

Improvements In Pesticides Screening Using A Latest-Generation LC/TOF-MS

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Overview

- Examine a pesticide screening method for possible improvement using latest-generation LC/TOF instrumentation
- Experiments used a "representative" set of pesticides, examined different instrument parameters, two mobile phase compositions, and data analysis parameters
- All pesticides in the study detected and identified at the level of 10 ppb in matrix, with cycle times for samples in a matrix, with analysis time of about ten minutes

Introduction

Pesticides as a group have been responsible for enormous beneficial gains in food productivity around the world. However, when present outside of their intended target, even in trace amounts, these same compounds may be harmful to the public health. For that reason, many organizations have developed analytical methods for identifying and quantifying these pesticides in foods and the environment. Our aim in this study was to apply recently-developed instrumentation to a method used for analysis of pesticides in foods or environmental samples, with the ultimate goal of improving analysis throughput, specificity, and/or detection limits.

Experimental

Samples
Some pesticides were selected to be representative of a wide range of responses. Others were selected based on published data indicating possible interference between analytes ("isobaric co-eluting species", abbreviated ICS [1]). Pesticide standards for method development were obtained from Chem Service (USA). The matrix used for method development was a methanolic extract of a desired concentration, with 8% methanol/92% water or 20% acetonitrile/80% water. Blank pepper matrix was prepared by a modified QUECHERS extraction of organic certified pesticide-free peppers [2]. This matrix solution in 100% acetonitrile was then spiked with the pesticide standard at the 10 ppb level. The spiked matrix was then spiked with water prior to analysis. The spiked tap water sample was obtained by adding the pesticide mixture to local tap water.

Instrument
Agilent Technologies 1290 Infinity LC binary pump, ALS with thermostat, thermostatted column compartment) and 6540 QTOF equipped with Agilent Jet Stream thermal gradient focusing technology. MassHunter software was used for acquisition and data analysis. Identification was performed using the Agilent ChemStation software. The data were analyzed using the Agilent ChemStation software. This algorithm also uses the data for the presence of the targeted isotopic pattern and spacing in addition to the accurate mass value of the monoisotopic peak.

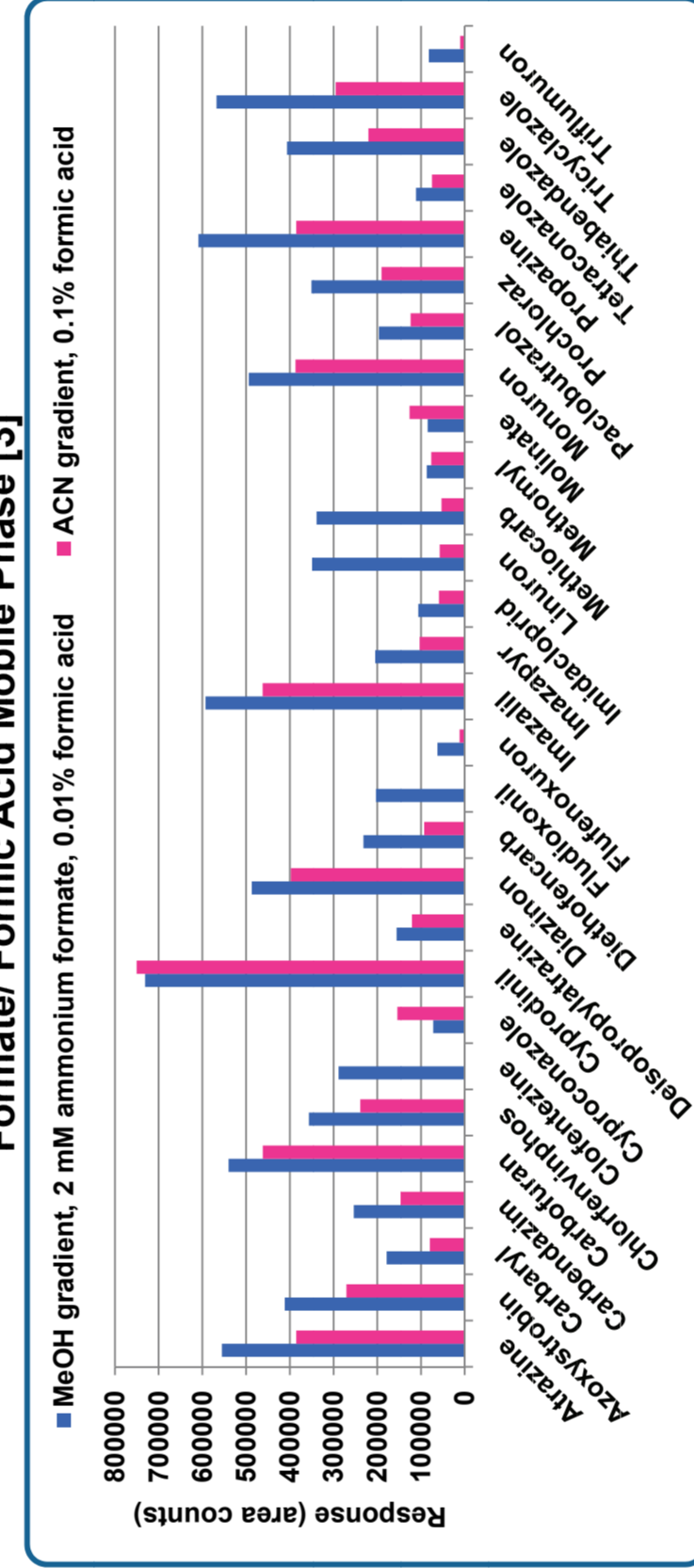
LC Conditions

Column: 2.1 x 50 mm Zorbax Eclipse Plus C18, 1.6 µm particle size, 40 °C
Mobile phase: A = 2 mM ammonium formate, 0.01% formic acid in water, B = 2 mM ammonium formate, 0.01% formic acid in methanol
Gradient: linear gradient from 5% B to 95% B from 0 min to 3 min, stop time 4 min (standard) or 10 min (samples with matrix)
Flow rate: 0.8 mL/min (maximum pressure attained was 727 bar)
Injection volume: 10 µL

MS Conditions
Scan mode: MS-only, 1700 m/z maximum mass, high resolution mode
Scan range: 100-1000 m/z
Scan rate: 3 scans/second
Agilent Jet Stream parameters: sheath gas = 12 L/min @ 400 °C, nozzle voltage = 0 V, drying gas = 10 L/min @ 325 °C
Capillary voltage: 4000 V
Fragmentor setting: 100 V
Internal reference mass correction: purine and HP-921 admitted continuously to the ionization region, producing 121.059873 and 122.059873 m/z ions for scan-by-scan correction

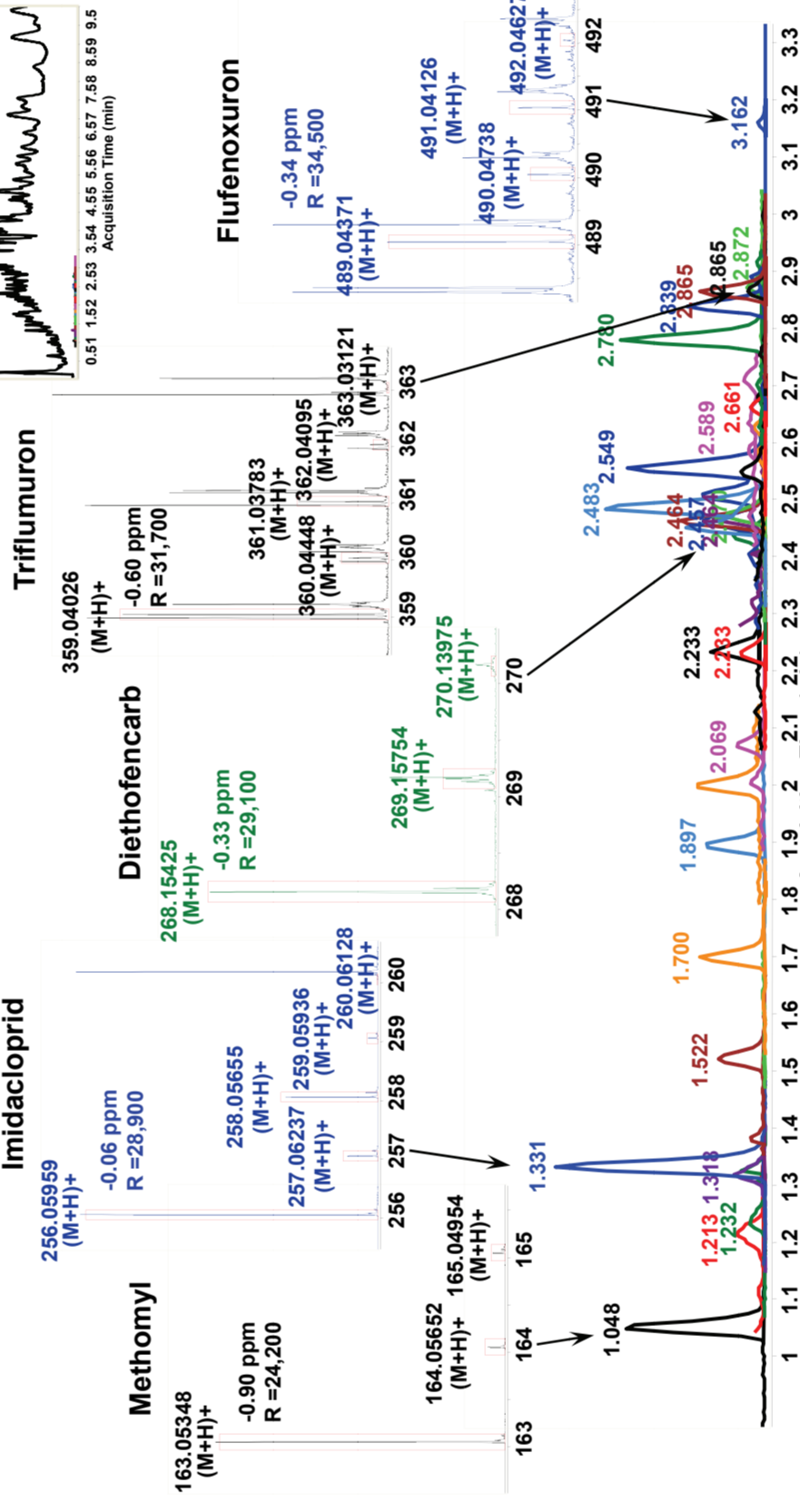
Results and Discussion

Improved Response For Standards with Methanol/Ammonium Formate/ Formic Acid Mobile Phase [3]



- Compared to the commonly-used, water:ACN gradient + 0.1% formic acid, water:methanol gradient yielded improved response [3]
- Response improved ~ 2X on average
- More than 5X improvement for Clodentazine, Fludioxonil, Flufenoxuron, Linuron, Methiocarb, and Imidacloprid
- Fludioxonil response was primarily from the ammonium adduct in the methanol mobile phase
- pH of mobile phase ~3.5 (or less) to avoid tailing and distortion of the Thibendazole peak
- Sodium adduct formation minor in all cases

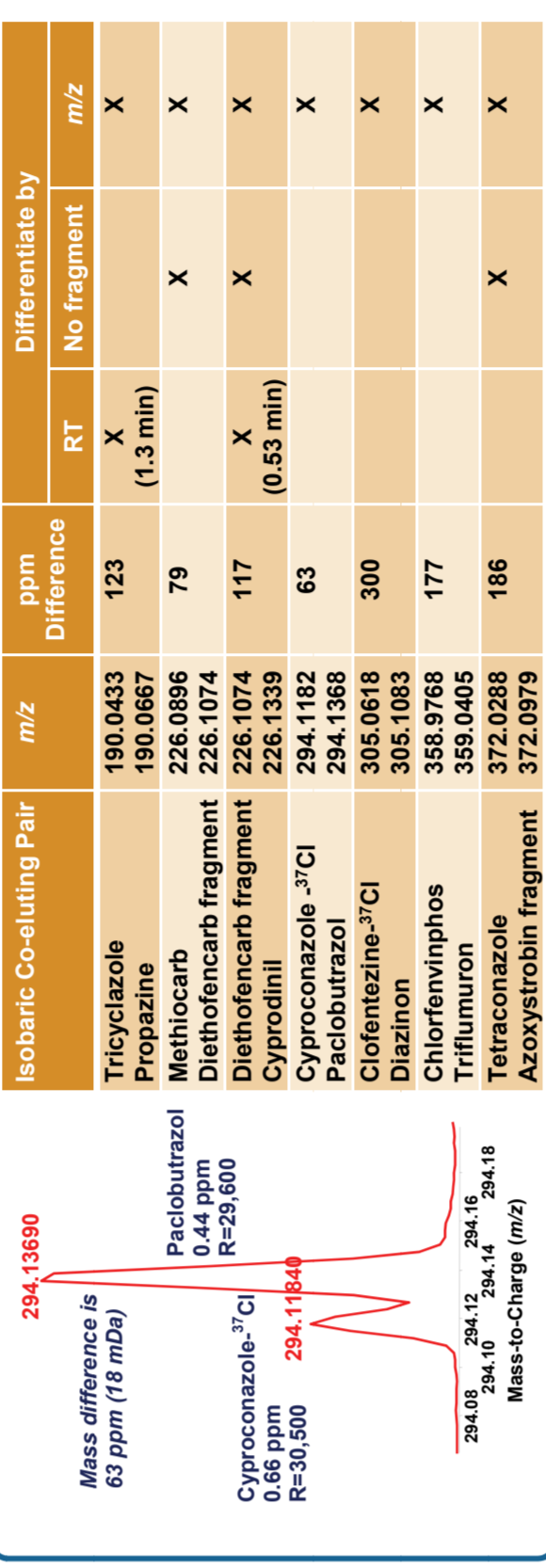
Pesticides in Pepper Matrix, 10 ppb



- All 28 analytes in the standard were found in the spiked pepper sample at the 20 µg on-column amount (10 ppb in the original produce assuming 100% recovery); red bars indicate isotopes located with Find by Formula algorithm
- Mass error of 1 ppm or less for all analytes
- Response for all analytes improved with the MeOH gradient, *vide supra*
- Trifluroxuron and Flufenoxuron, *vide supra*
- Potential matrix interferences (spectra of Diethofencarb, Trifluroxuron, and Flufenoxuron)
- Response for several pesticides was reduced in the pepper sample compared to the standard (e.g., Flufenoxuron)

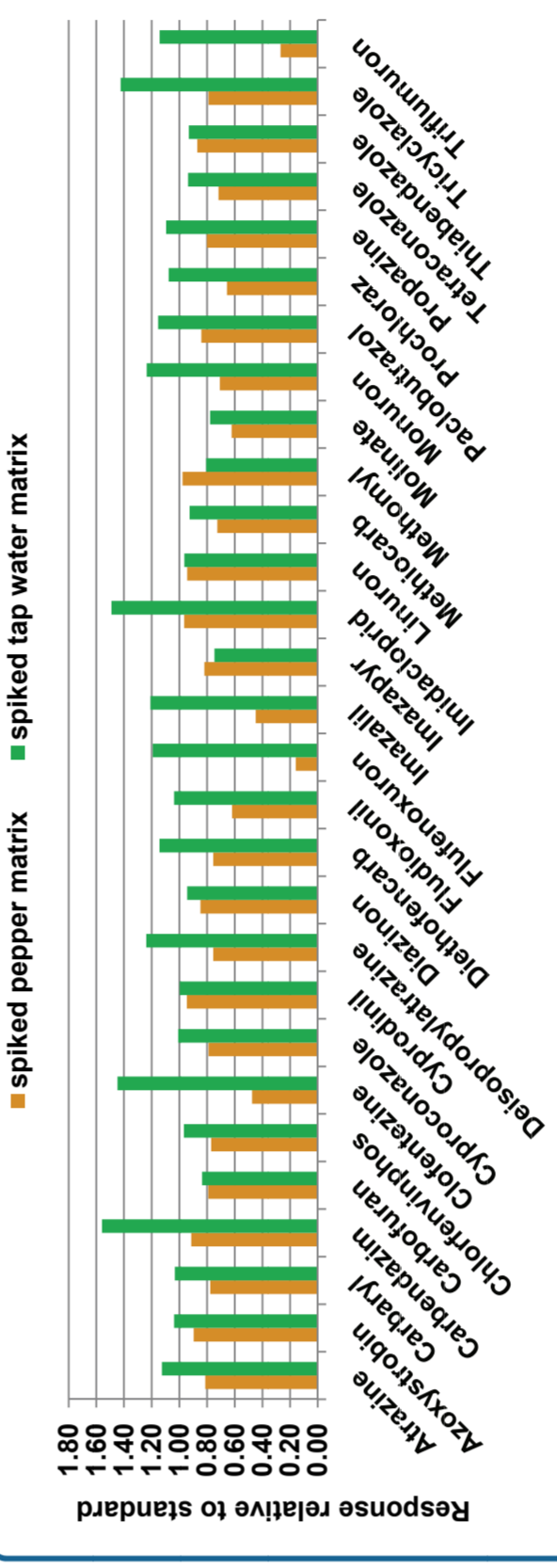
Results and Discussion

Isobaric Co-eluting Species



- Several ICS [1] were included in the samples, and all were resolved
- Two pairs were resolved by retention time differences (column and mobile phase in current work are different from those in previous work)
- Two pairs were resolved by fragmentor values to minimize fragmentation
- The mass spectrometry data were analyzed and differentiated solely by the resolving power of the mass spectrometer (such as the above case, spectrum taken from the region of overlapping peaks and identified using the Find by Formula algorithm)

Matrix Suppression Effect



- Generalization of the low response of Flufenoxuron in spiked pepper matrix—all analytes in pepper matrix show reduced response relative to the standard (usually corrected for using matrix-matched standards [4])
- Suppression effect minor or absent in tap water matrix

Conclusions

- Isobaric interferences down to ~60 ppm mass difference may be avoided by use of the higher resolution mass spectrometer
- Methanol mobile phase appears to have benefits for sensitivity and is easily implemented with an atmospheric pressure LC system, even at relatively high flow rates
- Possible future investigation topics: more pesticides, different matrices, sample preconcentration, improved sample preparation [5], reduction of suppression during ionization, matrix-matched standards [4]

References
1. M. Nishio, O. Garcia-Reyes, J.F. Molina-Diaz, A. Fernandez-Alba, R. And. Chem. 2008, 81, 915-929.
2. Ugarov, M., Stone, P.J., Perkins, P.D., J. AOAC Int. 2008, 89, 915-920.
3. Ugarov, M., Stone, P.J., Perkins, P.D., J. AOAC Int. 2008, 89, 915-920.
4. Fennel, L., Thurman, E. M., Fernandez-Alba, A. R. Anal. Chem. 2006, 77, 2916-2925.
5. Zhao, L., Schultz, D., Stevens, J., Agilent Technologies application note 5990-3075EN, August 2009.