# A UPLC/MS/MS Multi-Analyte Detection Method for Deleterious Organics in Drinking Water

Jim Krol, Sr. Applications Chemist, Waters Corporation, Milford, MA 01757 collaborating under CRADA agreement with
Larry Zintek, Sr. Chemist, and Josh Neukom, Chemist, USEPA Region 5 Chicago Regional Laboratory, Chicago, IL 60605

## Homeland Security

***Drinking Water a Public Safety Concern***

"Homeland security is a current issue and deliberate contamination, terrorist attack, of the drinking water supply is a major concern."

- Homeland Security Presidential Directive / HSPD-9

## Introduction

The EPA Region 5 Chicago Regional Laboratory (CRL) is championing an initiative to develop a robust and comprehensive monitoring protocol for drinking water quality in response to catastrophic events, intentional or unintentional. Routine drinking quality monitoring is the long term objective.

EPA Region 5 Chicago Regional Laboratory and Waters Corporation have formed a Cooperative Research and Development Agreement (CRADA) to build an LC-ESP library for deleterious organics. Currently, no transferable LC-ESP MS libraries are available, but GC/MS libraries (e.g. Wiley, NIST) are in widespread use.

This LC-ESP MS Library would provide laboratories with a tool to tentatively identify specific deleterious organics analytes in drinking water, or other environmental waters.

There are 280 deleterious organic analytes listed by the EPA as being of concern. They include commonly used agrochemicals, pharmaceuticals, and drugs of abuse, divided into 4 toxicity grouping. Groupings 1 and 2 are under development.

The appropriate strategy is a high resolution liquid chromatographic screening method with ESP MS. UPLC technology, with its 1.7 µm column particles, offers high throughput, high resolution chromatography making this screening method simple, practical, sensitive, and rapid.

To Download a Copy of this poster, Visit Waters.com/posters

## The MS/CID and MS/MS Dual Strategy for ESP Spectra

Traditional LC/MS/MS methods have been published for various multi-analyte subsets because its specificity and sensitivity, and ability to detect non-UV active analytes. The analyte identification is based upon specific analyte MS transitions and analyte retention time; but, this approach requires the use of a tandem quadrupole instrument.

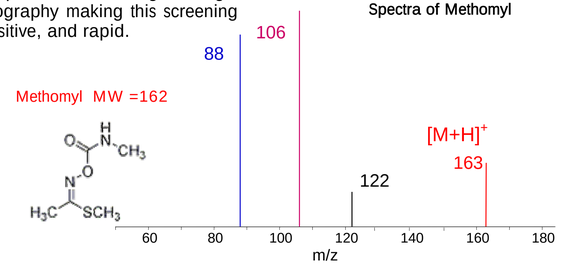
This high resolution UPLC approach provides the additional capability for a single quadrupole MS instrument to generate ESP analyte spectra along with retention time for identification similar to a tandem MS/MS instrument.

## MS Criteria for Library Spectra

For a single quadrupole instrument, the cone voltage is increased, called collision induced disassociation (CID), to cause the analyte to fragment into its product ions. The cone voltage is designed, using infusion of a 1 ppm standard in mobile phase, to give an analyte, precursor ion $[M+H]^+$ or $[M+NH_4]^+$, response at 10-25% the response of its major fragment, product ion, and if possible yield 2 or more product ions.

The full scan analyte spectra from 65 to 800 amu was acquired during a chromatographic run using a 1 ppm standard. The reagent blank spectra is subtracted out eliminate any variances due to DI water quality. This analyte ESP spectra and its retention time are entered into the library. Now analyte identification is similar to tandem analyte ESP spectra.

Figure 1: Example of MS / CID Spectra of Methomyl




## Multi-Analyte UPLC/MS/MS Method

| | |
|---|---|
| System*: | Waters ACQUITY UPLC™ System with Quattro micro™ API Triple Quadrupole, or with ZQ™ Single Quadrupole |
| Column: | Waters ACQUITY UPLC™ BEH $C_{18}$, 2.1 x 100 mm, 1.7 µm |
| Buffer A: | 5% AcCN / 5 mM $NH_4HCO_3$, pH 10 with $NH_4OH$ |
| Solvent B: | 95% AcCN / 5 mM $NH_4HCO_3$, pH 10 |
| Flow: | 300 µL / min |
| Col Temp: | 30°C |
| Gradient: | Hold at 100% A for 0.5 min<br>Linear Grad to 100% B over 10 min<br>Hold at 100% B for 2 min<br>Re-equilibrate for 5 min |
| Inj Vol: | 20 µL depending on LOQ requirements |
| Ionization: | +ESP |
| Data Proc: | Waters MassLynx™, ver 4, with ChromaLynx, QuanLynx, TargetLynx Options |

*Mention of Vendor Brands by the EPA Does Not Constitute Endorsement

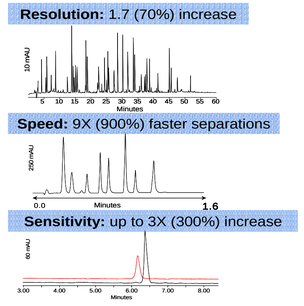
## MS/MS Criteria for Library Spectra

For an MS/MS instrument, the cone voltage of MS1 is designed, using infusion of a 1 ppm standard in mobile phase, for greatest $[M+H]^+$ or $[M+NH_4]^+$ response. The collision energy is determined giving a precursor response at 10-25% of the major product ion(s); analogous to MS/CID. The resulting analyte product ion spectra was acquired during a chromatographic run using MS/MS product ion functionality, and analyte retention time entered into the library. Analyte specific MS conditions are given below.

## Example of Analyte Specific MS And MS/MS Settings

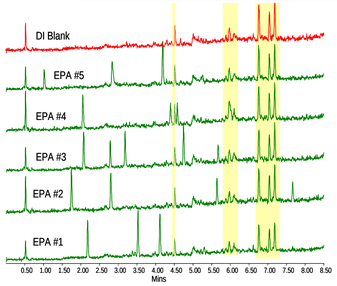
| Analyte | Mol. Wt. | Quantification Transition | MS/MS CV | MS/MS CE | MS/CID CV |
|---|---|---|---|---|---|
| Methamidophos | 141.10 | 142.14>94.02 | 24 | 17 | 44 |
| Aldicarb-Sulfoxide | 206.26 | 206.9>131.8 | 18 | 6 | 28 |
| Aldicarb Sulfone | 222.26 | 223.20>86.02 | 22 | 8 | 36 |
| Oxamyl | 219.30 | 237.0>71.8 | 12 | 6 | 15 |
| Methomyl | 162.20 | 162.8>87.8 | 12 | 6 | 25 |
| 3-Hydroxy Carbofuran | 221.26 | 220.0>162.9 | 22 | 9 | 42 |
| Aldicarb | 190.27 | 208.0>115.8 | 9 | 5 | 18 |
| Propoxur | 209.20 | 210.0>110.8 | 18 | 8 | 28 |
| Carbofuran | 221.26 | 221.9>164.8 | 21 | 12 | 38 |
| Carbaryl | 201.80 | 201.8>144.8 | 21 | 6 | 30 |
| Fensulfothion | 308.35 | 309.27>281.12 | 33 | 19 | 55 |

## UPLC™ Technology




The chromatographic VanDeemter HETP equation indicates as particle size decreases, enhance resolution can be maintained at higher flow rates. Enhanced resolution efficiency yields greater S/N sensitivity. Also, the ACQUITY UPLC ™ column technology is base resistant at pH 10 allowing for analysis of basic, cationic analytes as neutral analytes.

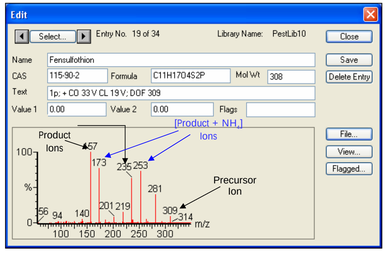
## UPLC Chromatograms Test Samples



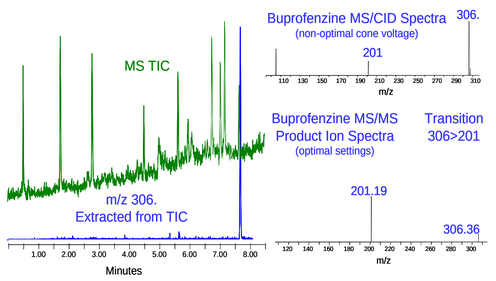

5-7 Analytes per sample at 2 ng on column

Yellow shaded area are TOC impurities from the reagents and DI Water

## Examples of Library Product Ion Spectra




## Library Spectra Example




## Current Status

The ESP Libraries currently have 100 deleterious analyte spectra which have been evaluated by several laboratories. The above buprofenzin MS/CID was scanned under a single, non-optimal cone voltage and still a first-hit library match was obtained. However, it is best to use the optimal settings for improved spectral match.

As in the case of MS scanning and library matching, MS/MS analysis will yield a confirmatory match if the scanning functions are tuned to the optimum settings, as given in the Library System, for particular analytes. Upon further confirmation via MS/MS, three levels of screening and identification data are now available:

(1) chromatographic retention time
(2) full scan mass spectrum
(3) product ion scan mass spectrum

Hence, the strategy using high-resolution UPLC/MS and UPLC/MS/MS library matching allows for a facility to screen for deleterious analytes, and incorporates present and future technologies. Analogous to the evolution from GC packed columns to GC capillary columns.

Lit Code Number 720001353EN