

Screening of Drinking Water for Deleterious Organics Using LC/MS and Automated Library Searching

Aisling M. O'Connor, Waters Corporation, Milford MA 01757

Lawrence B. Zintek, USEPA Region 5 Central Regional Laboratory, Chicago, IL 60605

INTRODUCTION

Safeguarding nation's water supply from deliberate contamination is a major issue of concern for the U.S. Department of Homeland Security. To respond to this need, the U.S. Environmental Protection Agency's (USEPA) Region 5 Central Regional Laboratory (CRL) in Chicago and Waters Corporation are working together under a Cooperative Research and Development agreement (CRADA) to develop a screening protocol for detection of deleterious organic contaminants in drinking water.

The majority of organic compounds which pose the greatest risk to human health are more amenable to LC/MS than GC/MS analysis. GC/MS spectral libraries have existed for many years but currently comprehensive transferable libraries do not exist for compounds not suitable for GC/MS analysis. The approach taken was to produce LC/MS and LC/MS/MS spectral libraries for 280 chemicals that are listed by the USEPA as being of concern. These include agrochemicals (pesticides, insecticides & herbicides), pharmaceuticals and drugs of abuse. The libraries created are transferable and compatible with all Waters single quadrupole, tandem quadrupole and TOF MS instruments.

The project goal is to provide laboratories with a quick and easy to use tool to tentatively identify these 280 compounds in drinking water without the use of sample preparation or standards. To date, the spectra of 128 compounds have been added to both the LC/MS and LC/MS/MS libraries, with the remaining 152 compounds to be added by the end of this year.

OVERVIEW OF METHODOLOGY

Part A: Library Creation

- Create LC/MS-CID Library
- Create LC/MS/MS Library

Part B: Sample Screening (LC/MS)

- LC/MS analysis of sample with MS scan functions at various cone voltages
- Manually or automatically search LC/MS-CID library for possible hits

Part C: LC/MS/MS Analysis

- LC/MS/MS analysis of sample with MS product ion scan functions for tentative hits
- Manually or automatically search LC/MS/MS library to corroborate or exclude compounds identified from the LC/MS analysis.

LC/MS LIBRARY CREATION

The use of in-source Collision Induced Dissociation (CID) allows the use of single quadrupole mass spectrometers to produce spectra that provide both precursor and product ion information. CID is carried out by increasing the cone voltage causing the analyte to fragment into its product ions.

For the creation of the LC/MS-CID library spectra, a solution of the compound was infused and the cone voltage optimized to create spectra which contained characteristic fragmentation while maintaining the integrity of the molecular ion at 10–25% to insure accurate tentative identification.

The spectra added to the library were generated by injecting 25ng of the compound on column and acquiring full scan spectral data from 70–800 m/z at the optimum cone voltage. Retention time (RT) and cone voltage (CV) information are added to the library entry, for use as filters for automated library searching. Figure 1 shows the CID spectrum of one of the library entries, nicotine.

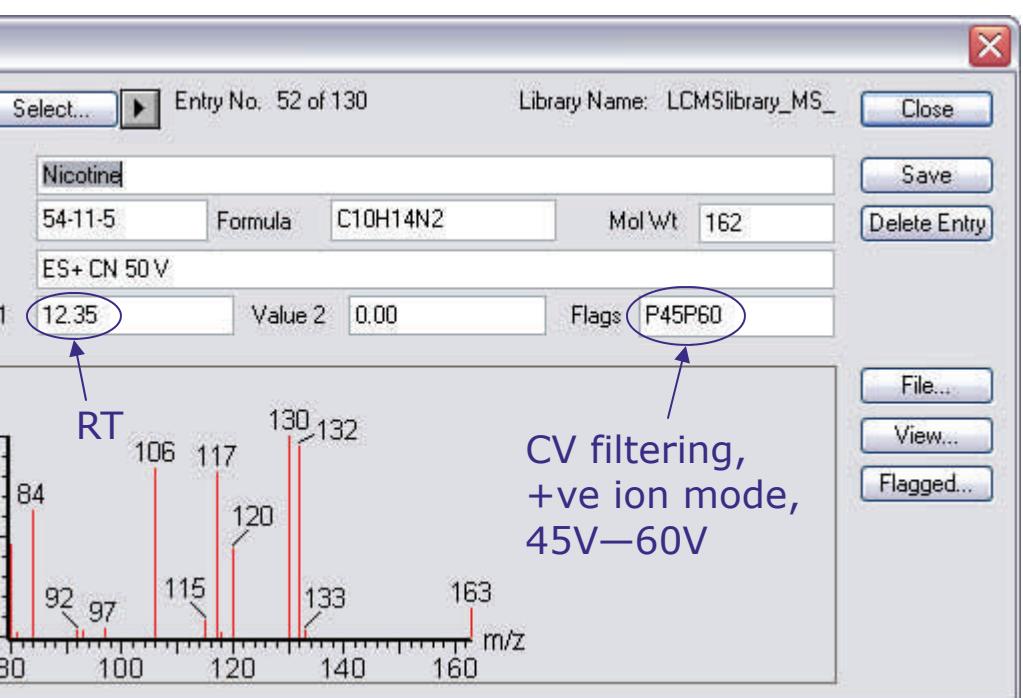


Figure 1: LC/MS library spectrum for Nicotine

LC/MS/MS LIBRARY CREATION

A solution of the compound to be added to the library was infused and the CV optimized to gain the greatest response for the molecular ion. The collision energy was optimized to create spectra with at least 2 product ions while maintaining the integrity of the molecular ion at 10–25%.

The spectra added to the library were generated by injecting 25ng of the compound on column and acquiring product ion spectral data from 70 m/z to 50 m/z > molecular ion at the optimum cone voltage and collision energy for that compound. The LC/MS/MS spectrum obtained for nicotine is shown in Figure 2 below.

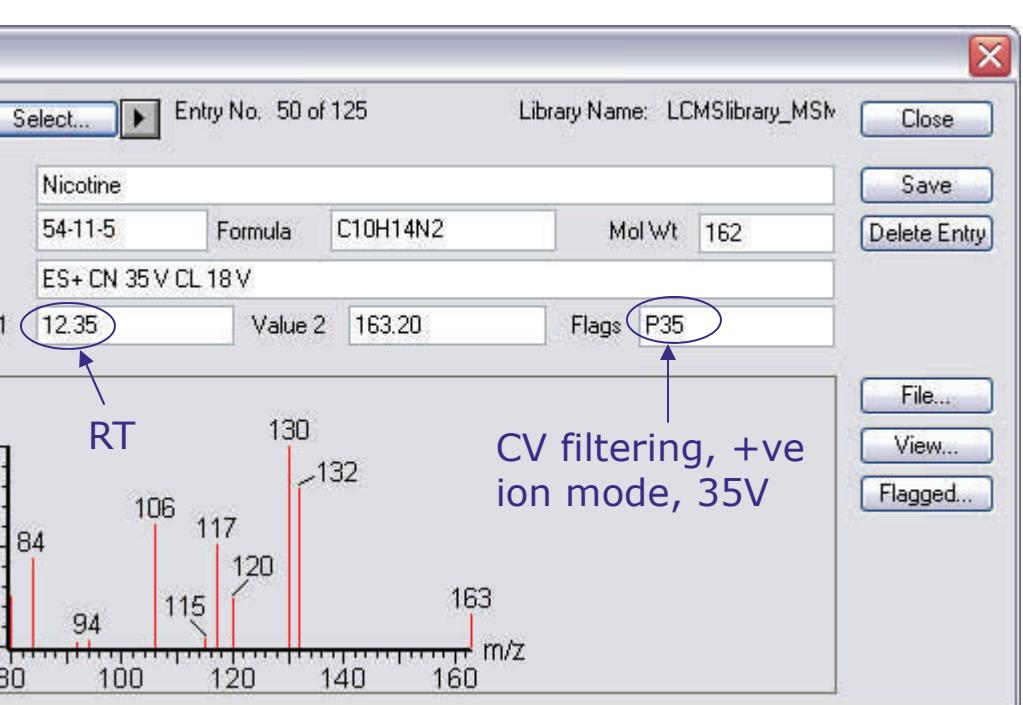


Figure 2: LC/MS/MS library spectrum for Nicotine

SAMPLE SCREENING

Water samples are filtered prior to analysis to remove particulate matter. LC/MS analysis of the water sample is carried out using MS scan functions at various cone voltage settings. LC and MS conditions are outlined below.

LC Conditions

- Waters XBridge™C18 3.5 μ m, 2.1x150mm Column
- Solvent A: 5% ACN/5mM NH₄HCO₃, pH= 10
- Solvent B: 95% ACN/5mM NH₄HCO₃, pH= 10
- Linear Gradient from 100% A to 100% B over 20 minutes. Hold at 100% B for 2 minutes and re-equilibrate for 10 minutes
- Flow rate: 0.3 mL/min
- Injection volume: 100 μ L

MS Conditions

- 7 scan functions, 70 – 800 m/z at the following cone voltages: ES- 30V and ES+ 15V, 30V, 45V, 60V, 75V & 90V

AUTOMATED LIBRARY SEARCHING: CHROMALYNX™

Manual searching of spectral libraries is a time consuming task and requires a knowledgeable user. Waters ChromaLynx software automates the processing of LC/MS/(MS) data. The ChromaLynx chromatographic deconvolution algorithm allows efficient peak location, peak detection and generation of clean mass spectra. The deconvoluted spectra can be automatically searched against libraries using retention time and cone voltage filtering. The candidate list of identified compounds, chromatograms, component spectra, library spectra and library match factors are all viewable in a customizable browser. The use of ChromaLynx has reduced the processing time from 30 minutes to 0.5 minutes per sample. Figure 3 shows a ChromaLynx dataset for the screening analysis of a spiked water sample. The match factors for all 5 compounds identified are >700 indicating good matches with the library spectra.

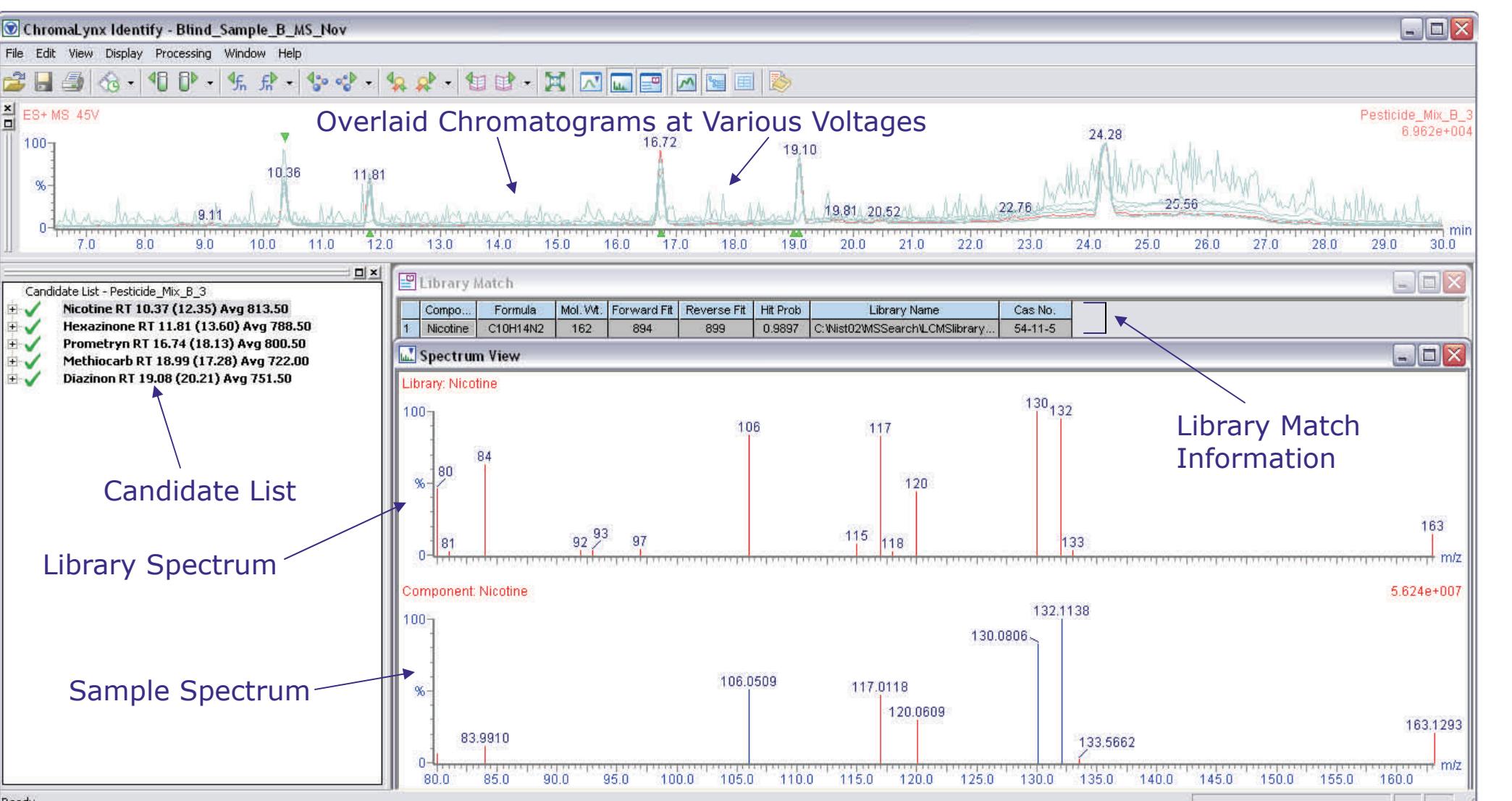


Figure 3: ChromaLynx dataset for LC/MS screening analysis of a water sample spiked with Nicotine, Hexazinone, Prometryn, Methiocarb and Diazinon

LC/MS/MS ANALYSIS

LC/MS/MS product ion scan analysis is carried out on compounds tentatively identified by the LC/MS analysis. This data can then be searched against the LC/MS/MS library, to corroborate or exclude compounds identified from the LC/MS analysis. LC and MS conditions are outlined below.

LC Conditions

- As for LC/MS analysis

MS Conditions

- Set up a product ion scan for each compound identified from the LC/MS analysis
- Scan start mass = 70 m/z and end mass approx. 50 m/z > precursor ion
- Use optimum cone voltage and collision energy for each compound
- Precursor ion should be accurate to 0.1 m/z
- Scan start and end times centered around RT from LC/MS analysis

CONCLUSIONS

- A protocol has been developed for screening deleterious organic compounds in drinking water
- Transferable LC/MS-CID and LC/MS/MS spectral libraries have been created for 128 of the 280 compounds listed as of concern by the USEPA. The remaining compounds will be added to the libraries later this year
- The spectral libraries are compatible with all Waters quadrupole and TOF mass spectrometers as well as HPLC and UPLC
- Sample screening is carried out by LC/MS and optional LC/MS/MS is used for exclusion or corroboration of identified analytes
- Automated searching of libraries using ChromaLynx software is faster and easier than manual searching