Development and Validation of a Large Multiresidue LC-MS/MS Method Using On-Line Dilution and Other Features Useful for Routine Analysis

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Abstract
The aim of this work was to develop and validate a pesticide multiresidue LC-MS/MS method for the analysis of about 450 analytes eluting in less than 10 min. The MS/MS acquisition method employs triggered multiple reaction monitoring (MRM), which provides increased confidence in analyte identification through triggered acquisition of additional MRMs when one of the primary MRMs exceeds a set abundance threshold (>2000 MRMs included in the method). The mobile phase gradient was optimized to spread the analytes evenly throughout the elution window, with special attention paid to the separation of critical pairs. The LC system employs an on-line dilution set-up, ensuring excellent peak shapes of early eluting (more polar) analytes. As a result, acetonitrile extracts (prepared using a QuEChERS-based extraction) are injected directly without a need for a dilution with an aqueous buffer/solution prior to the injection. The method was validated in three different routine laboratories in multiple food commodity types/matrixes, with 0.01 mg/kg method validated LOQ achieved for the majority analyte-matrix combinations.

Introduction
LC Problems for Early Eluting Analytes
Retention/peak focusing of early eluting compounds injected in strong solvents, such as relatively polar analytes injected in acetonitrile in reverse-phase separations (including pesticide multiresidue analysis).

Analyte Identification
SANTE/11945/2015 guidelines “Analytical quality control and method validation procedures for pesticide residue analysis in food and feed”
Identification using LC-MS/MS:
► retention time ± 0.1 min
► 2 product ions
► ≥ 30% maximal relative tolerance for ion ratios

Triggored MRM (MMRM)
► Data-dependent function in Agilent LC-QQQ instruments
► Triggored acquisition of additional (secondary) MRMs when one of the primary MRMs exceeds set threshold
► Up to 10 MRMs per analytes combined into a “spectrum”

Experimental
Pesticide Standards
Agilent LC-MS standard mixes 1.8-10 µg/mL in acetonitrile (Agilent Part # 5190-0551) were combined with several custom mixes to create a composite with >450 analytes.

Sample Preparation
Sample size:
► 10 g high-moisture samples
► 5 g low-moisture/low-fat samples
► 1 g ultra-low-moisture/complex samples
► QuEChERS extraction/partition (AOAC 2007.01)
► 10 mL acetonitrile with 11% acetic acid
► Addition of 3× low-moisture samples
► 4 g MgSO4 and 1 g NaOAc for phase separation/buffering
► No SPE clean-up and no dilution for LC-MS/MS analysis

Results and Discussion
Optimization of MS/MS Conditions
Optimization and selection of MS/MS transitions (typically 10 MRMs per analyte) using MassHunter Optimizer software, followed by a detailed review of the collected information.

Optimization of UHPLC Conditions and On-Line Dilution
► Optimum analyte separation and detection within a relatively short separation time
► On-line dilution and mixing using a serial combination of two high-pressure mixers to improve chromatography of early eluting compounds – an alternative setup without the second (quaternary) pump possible with the use of a 0.3µm high-pressure filter
► Improved retention and peak shape of early eluting, more polar analytes:

References