Matrix Effects in Multiresidue Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometric Analysis of Emerging Organic Pollutants

45th Florida Pesticide Residue Workshop

Paul Yang, Chunyan Hao, Xiaoming Zhao and Bick Nguyen

Laboratory Services Branch
Ontario Ministry of the Environment
Toronto, Canada

Outline

- Background information
- What are emerging organic pollutants?
- Analysis of emerging organic pollutants using liquid chromatography/electrospray ionization tandem mass spectrometry (LC/MS-MS)
- Phenomena of matrix effects
  - Sample type & volume
  - Extraction pH and storage
  - Mode of analysis
- Conclusion

Background − Ontario

- Ontario, Canada, is a province of:
  - densely populated urban centres
  - highly developed agricultural areas
  - cottage areas, and remote fishing and hunting
  - recreational waters with 1.5 million miles of shore line
- Population is approximately 13 million residents.
- Ontario has a total area of 420,000 square miles, 11% of the total of Canada.

Ontario − Geographic Makeup

TUNDRA

FORESTED AREA

AGRICULTURE

MANUFACTURING

Who Are We?

Laboratory Services Branch (LaSB)
Ontario Ministry of the Environment (MOE)

Our Vision

"To be an analytical science and service leader in the environmental community through the investment in our dedicated staff and the most efficient use of technology."

Our Mission

"The mission of the Laboratory Services Branch is to be a respected scientific leader and provider of analytical science and services to support environmental programs and regulations for the purpose of protecting the people and environment of Ontario."

Emerging Organic Pollutants (EOPs)

- Occur in the environment as a result of human and/or anthropogenic activities
- Minimal knowledge of their background levels and effect in the environment and no guidelines/regulations are set
- Anticipated risks for environment and human health – alone or synergistically

New pollutants
Unknown issues

New concerns
Old pollutants

EOPs Include

- Algal toxins and pathogens
- Brominated flame retardants
- Disinfection by-products
- Pesticides and their environmental metabolites
- Naphthenic acids
- UV stabilizers (benzotriazoles) and filters (benzophenone, cinnamate, and octocrylene)
- Perfluoroorganic compounds
- Perchlorate, organotins

Richardson, S. D.; Ternes, T. A. Water analysis: emerging contaminants and current issues. *Anal. Chem.* **2005**, *77*, 3807-3838.

Pharmaceuticals and Personal Care Products (PPCPs)

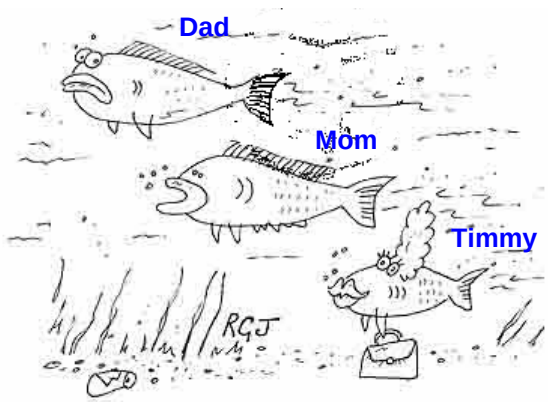
- Analgesics
- Lipid regulators
- Antibiotics
- Blood pressure
- Anti-depressants
- Anti-epileptics

Pharmaceuticals and **Personal Care Products**

- Fragrances
- Antimicrobials
- UV filters
- DEET
- Surfactants (e.g., laundry and dishwashing)

Endocrine Disrupting Compounds (EDCs)

- Synthetic estrogens
- Bisphenol A
- Phthalates
- 69 pesticides and four pesticide formulation synergists (*draft list for EDC screening, Federal Register (2007), 72 (116) pp. 33486-33503)*



Major Pathways of Entry into Environment

Reported Effects of PPCPs and EDCs

- Kidney failure in fish
- Inhibition of xenobiotic excretion in mussels
- Reduced egg viability in fish
- Changes in algae and cyanobacteria populations
- Fish and vulture population decline

To Advance the Understanding of the Environmental Effects of EOPs

In need of an efficient, reliable multiresidue method to address the occurrence, concentration, fate, and support toxicity studies of EOPs

Challenges in EOP analysis

- Relatively new and can form conjugates and/or metabolites during the analysis
- Published methods usually focused on a limited number of compounds
- Limited isotope-labeled compounds available as method surrogates
  - Limited method validation data
  - Limited quality control procedures to ensure data quality
- Matrix effects (phenomena in LC/MS EOP analysis)

Hao, C.; Clement, R.; Yang, P. Liquid chromatography–tandem mass spectrometry of bioactive pharmaceutical compounds in the aquatic environment—a decade's activities. *Anal. Bioanal. Chem.* **2007**, *387*, 1247–1257

LaSB EOP Analytical Method

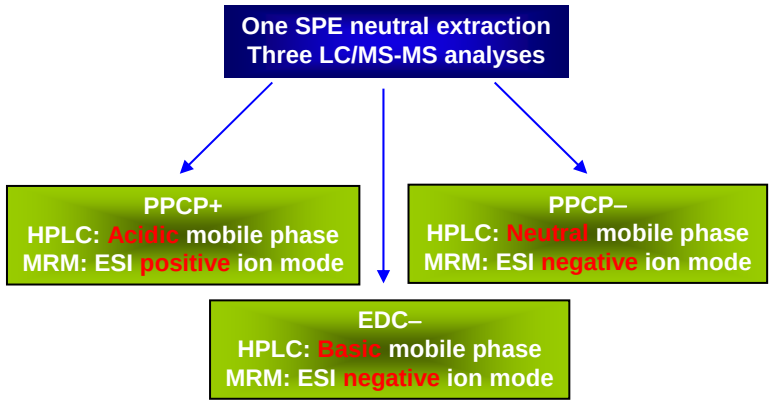
- **PPCPs (Pharmaceuticals and Personal Care Products):**
  - Defined by chemical classes
  - Human, veterinary drugs, diagnostic agents, biologics, nutraceuticals, fragrances, sun-screen agents, etc.
- **EDCs (Endocrine Disrupting Chemicals):**
  - Defined by biological effects or mechanisms
  - Substances that can act like natural hormones
- **Surfactants:**
  - Nonylphenol and its ethoxylate isomers
  - Perfluorinated surfactants PFOS, PFOA, PFBS, etc.

Chunyang Hao, Xiaoming Zhao, Shahram Tabe and Paul Yang
Environ. Sci. Technol., 42 (11), 4068–4075, 2008.

Parameters Analyzed

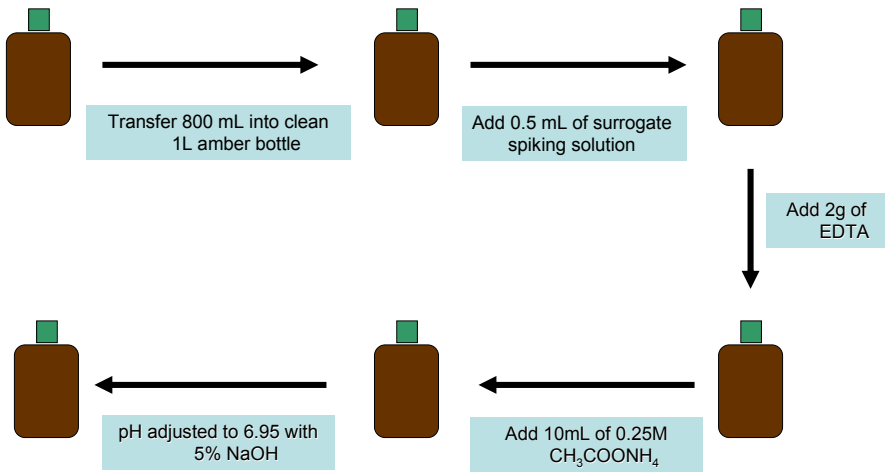
1. Antibiotics − human (19) & veterinary (5)
2. EDCs/hormones (9)
3. PCPs (5) − surfactants & plasticizers
4. Perfluoroorganic compounds (3)
5. Pharmaceuticals (10) – analgesics, lipid regulators, antiepileptic

MOE Multi-Residual Method (E3454)



SPE: Solid Phase Extraction
MRM: Multiple Reaction Monitoring
ESI: Electrospray Ionization

Sample Preparation:



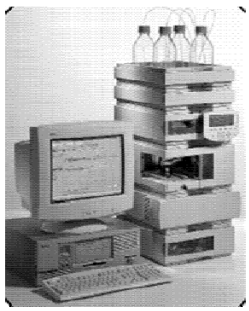
Sample Extraction/Elution:

- 800 mL water samples were extracted using solid-phase extraction (SPE) (*i.e.* Waters® HLB cartridges)
- methanol was used to elute the analytes from the cartridges

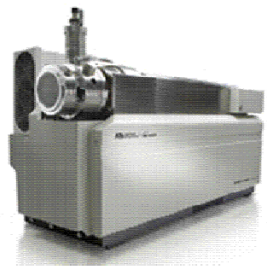
LC/MS-MS Analysis of EOPs

Instrument

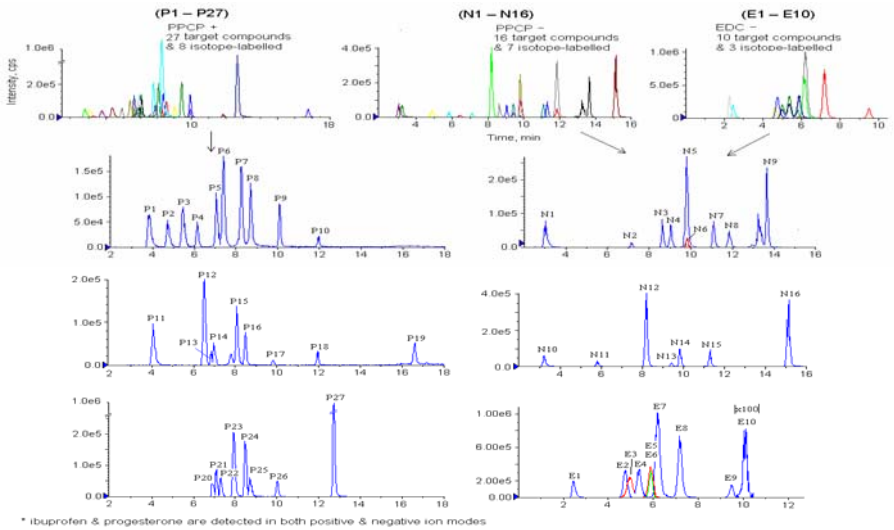
HPLC: Agilent HP1100 chromatogram separation



MS: MDS Sciex 4000QTRAP, capable of multiple reaction monitoring (MRM) detection & enhanced product ion scan (EPI, ion trap full spectral scan)



Typical Chromatograms



* ibuprofen & progesterone are detected in both positive & negative ion modes

Method Detection Limits (MDLs)

PPCP+

Compound Name	MDL (µg/L)
Monensin	0.10
Meclocycline	0.20
Tetracycline	0.23
Lincomycin	0.04
Erythromycin	0.17
Roxithromycin	0.11
Trimethoprim	0.02
Chlorotetracycline	0.81
Sulfadimethoxine	0.05
Sulfamerazine	0.11
Sulfamethazine	0.05
Sulfathizaole	0.17
Oxytetracycline	0.30
Sulfamethizole	0.08
Sulfachloropyridazine	0.18
Sulfamethoxazole	0.09
Carbamazepine	0.01
Penicillin G	2.15
Doxycycline	0.13
Ciprofloxacin	0.12
Tylosin Tartrate	0.31
Naproxen	0.03
Ketoprofen	0.02

PPCP-

Compound Name	MDL (µg/L)
Chloramphenicol	0.02
Gemfibrozil	0.04
Ibuprofen	0.14
Bezafibrate	0.01
Diclofenac	0.04
Indomethacin	0.66
Virginiamycin M1	0.75
Sulfadiazine	0.26
Carbodox	0.20
Clofibirc Acid	0.06
4-Acetamidophenol	0.09
Warfarin	0.03
Amoxicillin	0.47
Lasaloid A	0.28
PFBS	0.02
PFOS	0.03
PFOA	0.04

EDC

Compound Name	MDL (µg/L)
Bisphenol A	0.11
Diethylstilbestrol	0.85
17-α-Estradiol	0.40
Esterone	0.41
Equiline	0.30
19-Norethersterone	0.37
17-α-Ethynylestradiol	0.40
Progesterone	15.28
Estriol	0.27
17-β-Estradiol	0.20

Characteristics of the MOE Method

- Method optimized for sample extraction and analysis
  - Preservatives, extraction pH, storage
  - Optimized separation and electrospray ionization modes
- Use a stringent quality control (QC) protocol to ensure the delivery of good and consistent quality assurance data
  - QC samples account for more than 35% of workload
  - Use duplicate sample matrix spikes to control method recoveries and within run precision
- Use isotope dilution mass spectrometry based quantitation whenever possible

Isotope-labeled Standards

$^{2}H_{10}$-carbamazepine
$^{13}C^{15}N$-ciprofloxacin
$^{13}C_{3}$-ibuprofen
$^{2}H_{3}$-ibuprofen
$^{13}C^{2}H$-naproxen
$^{2}H_{9}$-progesterone
$^{13}C_{6}$-sulfamethazine
$^{13}C_{6}$-sulfamethoxazole
$^{2}H_{4}$-acetaminophen
$^{2}H_{4}$-clofibric acid
$^{2}H_{4}$-diclofenac
$^{2}H_{6}$-gemfibrozil
$^{2}H_{4}$-indomethacin
$^{2}H_{16}$-bisphenol A
$^{2}H_{4}$-equilin
$^{2}H_{4}$-estrone

Applications of the MOE Method

1. The MOE has been engaged in research initiatives involving PPCPs since 2002
2. Low levels of PPCPs have been detected in the Ontario environment in all environmental media, biosolids, manures, surface water, drinking water, wastewater influent and effluent
3. Currently supporting several research projects on PPCPs in wastewater, surface water, drinking water treatment technologies, and PPCP metabolites from treatment process

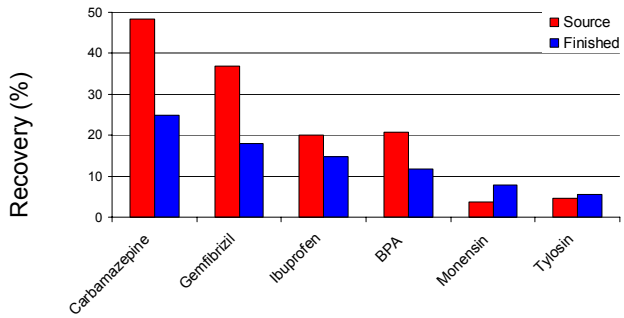
Sampling Sites

$Na_2S_2O_3$ used as preservative

Preserved by $Na_2S_2O_3$ + Formaldehyde

Detected Parameters

- 26 of the 51 parameters were detected in either source or finished waters in low ng/L range.
- The most frequently detected compounds



Parameters not detected

- 26 of the 51 parameters were detected in either source or finished waters.

17a-ethynyl estradiol
17b-estradiol
Estrone
Diethyldtilbesterol
Warfarin
Chloramphenicol
Chlortetracycline
Ciprofloxacin
Doxycycline
Penicillin G
Lasalocid A (V)
Sulfadimethoxine (V)
17a-estradiol
Estriol
Progesterone
Indomethacin
Amoxacillin
Sulfadiazine sodium
Sulfamethizole
Sulfamerazine
Virginiamycin M1
Carbadox (V)
Sulfosalinicylate

Matrix Effects

target compound signal

ESI ionization efficiency (±)

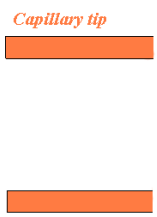
sample type
sample volume
sample pH
storage

matrix component
composition
& amount

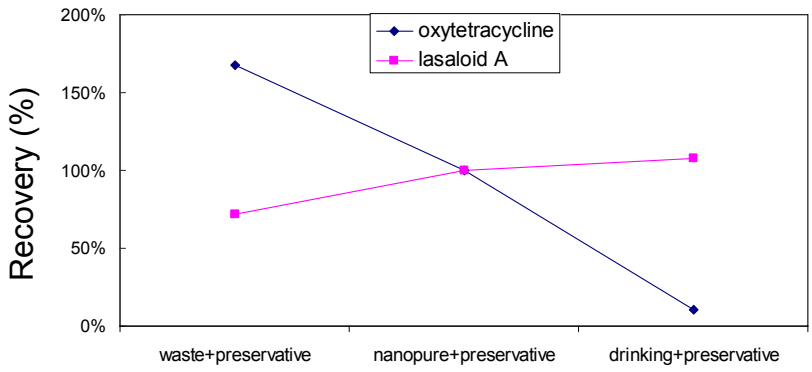
chemical
environment

Electrospray Ionization Interface of LC/MS-MS

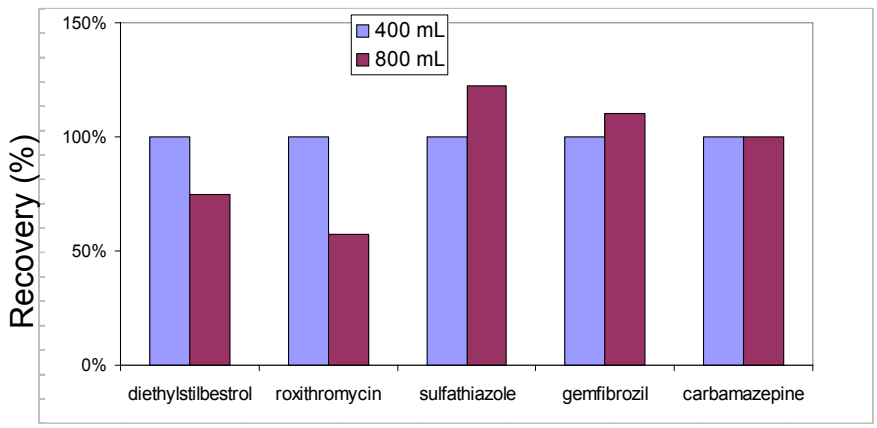
- Soft ionization: transfer ions from solution to gas phase
- Charged droplets formed under the effect of a high potential; droplets shrink: solvent evaporation and uneven fission; generation of gas phase ions: charge residue or ion evaporation model
- Ionization efficiency affected by voltage & chemical environment, e.g., pH, solvent, chemical composition, etc.



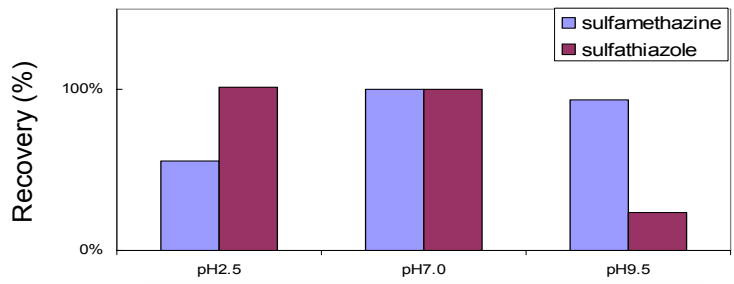
Phenomenon 1: sample type effect

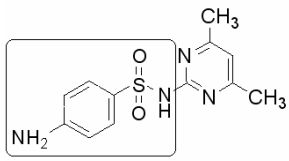


Phenomenon 2: sample volume effect

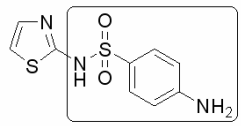


Phenomenon 3: sample pH effect



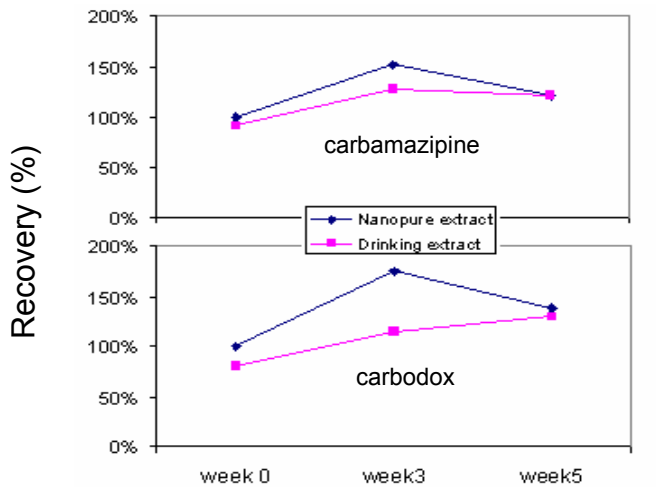


sulfamethazine

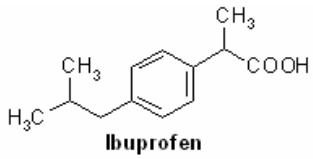


sulfathiazole

Phenomenon 4: sample extract storage



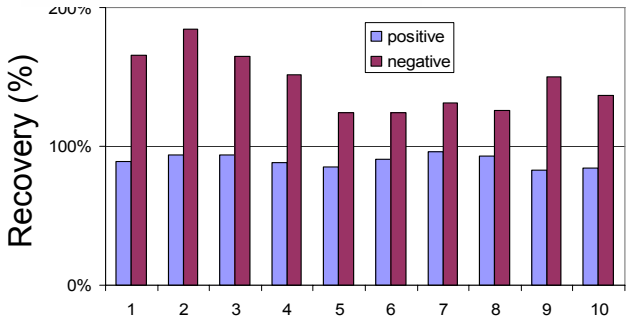
Phenomenon 5: ionization mode



Ibuprofen
MW 206.29

205/161 $[M–H]^-$/ $[M–H–COO]^-$

207/161 $[M+H]^+$/ $[M+H–HCOOH]^+$



Ibuprofen,
Sept–Dec'05

Eliminate Matrix Effects during ESI

- Sample cleanup
- Better separation
- Standard addition: standards under same effect

All need extra work, except

- Isotope dilution mass spectrometry which uses isotope-labeled (^{15}N, ^{13}C & ^{2}H) standards to compensate for matrix effect

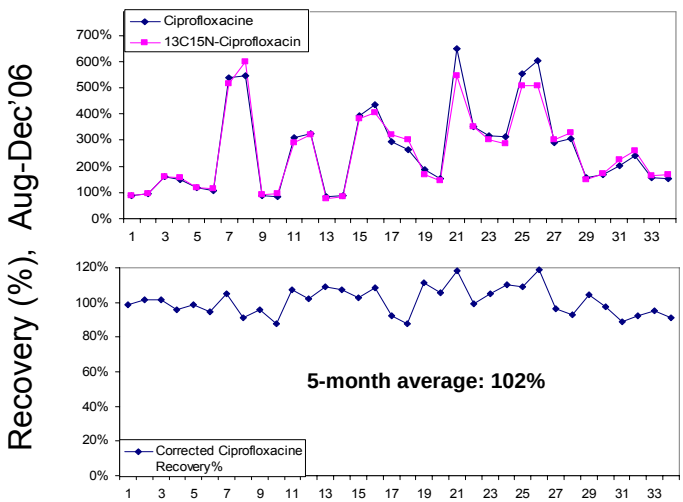
Chunyan Hao, Xiaoming Zhao, and Paul Yang; Trends in Analytical Chemistry, **2007**, 26 (6):569-580.

Isotope-labeled (^{15}N, ^{13}C & ^{2}H) Standards

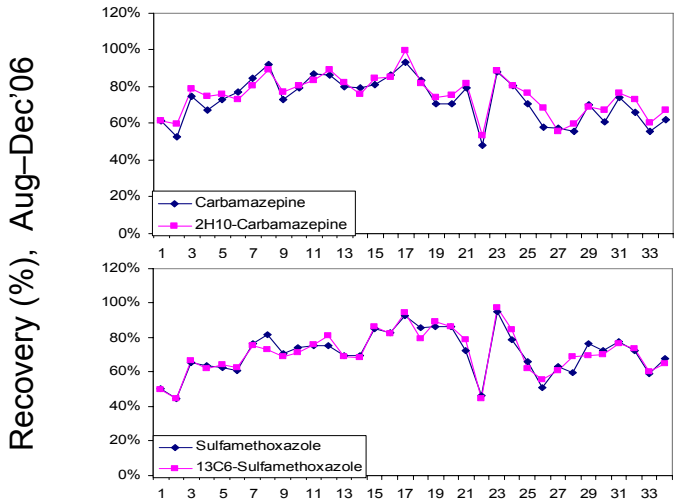
- Same chemical nature as native compounds, but different mass
- Same retention time, different MRM channel

$^{2}H_{10}$-carbamazepine, $^{13}C^{15}N$-ciprofloxacin, $^{13}C_{3}$-ibuprofen, $^{2}H_{3}$-ibuprofen, $^{13}C^{2}H$-naproxen, $^{2}H_{9}$-progesterone, $^{13}C_{6}$-sulfamethazine, $^{13}C_{6}$-sulfamethoxazole, $^{2}H_{4}$-acetaminophen, $^{2}H_{4}$-clofibric acid, $^{2}H_{4}$-diclofenac, $^{2}H_{6}$-gemfibrozil, $^{2}H_{4}$-indomethacin, $^{2}H_{16}$-bisphenol A, $^{2}H_{4}$-equilin, $^{2}H_{4}$-estrone

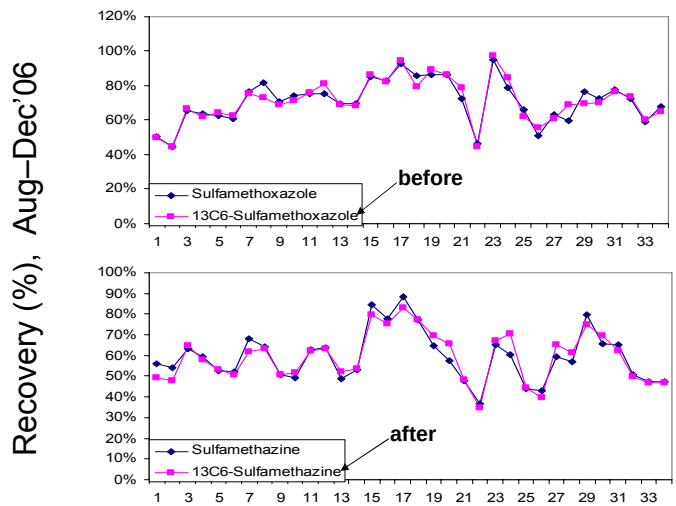
Isotope Dilution Correction



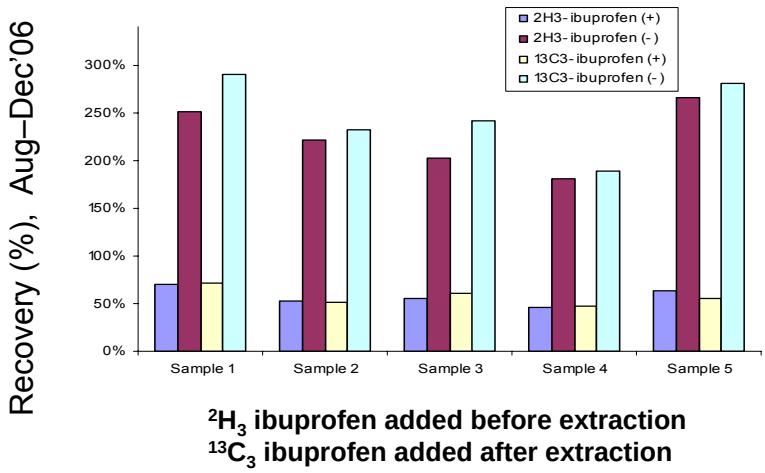
^{13}C or ^{2}H ?



Before or After Extraction

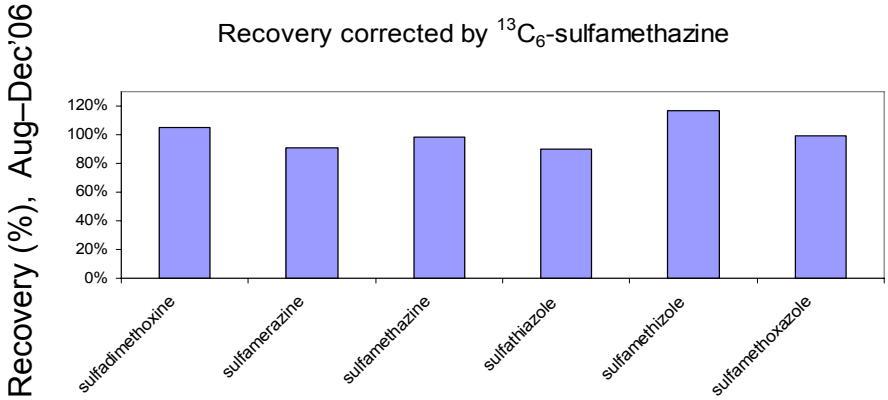


Before or After & Positive or Negative



$^{2}H_3$ ibuprofen added before extraction
$^{13}C_3$ ibuprofen added after extraction

Same or Similar Structure



Summary of Quality Assurance Data

Compound Name	N	Before Correction		After Correction	
		%R	%RSD	%R	%RSD
Carbamazepine	78	73.3	16.3	96.0	5.9
Ciprofloxacin	34	263.7	62.3	100.7	8.2
Ibuprofen	76	89.1	12.0	104.6	11.1
Ibuprofen	76	89.1	12.0	106.1	10.3
Naproxen	76	87.2	10.5	99.9	5.3
Sulfamethazine	78	59.6	26.7	100.8	6.1
Sulfamethoxazole	34	71.2	17.6	100.1	5.3
Acetaminophen	78	32.3	29.7	102.9	12.4
Diclofenac	76	114.6	15.6	106.0	15.1
Gemfibrozil	78	108.5	11.3	107.6	7.9
Ibuprofen	78	111.7	18.9	101.9	10.2
Ibuprofen	78	111.7	18.9	102.4	8.2
Indomethacin	34	67.9	31.7	100.1	9.7
Bisphenol A	72	87.7	20.1	107.5	10.5
Equilin	72	89.7	17.1	104.8	8.7
Estrone	72	96.3	19.1	100.8	9.7

Chunyan Hao, Xiaoming Zhao, Shahram Tabe, and Paul Yang, Environ. Sci. Technol., 42 (11), 4068–4075, 2008.

Conclusion

- Using LC/MS allows the opportunity to develop high efficiency methods for true-positive identification of target compounds
- Isotope-labeled standards can effectively compensate matrix effect during ESI
- ^{13}C & ^{2}H-labeled standards behave similarly, ^{2}H-labeled standards are economical choice because of the cost
- In a multi-residue method, isotope-labeled standards can be used to provide more precise and accurate results for compounds with similar structure
- Future development in mass spectrometry hardware and software will improve the ability of LC/MS and data quality and operational efficiency.

Acknowledgements

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