





**Quantitative Methodologies for the Analysis of 75 Endocrine Disrupting Compounds, Pharmaceuticals, and Personal Care Products in River Water Using LC/MS/MS**

LCMS Applications Group  
 Applied Biosystems/MDS Sciex

Endocrine Disruptors




### Introduction – Why Endocrine Disruptors?

- Endocrine disruptors class of environmental contaminants of growing concern to the health of humans and aquatic ecosystems.
- Many pesticides, industrial waste, pharmaceuticals, and personal care products have been shown to act as hormone mimics in the environment.
- Hormone are endogenous molecules with very noticeable and potent effects on development and thus the mimics of these molecule can also trigger many adverse effects, even at seemingly low levels.
- Criteria for acceptable levels in the environment are not set or in some cases were set by less sensitive, lower throughput techniques.
- Recent concerns have been raise over transgenerational (fetal accumulation) effects of certain compounds and bioaccumulation.
- Population growth will place further restraints on the already limited supply of water for agriculture and use by humans.
- New tools and techniques will enable us to quickly identify threats to the environment and take action before adverse health effect are seen in organisms.

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### Objectives

- To demonstrate the feasibility and advantages of using LC/MS/MS to screen, quantitate and confirm the presence of endocrine disruptors in environmental water samples.
- Hardware and software automation was used to screen for a very large number of analytes.
- Reversed phase SPE was used to clean the samples as well as concentrate the samples.
- Direct detection with no derivatization and a minimum of sample preparation was used.
- To cover the diverse set of analytes, several approaches using ESI, APCI, polarity switching, and up to four injections were evaluated for performance and practicality.
- Software was used to reduce the large bodies of data down to a manageable form so that conclusions can be drawn quickly and with confidence.

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### Experimental

- HPLC
  - Shimadzu LC system
  - Column: Phenomenex Ultracarb C18(20)m 250x4.6mm (alternative: Phenomenex Ultracarb 3u 150x2.1mm)
  - Eluent A: water + 0.01% formic acid
  - Eluent B: acetonitrile + 0.01% formic acid
  - Column oven temperature 28°C
  - Flow of 600µL/min
  - Injection of 50µL (up to 100µL possible)

Time	Module	Event	Parameter
1.00	Pump	Val	5
2.00	Pump	Val	50
25.00	Pump	Val	56
43.00	Pump	Val	56
47.00	Pump	Val	5
53.00	Pump	Val	5
55.10	Controller	Stop	

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### Experimental

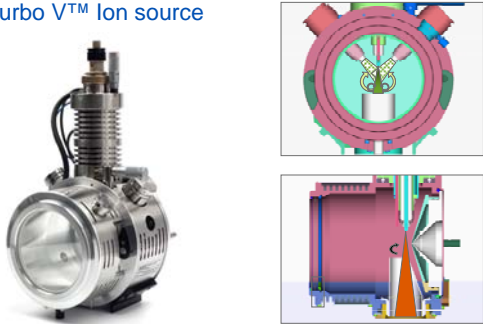
- MS/MS
  - API 4000™ triple quadrupole LC/MS/MS System
  - Turbo V™ source (Electrospray Ionization)
  - Heater temperature of 600°C
  - Polarity Switching
  - 2 MRM transitions per compound
  - Total run time of 55min
  - 1 period ESI with Positive-Negative switching was more pragmatic than ESI-APCI switching using DuoSpray™ source *in this case* because ESI equal to or better than APCI

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### Turbo V™ Ion source



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### Sample Prep (Solid Phase Extraction)

1. Collect 1.0 L of river water.
2. Condition Strata X cartridges,
3. Filter water samples to remove
4. Load sample onto SPE at a
5. To elute, add 5.0 mL of ACN
6. After 10 minutes collect the
7. Add water to a final volume of
8. Inject 50  $\mu$ L to LCMS.

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### Sample Prep Recovery(SPE) - Comparison of Pre and Post Spiked Water.

Analyte	% Recovery	Analyte	% Recovery	Analyte	% Recovery
Acetaminophen 1	59.2	Sulfamethoxazole 1	100	Sulfamethoxazole 1	143
Ibuprofen 1	37.2	Ketoprofen 1	68.4	Ciprofloxacin 1	75.2
Ibuprofen 2	36.7	Estradiol 17 $\beta$ 1		Chloramphenicol 1	107
		Flucloxacillin 1	79		

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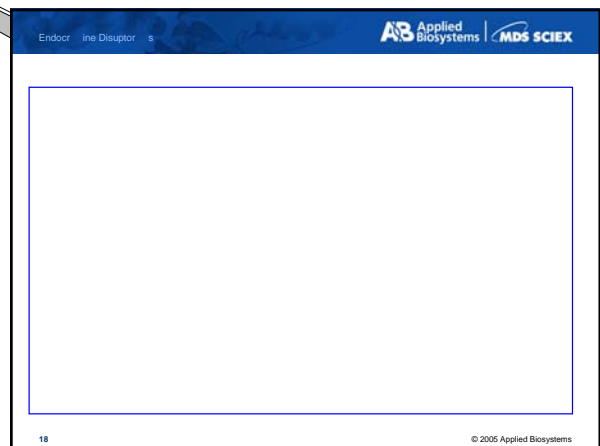
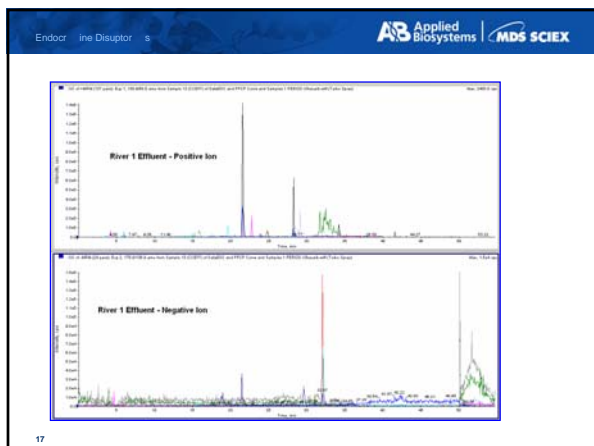
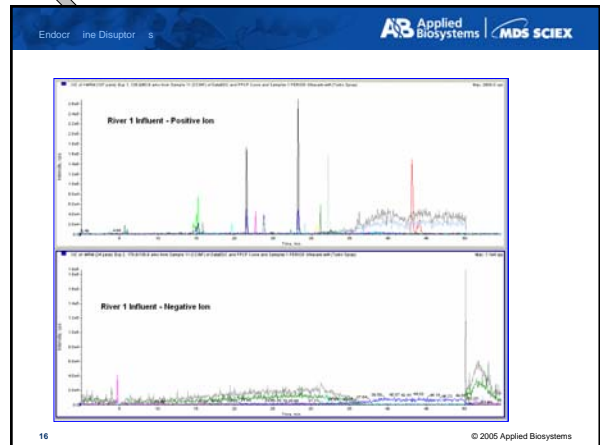
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### Sample Collected

- Samples collected:
  - River 1 – Influent and Effluent Samples of a suburban area
  - River 2 – Influent and Effluent of a river running through a major city

Method Detection Limits – 30.0 ng/L or less. Many compounds have a detection limit of 250 pg/L!

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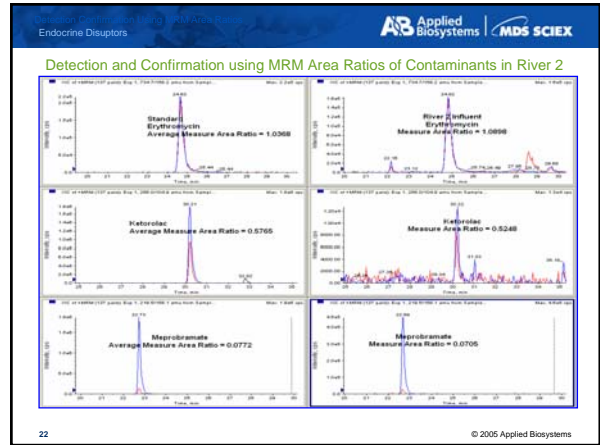
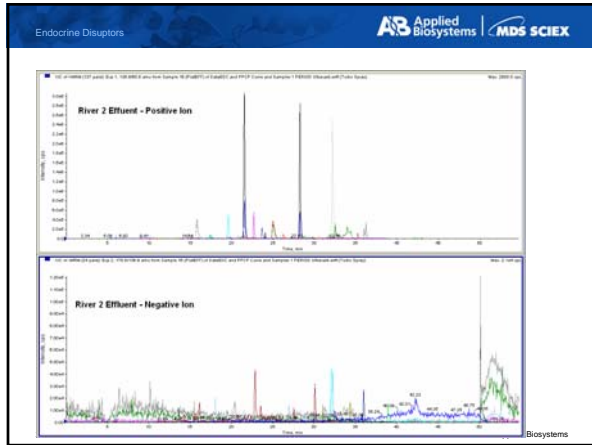
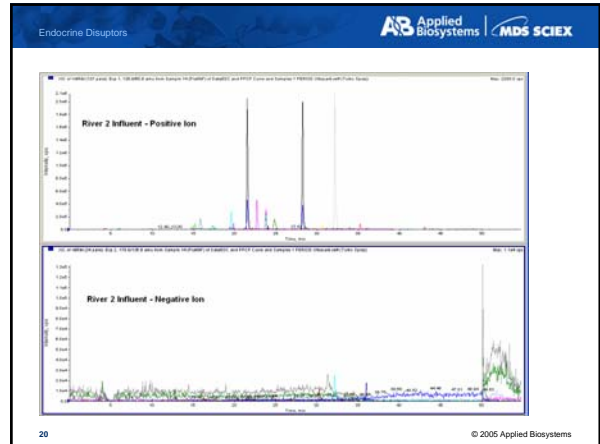
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### Results River 1 (suburban influent and effluent)

Influent		Effluent	
Analyte	Sample Concentration (ng/L)	Analyte	Sample Concentration (ng/L)
Cotinine	2.05		
DEET	1.49	DEET	1.67
Caffeine	40.95	Caffeine	23.5
Carbamazepine	65.5	Carbamazepine	151.5
Sulfamethoxazole	13.15	Sulfamethoxazole	13.3
Ciprofloxacin	3.805		
Erythromycin	3.08	Erythromycin	53.5
		2,4-D	9.35

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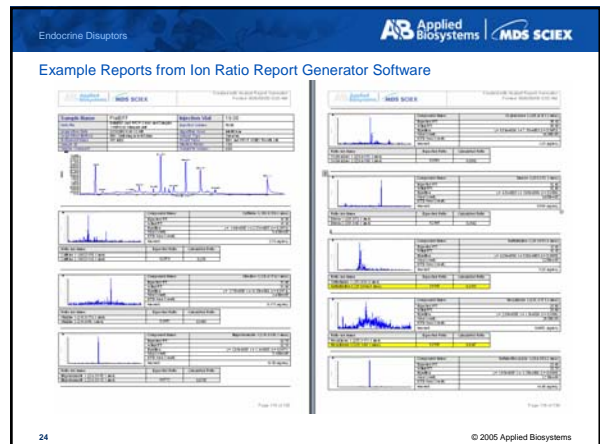


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River 2 Influent		River 2 Effluent	
Analyte	Sample Concentration (ng/L)	Analyte	Sample Concentration (ng/L)
Cotinine	14.4		
DEET	24	DEET	29.9
Caffeine	57	Caffeine	13.5
Simazine	1.01		
Atrazine	1.075	Atrazine	0.875
Meprobamate	85.5	Meprobamate	97.5
		Oxycodone	6.25
Duron	1.375	Duron	4.345
Carbamazepine	870	Carbamazepine	1.52
Sulfamethoxazole	95.5	Sulfamethoxazole	74.5
Ketorolac	2.49	Ketorolac	3.06
		Bromacil	7.4
Pentoxifyline	6.6	Pentoxifyline	3.385
		Diazepam	0.3875
Trimethoprim	58.5	Trimethoprim	122.5
Norethisterone	1.15		
Codine	17.05	Codine	77.5
Lincomycin	1.525	Lincomycin	3.015
Erythromycin	134.5		
Tylosone Tartrate	4.275		
2,4-D	3.24		
Triclosan (Irgasan)	5.9	Triclosan (Irgasan)	31.4
		Warfarin	0.93

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Example Reports from Ion Ratio Report Generator Software



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### Triple Quad MRM – Multiple reaction Monitoring (MSMS)

**Steps MS2:** 1 2 3 & 4 Exit lens

Ion accumulation

Q0 Q1 Q2 Q3

Precursor ion selection Fragmentation  
N<sub>2</sub> CAD Gas

1. Precursor ions selection in Q1-No Isolation time.
2. Fragmentation in Q2-for a richer fragmentation pattern, no low mass cutoff
3. Mass scan.

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### Q TRAP® System Enhanced Product Ion Scanning

**Steps MS2:** 1 2 3 & 4 Exit lens

Ion accumulation

Q0 Q1 Q2 Q3

Precursor ion selection Fragmentation  
N<sub>2</sub> CAD Gas

linear ion trap  
3x10<sup>-6</sup> Torr

1. Precursor ions selection in Q1-No Isolation time.
2. Fragmentation in Q2-for a richer fragmentation pattern, no low mass cutoff
3. Trap products in Q3-short, efficient fill
4. Mass scan.

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### Automatically Generated EPI Spectra for ID / Structural Elucidation

Triazophos

DEET

Atrazine

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### Automatically Generated EPI Spectra for ID / Structural Elucidation

Triazophos

DEET

Atrazine

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### Library Search

Name	Formula	Molecular Weight	FW	Boiling Point	Purity	CE
1	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
2	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
3	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
4	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
5	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
6	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
7	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
8	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
9	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
10	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00

COMPOUND INFORMATION  
Compound Name: DEET  
Formula: C<sub>11</sub>H<sub>15</sub>N  
Molecular Weight: 177.21  
CAS Number: 122-66-7

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### Library Search

Name	Formula	Molecular Weight	FW	Boiling Point	Purity	CE
1	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
2	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
3	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
4	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
5	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
6	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
7	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
8	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
9	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00
10	C <sub>11</sub> H <sub>15</sub> N	177.21	177.21	177.21	100.00	41.00

COMPOUND INFORMATION  
Compound Name: DEET  
Formula: C<sub>11</sub>H<sub>15</sub>N  
Molecular Weight: 177.21

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## Library Search

The screenshot displays a software interface for library search. It features three mass spectra plots at the top, each with a base peak at m/z 193.0. Below the spectra is a table with columns for Name, Formula, Molecular weight (amu), and a checkbox for Search. The table lists one entry: **Testosterone** with the formula  $C_{19}H_{28}O_2$  and a molecular weight of 288.44. To the right of the table is a chemical structure diagram of testosterone. Below the table is a 'COMPOUND INFORMATION' section with fields for Compound Name (Testosterone), Source (LIDARMS010), Molecular Weight (amu) (288.44), and Pub Number.

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## Thank you for your time.

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*Loren Y Olson, Christopher D Borton, Hesham Ghobarah, Elliott B Jones, Robert Ellis and Andre Schreiber*

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## Legal acknowledgements

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