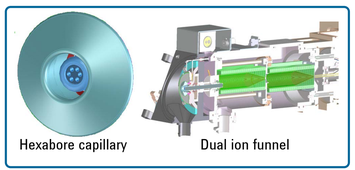
Agilent 6490 Triple Quadrupole MS:

Scan type: Dynamic MRM (DMRM). **Polarity:** Positive/Negative switching. **Resolution:** MS1/MS2: Unit/Unit (Q1: 0.7 m/z / Q2: 0.7 m/z).

Agilent Jet Stream Parameters: Drying gas temp.: 200°C, Drying gas flow: 24 L/min, Sheath gas temp.: 300°C, Sheath gas flow: 12 L/min, Nebulizer pressure: 25 psi, Nozzle voltage: 0 V (+/-), Capillary voltage: 3500 V (+/-).

Funnel RF voltages: Low pressure funnel RF: 60 V (+), 40 V (-), High pressure funnel RF: 150V (+), 70 V (-)

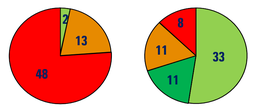
iFunnel Technology: Agilent Jet Stream (AJS) technology consists of the addition of a concentric super-heated nitrogen sheath gas to the nebulizer. The super-heated sheath gas collimates the nebulizer spray producing efficient desolvation and ion generation. The use of a hexabore capillary increases the interface area of the capillary inlet within the AJS thermal ion rich zone. The bores spread across the central, ion rich part of the AJS thermal confinement zone. In this way the multibore capillary captures more ions but also more gas. This gas load would normally overwhelm the vacuum system and therefore a novel dual ion funnel was added to efficiently remove the gas, while focusing the ions into the entrance of the first quadrupole.



Matrix effects in non-diluted and 1:20 diluted black tea samples

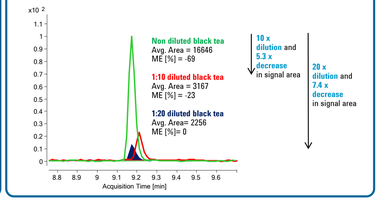
Non-diluted black tea exhibited strong matrix effects for 48 from 63 detected pesticides. However only 8 from 63 pesticides exhibited strong matrix effects after 1:20 dilution. Dilution had a positive impact on signal response as shown in the figure below for 0.5 ppb prochloraz in non-diluted, 1:10 diluted and 1:20 diluted black tea. A 10-fold dilution led to only a factor 5 decrease in signal area and a 20-fold dilution led to only a factor 7 decrease in signal area, indicating that the signal was suppressed in the non-diluted sample.

Distribution of Pesticides with Zero, Low, Medium or Strong Matrix Effects



Zero: Matrix effects at LLOQ = 0%
Low: Matrix effects at LLOQ < ± 20%
Medium: ± 20 < Matrix effects at LLOQ < ± 50%
Strong: Matrix effects at LLOQ > ± 50%

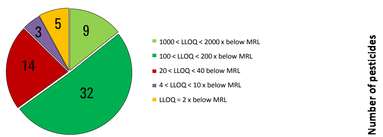
Effect of Dilution on Signal response and Matrix Effects
Selected Example: 0.5 ppb Prochloraz in black tea



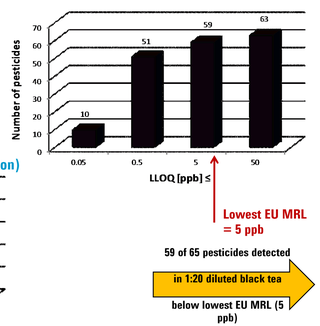
1:20 dilution enabled by improved sensitivity

After 20-fold dilution, all pesticides were detected at a LLOQ at least 2 x below the EU MRL and 55 from 65 pesticides were detected at a LLOQ at least 20 x below the EU MRL. At LLOQ, all pesticides exhibited Area RSD [%] < 20% with most of the pesticides showing RSD [%] values lower than 15%, which is in good agreement with SANCO criteria of area RSD [%] < 20% at LLOQ.

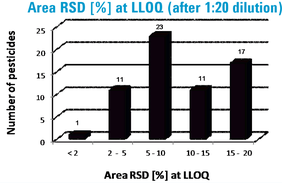
Distribution of pesticides with LLOQ x times below EU MRL (after 1:20 dilution)



Frequency of pesticides with LLOQ < 0.05 ppb, 0.5 ppb, 5 ppb or 50 ppb (after 1:20 dilution)



Area RSD [%] at LLOQ (after 1:20 dilution)

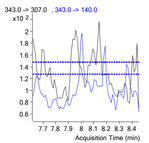


Quantitative performance of selected pesticides after 1:20 dilution

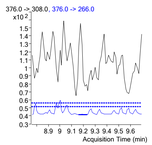
The following figures show DMRM chromatograms for blank and LLOQ as well as quantitative performance (area RSD [%], accuracy and S/N) after 1:20 dilution for selected pesticides with high risk relevance, i.e. listed as high in "Check your scope" ranking. The ranking lists more than 1100 compounds, including pesticides currently used or used in the past and pesticide metabolites of importance. Ranking of pesticides is based on toxicological data, residue situation in crops (reporting from labs in Europe and RASFF notifications) and agricultural usage, including potential for misuse and persistent pesticides.

Matrix effects [%], area precision, accuracy and S/N (Peak to Peak) were calculated based on 5 replicate injections

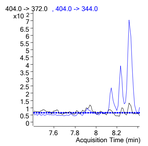
Boscalid, matrix blank



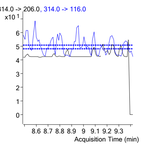
Prochloraz, matrix blank



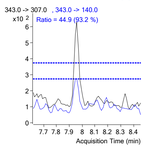
Azoxystrobin, matrix blank



Kresoxim methyl, matrix blank

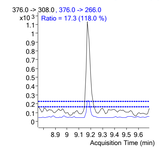


Boscalid, LLOQ = 0.5 ppb



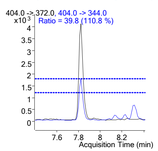
Ranking points = 374
LLOQ is 1000 x below EU MRL
ME without dilution [%] = -60
ME after 1:20 dilution [%] = 0
Area RSD [%] = 18
Avg. Accuracy [%] = 103.8
Avg. S/N (Peak to Peak) = 11

Prochloraz, LLOQ = 0.5 ppb



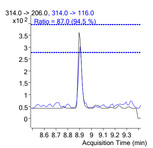
Ranking points = 343
LLOQ is 200 x below EU MRL
ME without dilution [%] = -69
ME after 1:20 dilution [%] = 0
Area RSD [%] = 17
Avg. Accuracy [%] = 96.7
Avg. S/N (Peak to Peak) = 20

Azoxystrobin, LLOQ = 0.5 ppb



Ranking points = 333
LLOQ is 200 x below EU MRL
ME without dilution [%] = -40
ME after 1:20 dilution [%] = 0
Area RSD [%] = 4
Avg. Accuracy [%] = 101
Avg. S/N (Peak to Peak) = 81

Kresoxim methyl, LLOQ = 0.5 ppb



Ranking points = 202
LLOQ is 200 x below EU MRL
ME without dilution [%] = -69
ME after 1:20 dilution [%] = 0
Area RSD [%] = 20
Avg. Accuracy [%] = 108.7
Avg. S/N (Peak to Peak) = 201

Conclusions

1. Taking full advantage of dual ion funnel technology, it was possible to use a 20-fold dilution to minimize matrix effects while still achieving a LLOQ below the EU MRL for all detected pesticides in black tea. Moreover using sample dilution an improved robustness of the method can be expected due to the lower matrix amount introduced into the LC/MS system which leads to better chromatography, reduced chemical noise and less matrix effects in the API source.
2. A generic set of Agilent Jet Stream (AJS) settings was found that allowed reliable quantitation of multiple pesticides of different chemical classes below the European Union Maximum Residue Limit (EU MRL) even after 1:20 dilution. 2 pesticides that were not detected using the generic AJS conditions achieved the EU MRL using lower drying gas and sheath gas temperature conditions
3. Precision at LLOQ after 1:20 dilution was excellent with most of the pesticides exhibiting area RSD [%] < 15%