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A Rapid LCMS Method for Evaluation of EPA 1694 and 6810 Contaminants in Drinking Water

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NOVEL ASPECT

An ultra-fast polarity switching five minute method to analyze and quantify many different contaminants of emerging concern (CECs) by LCMS-MS.

INTRODUCTION

Contaminants of emerging concern (CECs) are chemicals such as endocrine disrupting compounds, pharmaceuticals and personal care products that have been detected in the environment. Most of these chemicals enter the environment through treated and raw sewage and are detrimental to the aquatic organisms at picogram per milliliter levels. Early detection for these CECs was performed using gas chromatography mass spectrometry (GC-MS) but more recently liquid chromatography mass spectrometry (LC-MS) has become more popular for detecting low levels of these contaminants in wastewater. This poster describes a comprehensive method for evaluating multiple steroids, pharmaceuticals and personal care products at picogram per milliliter levels in drinking water using ultra-fast LCMS-MS.

METHODS

A mixture of steroids (estrone, progesterone, testosterone, 17α-ethynylestradiol, aldosterone, 11-deoxycortisol, 11-deoxycorticosterone, cortisol, cortisone, 17-hydroxyprogesterone, androstenedione, DHEA), pharmaceuticals and personal care products (ibuprofen, triclosan, sulfamethoxazole, caffeine, acetaminophen, erythromycin, fluoxetine, gembfibrozil, diclofenac, ciprofloxacin, carbamazepine, primidone, trimethoprim) have been analyzed using ultra-fast liquid chromatography mass spectrometry. Heated electrospray ionization (hESI) in both positive and negative modes with polarity switching was used for ionization of the compounds. MRM transitions for each analyte were optimized and separated using reversed phase chromatography in a single five minute method. Tap water was directly injected in triplicate for the drinking water samples.

Chromatography Conditions	
Mobile Phase A	: Water with additive
Mobile Phase B	: 0.3% formic acid in Methanol
Column	: Restek Raptor Biphenyl (2.7 μm x 100 x 2.1mm)
Gradient	: Linear from 34% - 100% B.
Flow Rate	: 0.6 mL/min
Column oven	: 40°C
Injection volume	: 1 μL

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Calibration levels

Level	ng/mL (ppb)		Level	ng/mL (ppb)
1	100		11	1.25
2	75		12	0.7813
3	50		13	0.6250
4	37.5		14	0.2930
5	25		15	0.1953
6	18.75		16	0.1465
7	12.5		17	0.0977
8	10		18	0.0781
9	5		19	0.0488
10	2.5		20	0.0391

RESULTS - CHROMATOGRAMS

A representative chromatogram for a 10 ppb standard of the analyte mix is shown. Carbamazepine has the highest response as shown in the top figure. All other analytes are shown in the zoomed in region from 0 - 100000 intensity in the bottom figure. The retention times for each of the analytes are listed in the table above.

Retention times for all analytes analyzed in the mixture.

Analyte	Ret. Time		Level	ng/mL (ppb)
Acetaminophen	0.562		Aldosterone	3.32
Trimethoprim	0.991		Diclofenac	3.538
Ciprofloxacin	1.443		11-Deoxycortisol	3.552
Primidone	1.445		DHEA	3.635
Sulfamethoxazole	1.598		Estrone	3.635
Caffeine	1.609		Gemfibrozil	3.655
Fluoxetine	2.541		Triclosan	3.768
Erythromycin	2.581		Testosterone	3.869
Carbamazepine	2.869		Androstenedione	4.214
Cortisol	2.87		11-Deoxycorticosterone	4.233
Cortisone	3.053		17-hydroxyprogesterone	4.233
17a-Ethynylestradiol	3.146		Progesterone	4.558

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Chromatogram for EPA Analysis

Zoom
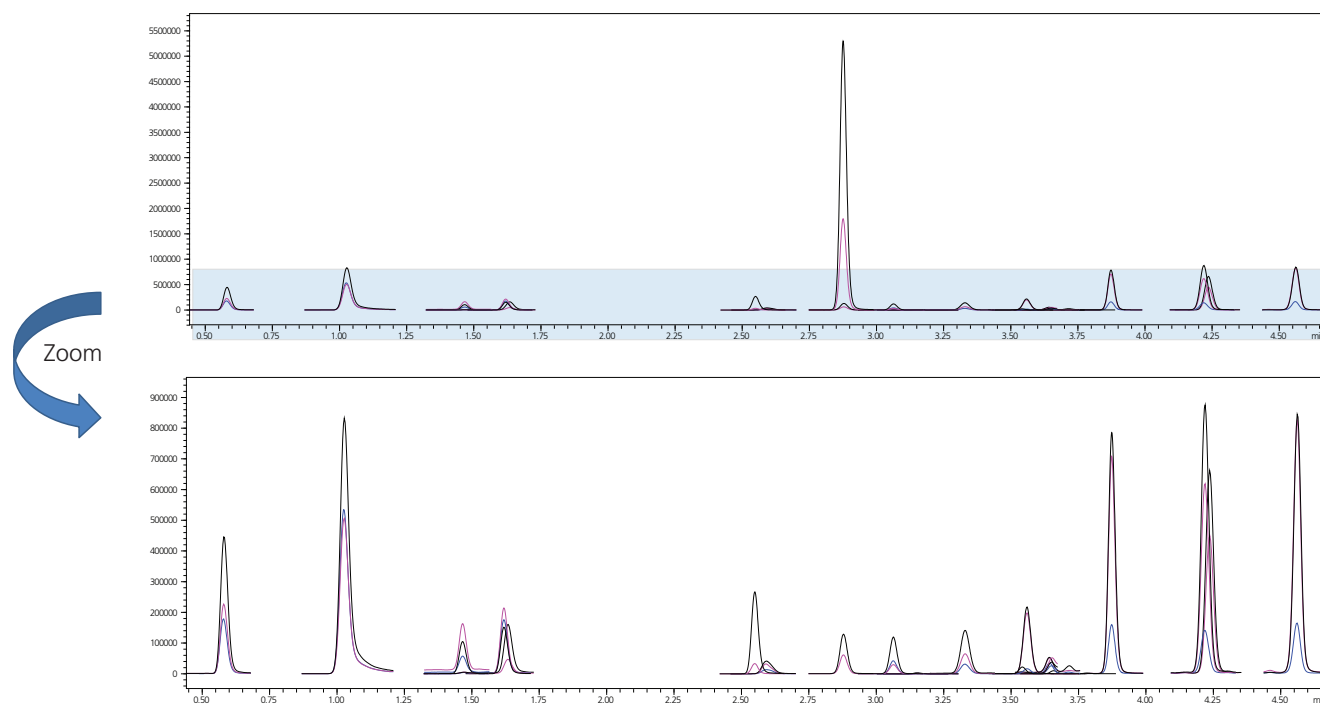
LC Chromatogram at 10 ppb for all twenty four compounds in under 5 minutes.

LCMS-8060 Parameters

- Ultrafast MRM methods
- Up to 555 MRM transitions per sec
- Heated electrospray source
- Scan speeds up to 30,000 u/sec
- Polarity switching 5 msec

LCMS-8060 Parameters	
Nebulizing Gas Flow	: 3 L/min
Heating Gas Flow	: 13 L/min
Drying Gas Flow	: 7 L/min
Interface Temperature	: 350 °C
DL Temperature	: 125 °C
Heat Block Temperature	: 350 °C

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| Analyte	m/z	Reference Ions	Ret. Time
Acetaminophen	152.25>110.15	152.25>64.95;	0.562
		152.25>93.05	
Trimethoprim	291.15>230.20	291.15>261.05;	0.991
		291.15>123.20	
Ciprofloxacin	332.15>288.15	332.15>231.10;	1.443
		332.15>314.20	
Primidone	219.25>91.05	219.25>162.30;	1.445
		219.25>119.05	
Sulfamethoxazole	254.20>92.05	254.20>108.10;	1.598
		254.20>156.20	
Caffeine	195.30>138.10	195.20>110.05	1.609
Fluoxetine	310.30>44.10	310.30>148.30	2.541
Erythromycin	734.50>576.35	734.50>116.15	2.581
Carbamazepine	237.30>194.20	237.30>193.05	2.869
Cortisol	363.30>121.10	363.30>327.10	2.87
Cortisone	361.20>163.10	361.20>121.20;	3.053
		361.20>105.15	
17a-Ethynylestradiol	295.20>144.95	295.20>159.20;	3.146
		295.20>267.15	
Aldosterone	361.20>343.25	361.20>315.25;	3.32
		361.20>325.00	
Diclofenac	294.00>250.10		3.538
11-Deoxycortisol	347.35>109.10	347.35>97.00;	3.552
		347.35>253.25	
DHEA	271.25>133.05	271.25>159.20;	3.635
		271.25>253.15	
Estrone	269.20>145.10	269.20>143.10;	3.635
		269.20>159.15	
Gemfibrozil	249.30>121.00		3.655
Triclosan	287.10>34.95		3.768
Testosterone	289.25>97.05	289.25>109.15;	3.869
		289.25>79.15	
Androstenedione	287.35>97.10	287.35>109.15;	4.214
		287.35>79.20	
11-Deoxycorticosterone	331.20>97.10	331.20>109.10	4.233
17-hydroxyprogesterone	331.20>97.00	331.20>109.10	4.233
Progesterone	315.40>109.10	315.40>97.05;	4.558
		315.40>79.15	

RESULTS - DRINKING WATER

Analysis of the analytes in drinking water sample. Direct injection of a 1 μL sample with no sample preparation.
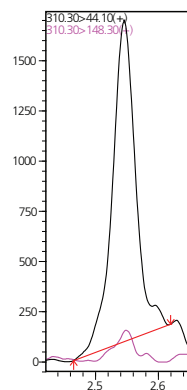Most analytes are not detected, however, erythromycin, trimethoprim and fluoxetine are detected.
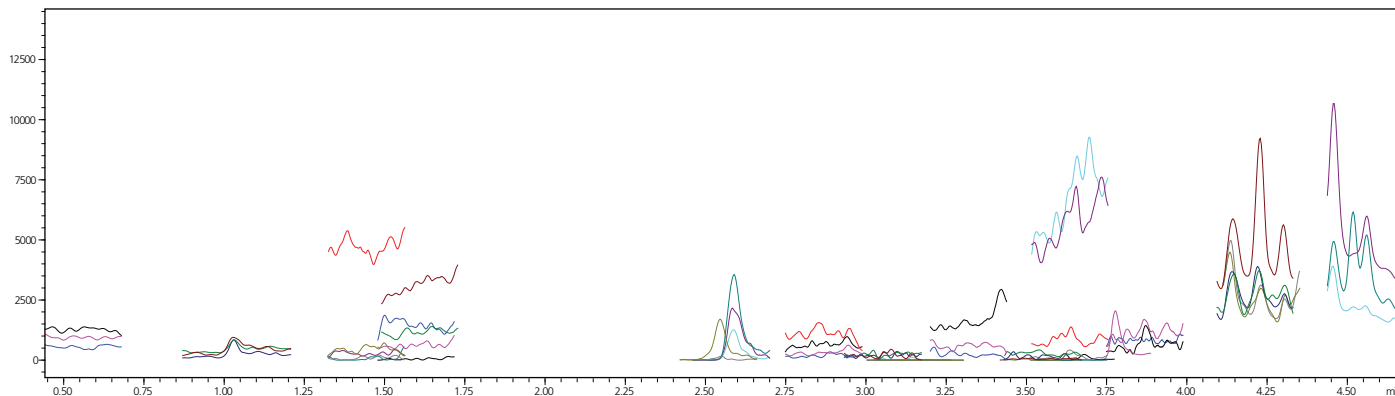
LC Chromatogram for drinking water sample. Most analytes are not detected.
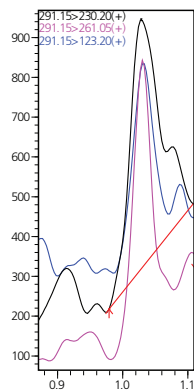
**Trimethoprim
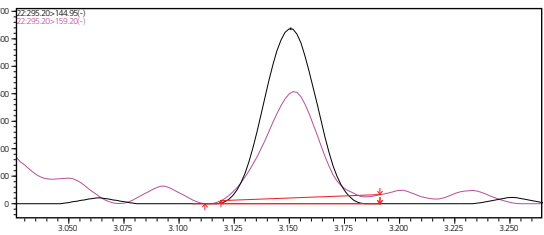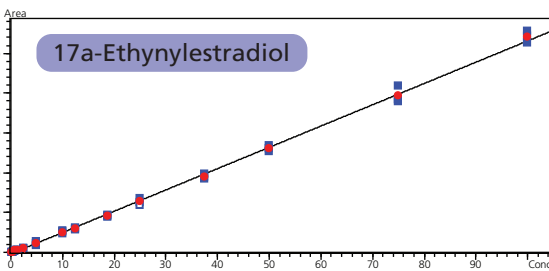1.6 ppb**
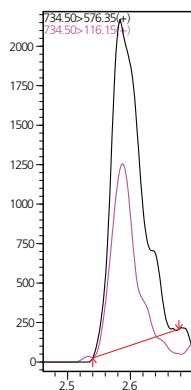
**Erythromycin
3.4 ppb**

**Fluoxetine
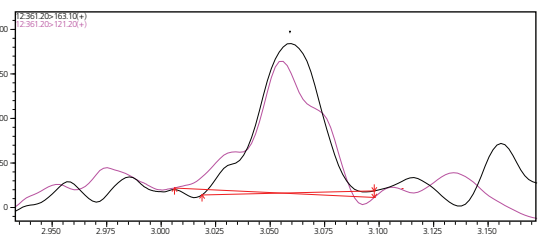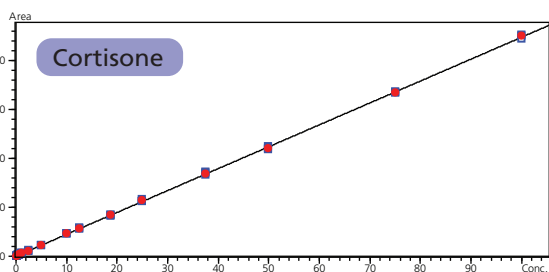3.2 ppb**

RESULTS – CALIBRATION CURVES

Calibration Curve and MS Chromatograms
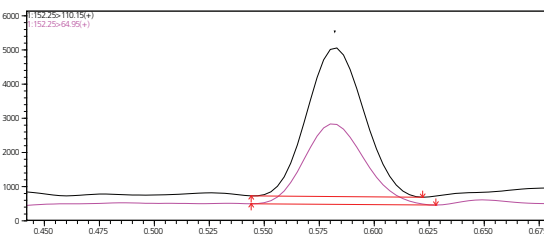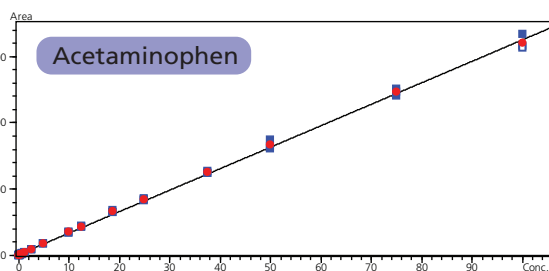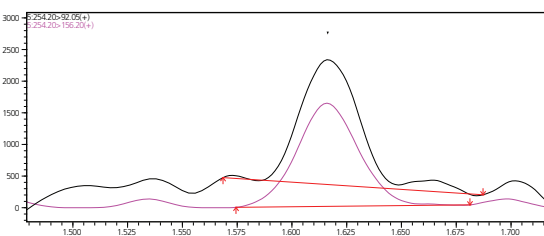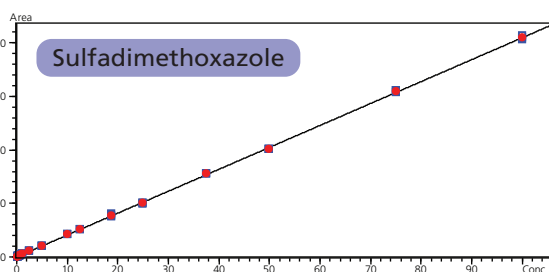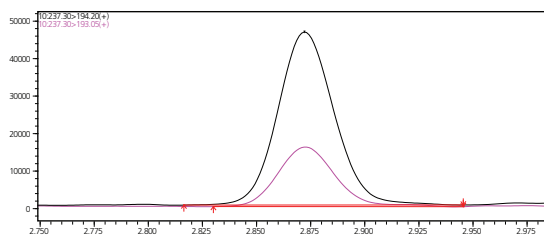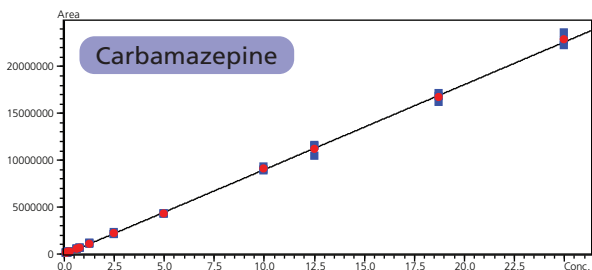
Limit of Quantitation* - All Analytes in Neat Standard

Analyte	Ret. Time	ng/mL (ppb)
Acetaminophen	0.562	100 - 0.0977
Trimethoprim	0.991	100 - 0.625
Ciprofloxacin	1.443	100 -10
Primidone	1.445	100 - 0.625
Sulfamethoxazole	1.598	100 - 0.0977
Caffeine	1.609	100 - 0.0977
Fluoxetine	2.541	37.5 - 2.5
Erythromycin	2.581	100 - 5
Carbamazepine	2.869	25 - 0.0391
Cortisol	2.87	100 - 0.0977
Cortisone	3.053	100 - 0.0977
17a-Ethynylestradiol	3.146	100 - 0.781

Analyte	Ret. Time	ng/mL (ppb)
Aldosterone	3.32	100 - 0.0977
Diclofenac	3.538	100 - 0.0977
11-Deoxycortisol	3.552	100 - 0.0977
DHEA	3.635	100 - 2.5
Estrone	3.635	100 - 0.292
Gemfibrozil	3.655	100 - 0.292
Triclosan	3.768	100 - 0.781
Testosterone	3.869	100 - 0.0488
Androstenedione	4.214	100 - 0.0977
11-Deoxycorticosterone	4.233	100 - 0.0977
17-hydroxyprogesterone	4.233	100 - 0.0977
Progesterone	4.558	100 - 0.0977

*LOQ for the analytes measured in neat standard. More work needs to be completed to give comprehensive results in matrix.

CONCLUSIONS

A robust and rapid five minute method for evaluating multiple endocrine disrupting compounds, pharmaceuticals and personal care products was developed using ultra-fast liquid chromatography mass spectrometry. Most analytes were detected in the parts per trillion (pg/mL) range in neat standard. Low levels of a few compounds were detected in the drinking water sample. Furthermore, this method can be applied to other environmental water samples.

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