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An LC/MS/MS Multi-Analyte Detection Method for Deleterious Organics in Drinking Water

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Homeland Security
Dealing with our Drinking Water

"The focus is on catastrophic terrorism—threats to the security of our homeland that could result in large-scale loss of life and major economic impact."
http://www.dhs.gov/dhspublic/theme_home5.jsp

"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

Initial EPA Region 5 60 Deleterious Analyte List

Acidic Analytes:

2,4-D	Cacodylic Acid	Dalapon	Endothall
Fenamiphos	Glyphosate	Methidathion	Picloram	Silvex

Neutral Analytes:

1-Naphthol	3-Hydroxycarbofuran	Alachlor	Aldicarb
Aldicarb Sulfone	Aldicarb Sulfoxide	Azinphos-Methyl	Bromadiolone
Buprofenzin	Carbaryl	Carbendazim	Carbofuran
Chlorpyrifos-methyl	Coumaphos	Cyclohexamide	Dichlorvos
Dimethoate	Diuron	EPN	Ethoprophos
Fenhexamid	Fensulfothion	Methamidophos	Methiocarb
Methomyl	Mevinphos	Oxamyl	Propoxur
Pyridaben	Pyridaphenthion	Scilliroside	Tebufenpyrad
Tebufos	Thiobencarb		

Basic Analytes:

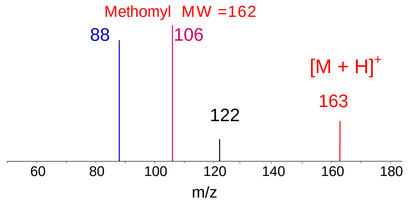
2-Aminobenzimidazole	Cyanizine	Cyprodinil	Dicrotophos
Imazalil	Imidacloprid	Nicotine	Pendimethalin
Pirimiphos-methyl	Propamocarb	Strychnine	Tebuconazol
Terbumeton	Terbuthylazine	Thiabendazole	Toxaphene
Chloropicrin			

Homeland Security Directive HSPD-9 EPA Initiative

- EPA Region 5 CRL is undertaking an initiative to develop a robust, comprehensive, and fully coordinated surveillance and monitoring system for drinking water quality that provides early detection and awareness of poisonous (deleterious) agents.
- EPA Region 5 CRL and Waters Corporation entered into a Cooperative Research and Development Agreement (CRADA) to develop a HPLC method utilizing Mass Spectrometry using MS/MS Spectral Library Confirmation.
  - –No transferable LC/MS Libraries are available for widespread use
- LC/MS method to provide laboratories with a screening tool to quickly identify deleterious organic contaminants in drinking water, and final quantification using LC/MS/MS to confirm identification.
  - –280 analytes are listed into 4 phases based on toxicity;
    - Commonly used agrochemicals, pesticides and insecticides
    - Drugs of abuse, nicotine, LSD, methamphetamine, ecstasy

MS Criteria for Library Spectra

- The cone voltage for a single quadrupole system was determined as giving a precursor ion $[M+H]^+$ response at 10-25% response of the major product ion, and, if possible, yield 2 or more product ions.
- MS/CID Analyte full scan Spectra acquired during a chromatographic run of 1 ppm analyte standard in mobile phase.
- MS/CID Full Scan Spectra and relative retention time entered into library entry for 2 variable analyte confirmation.
- Results for single quadrupole yield equivalent results, and should be considered as a lower cost system alternative.
  - –Single quadrupole full scan analysis is however less sensitive than product ion MS/MS



The MS/CID and MS/MS Dual Strategy

- LC/MS/MS methods have been established for various multi-analyte subsets
  - –MS/MS specificity and sensitivity in complex matrices
  - –Detection of non-UV active analytes
- Subset Analytes into Acidic, Neutral, and Basic Groupings
  - –Acidic pH for acidic analytes with -ESP
  - –Neutral pH for neutral analytes with ±ESP
  - –Basic pH for basic analytes with +ESP
  - –Minimize other ionization techniques, such as APCI, APPI, etc
- Analyte identification based on chromatographic retention time and MS/MS ESP Product Spectra, i.e., comparison of unknowns against a full scan analyte MS/MS product spectra for each analyte
  - –Similar to Photodiode Array spectral processing
  - –Analyte confirmation using conventional MS/MS transitions possible
- LC/MS Screening method
  - –Create full scan Libraries with Single Quadrupole MS/CID
  - –Establish cone voltage settings for analysis, and processing method parameters
- Analysis time less than 25 minutes with little sample preparation

MS/MS Criteria for Library Spectra

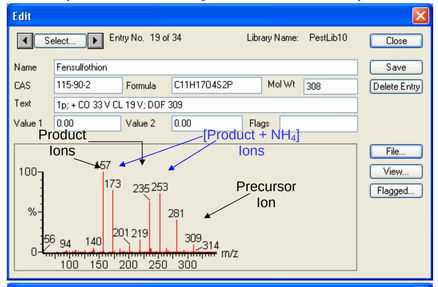
- The cone voltage for MS1 was determined at greatest $[M+H]^+$ or $[M+NH_4]^+$ response using a 1 ppm pure standard prepared in mobile phase.
- The collision energy was determined giving a precursor ion [M+H] response at 10-25% response of the major product ion, and, if possible, yield 2 or more product ions.
- MS/MS Analyte Spectra acquired during a chromatographic run of 1 ppm analyte standard in mobile phase using daughter ion MS/MS functionality.
- MS/MS Product Ion Spectra and relative retention time entered into library entry for 2 variable analyte confirmation.

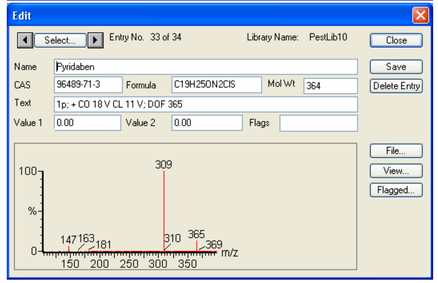
Multi-Analyte LC/MS/MS Method Neutral pH "Universal Gradient"

System*:	Waters Alliance® 2695 System with Quattro micro™ API Triple Quadrupole, or with ZQ™ Single Quadrupole
Column:	Waters Atlantis™ C_{18}, 2.1 x 150 mm, 3.5 µm
Buffer A:	5% AcCN / 5 mM NH_4OAc, natural pH
Solvent B:	95% AcCN / 5 mM NH_4OAc
Flow:	300 µL / min
Col Temp:	30°C
Gradient:	Hold at 100% A for 1 min Linear Grad to 100% B over 20 min Hold at 100% B for 5 min Re-equilibrate for 10 min
Inj Vol:	100 µL depending on LOQ requirements
Ionization:	ESP
Data Proc:	Waters MassLynx™, ver 4, with ChromaLynx, QuanLynx, TargetLynx Options

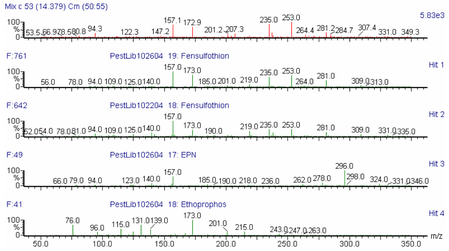
*Mention of Vendor Brands by US EPA Does Not Constitute Product Endorsement

Examples of Library Product Ion Spectra





Low Level 1 ppb Fensulfothion in Drinking Water



Library Evaluation Summary

- The created ESP MS/MS and MS/CID Library have been evaluated by 2 outside laboratories using the same brand of triple quadrupole instrument.
  - MS/MS (Product Ion) Spectra using Triple Quadrupole
    - –All of the analytes can be successfully detected at 100 ppb
    - –All of the analytes can be successfully detected at 25 ppb
    - –10 of 29 can be successfully detected at 1 ppb
  - MS/CID Spectra Library
    - –25 out of 29 analytes can be successfully detected at 100 ppb
    - –Detection limit is compound dependent and varies
    - –1 ppb is below detection limit for all MS/CID Library work
    - –Analyte co-elution will bias the MS/CID Spectra because of additional co-elution ion fragments.
      - To be addressed using ChromaLynx deconvolution spectral processing successfully used for GC/MS.
- Improvements and additions to the libraries are continually being made as more method research is conducted.
  - –Adding additional analytes; acidic and basic analytes
    - Goal to have 200-300 analytes of homeland security concern
  - –Adapt method protocol and libraries to single quadruple instruments using in-source CID
    - Lower cost alternative for field labs
- Improvement in detection sensitivity and spectral quality
  - –Analyte concentrations in drinking water are dependent upon the action limits defined as threat agent concentration.
  - –Analyte enrichment using SPE may be necessary