

# Trace analysis of pharmaceuticals and organic contaminants in water

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## Key words

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## Goal

**To demonstrate the reliable and accurate quantitative analysis of contaminants at the pg/mL level in drinking water using the Thermo Scientific™ EQuan MAX Plus™ LC-MS system coupled to the Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer.**

## Introduction

The presence of endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) in surface water and ground water sources has been known for many years. Some of these emerging contaminants are hard to remove from the source water by current drinking water treatment techniques. Municipal water could contain trace amounts, typically part per trillion (ppt) level to part per billion (ppb) level, of certain EDCs and PPCPs. While no research results yet show that these



emerging contaminants constitute a health risk at these low levels, their presence is a concern to consumers. Thus, the industry is trying to make new point-of-use drinking water treatment products that can effectively remove these contaminants from municipal water. Reliable analytical methods and instrumentation to provide qualitative and quantitative analyses of these emerging contaminants at low ppt levels are of the utmost importance. In this application note, the reporting limit (RL) of a compound is about one-sixth to one-fourth of the Maximum Effluent Concentration (MEC) of the compound in potable water.<sup>1</sup>

<sup>1</sup>Maximum Effluent Concentration (MEC) from NSF/ANSI Standard 401. The NSF/ANSI Standard sets challenge concentrations (influent concentrations) for each compound based upon the occurrence level of the contaminant in drinking water (municipal water) across the United States. In order to meet the standard, a point-of-use drinking water treatment product must remove at least 85% of the contaminant in the challenge water to meet the MEC in effluent water.

The EQuan MAX Plus LC-MS system combines a highly sensitive, online pre-concentration liquid chromatography system with the TSQ Endura triple quadrupole mass spectrometer to achieve low pg/mL level limits of quantitation with excellent quantitative reproducibility. Online pre-concentration and solid phase extraction (online SPE) avoids the disadvantages of offline SPE, including large sample volumes and preparation time, by utilizing a smaller sample volume collected in the field and eliminating the manual offline SPE step. Using this approach for analyzing for contaminants in drinking water can reduce the sample preparation time from many hours to a few minutes and still achieve ppt sensitivity.

## Experimental

The EQuan MAX Plus LC-MS system was coupled to the TSQ Endura mass spectrometer.

### Sample preparation

Analytical standards obtained from Restek (Catalog 569687, 569688, and 569689; Table 1) were mixed in equal proportions (Table 2) and then diluted directly into tap water from the San Jose Municipal Water System, San Jose, CA (Table 3). All dilutions to form the standard curves were made from the same San Jose tap water. No additional filtering was applied before analysis with the EQuan MAX Plus LC/MS/MS system.

**Table 1. Reference standards.**

Restek Standard	Compound	Stock (µg/mL)	2.5× MEC (pg/mL)	0.5× MEC (pg/mL)
569687 - Group A Standard	Ibuprofen	153.5	153.5	30.7
	Nonylphenol	534.5	534.5	106.9
	Naproxen	53.5	53.5	10.7
569688 - Estrone Standard	Estrone	53.4	53.4	10.68
569689 - Group B Standard	Atenolol	76.6	76.6	15.32
	Trimethoprim	53	53	10.6
	Phenytoin (Dilantin)	76	76	15.2
	Linuron	53	53	10.6

**Table 2. Reference standards stock mixture.**

Compound (-)	Stock (µg/mL)	Compound (+)	Stock (µg/mL)
Ibuprofen	51.17	Atenolol	25.53
Nonylphenol	178.17	Trimethoprim	17.67
Naproxen	17.83	Phenytoin (Dilantin)	25.33
Estrone	17.80	Linuron	17.67

**Table 3. Dilutions of stock solutions in San Jose, CA, tap water.**

Dilution	Stock (µg/mL)	10× MEC (pg/mL)	3.33× MEC (pg/mL)	1.11× MEC (pg/mL)	0.37× MEC (pg/mL)	0.19× MEC (pg/mL)
<b>Compound (-)</b>						
Ibuprofen	51.17	614.00	214.90	71.63	23.88	11.94
Nonylphenol	178.17	2138.00	748.30	249.43	83.14	41.57
Naproxen	17.83	214.00	74.90	24.97	8.32	4.16
Estrone	17.80	213.60	74.76	24.92	8.31	4.15
<b>Compound (+)</b>						
Atenolol	25.53	306.40	107.24	35.75	11.92	5.96
Trimethoprim	17.67	212.00	74.20	24.73	8.24	4.12
Phenytoin (Dilantin)	25.33	304.00	106.40	35.47	11.82	5.91
Linuron	17.67	212.00	74.20	24.73	8.24	4.12

Eight target compounds were selected for the analysis (Figure 1). Of these, estrone, ibuprofen, nonylphenol and naproxen are suited to negative ion LC/MS/MS analyses, and trimethoprim, phenytoin, linuron, and atenolol are suited to positive ion LC/MS/MS analyses. Samples were prepared as described in Table 4 at several concentration levels based on the target MEC.

## HPLC

Water samples of 1 mL were directly injected onto a Thermo Scientific™ Hypersil GOLD™ aQ pre-concentration trapping column (2.1 × 20 mm, 12 μm, P/N 25302-022130)

at 1.5 mL/min with water + 0.1% formic acid for positive ion analysis and 1.5 mL/min with water for negative ion analysis. After sufficient washing on the pre-concentration column, the target compounds were transferred at 0.4 mL/min either to a Thermo Scientific™ Accucore™ aQ analytical column (2.1 × 100 mm, 2.6 μm, positive ion analysis, P/N 17326-102130) or a Thermo Scientific™ Hypersil GOLD™ aQ analytical column (2.1 × 100 mm, 3.0 μm, negative ion analysis, P/N 25302-102130) for chromatographic separation by gradient elution prior to introduction into the mass spectrometer (Table 5).

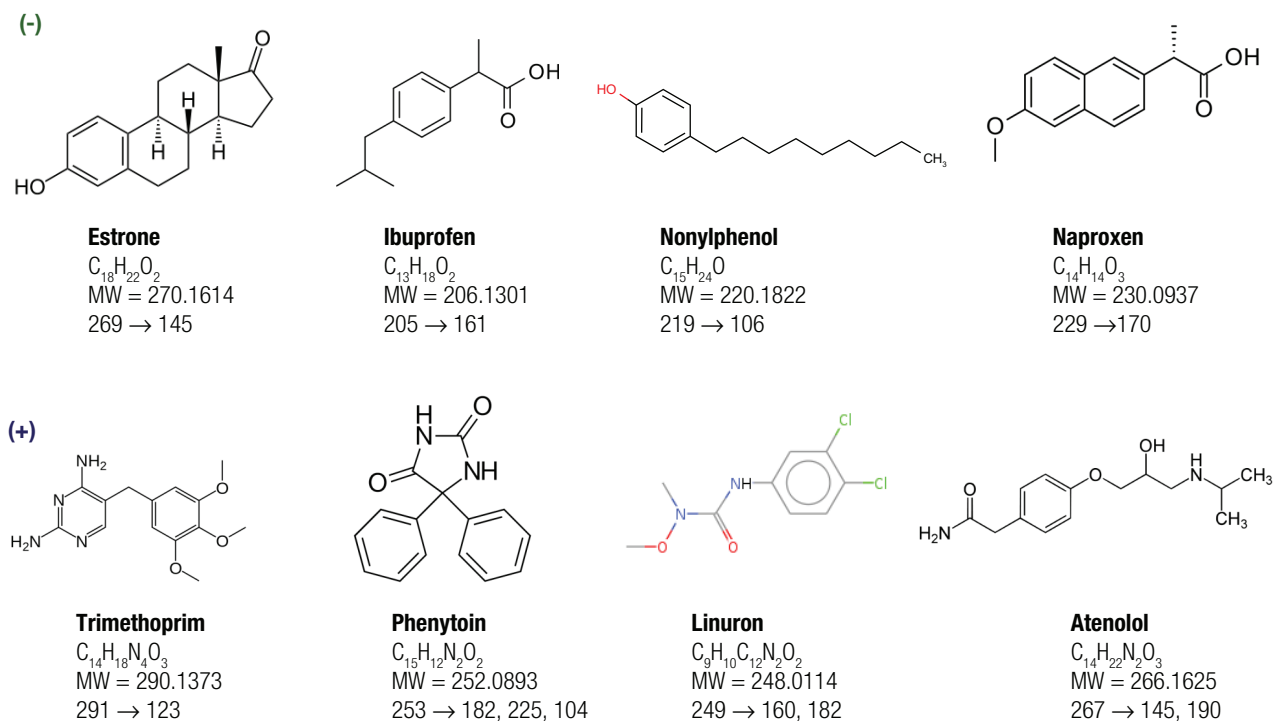


Figure 1. Target compounds.

Table 4. Sample composition.

	Compound Name	CAS #	Sample Concentration (pg/mL)						
			Sample #1 (Blank)	Sample #2 (0.5× MEC Level)	Sample #3 (2.5× MEC Level)	Sample #4 (5× MEC Level)	Sample #5 (0.5× MEC Level)	Sample #6 (2.5× MEC Level)	Sample #7 (5× MEC Level)
Group A	Estrone	53-16-7	0	11	53	106	0	0	0
	Ibuprofen	15687-27-1	0	30	152	304	0	0	0
	Nonylphenol	104-40-5	0	106	532	1064	0	0	0
	Naproxen	22204-53-1	0	11	53	106	0	0	0
Group B	Trimethoprim	738-70-5	0	0	0	0	11	53	106
	Phenytoin (Dilantin)	57-41-0	0	0	0	0	15	76	152
	Linuron	330-55-2	0	0	0	0	11	53	106
	Atenolol	29122-68-7	0	0	0	0	15	76	152

Table 5. Gradient method.

Positive Ions			
Mobile Phase		A: 0.1% formic acid in water B: 0.1% formic acid in methanol	
Gradient	Time	%A	%B
	0.00	100	0
	1.00	100	0
	5.00	0	100
	6.50	0	100
	6.60	100	0
	8.50	100	0
Negative Ions			
Mobile Phase		A: 0.1% ammonium hydroxide in water B: 0.1% ammonium hydroxide in methanol	
Gradient	Time	%A	%B
	0.00	90	10
	1.00	90	10
	3.50	0	100
	6.50	0	100
	6.60	90	10
	8.50	90	10

## MS

MS analysis was carried out on a TSQ Endura triple quadrupole mass spectrometer equipped with a heated-electrospray ionization interface (H-ESI). Two selected reaction monitoring (SRM) transitions per compound were acquired: one for quantitation and the other for positive confirmation.

The MS conditions were as follows:

Parameter	Setting
Spray voltage	Positive: 3000 V Negative: 2000 V
Sheath gas	60
Aux gas	15
Sweep gas	1
Ion transfer tube temperature	300 °C
Vaporizer temperature	375 °C
Cycle time	0.35 s
Q1/Q3 resolution	0.7 amu
CID gas	2 mTorr
SRM transitions	Tables 6 and 7

Quantitative analysis was performed using Thermo Scientific™ TraceFinder™ software.

Table 6. SRM transitions for positive ions.

Compound	Start Time (min)	End Time (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Linuron	1	6.5	Positive	249.02	160	17	100
Linuron	1	6.5	Positive	249.02	182.02	16	100
Phenytoin	1	6.5	Positive	253.1	104.05	20	120
Phenytoin	1	6.5	Positive	253.1	182.1	15	120
Atenolol	1	6.5	Positive	267.17	145.07	25	115
Atenolol	1	6.5	Positive	267.17	190.09	19	115
Trimethoprim	1	6.5	Positive	291.15	123.07	26	125
Trimethoprim	1	6.5	Positive	291.15	230.12	24	125

Table 7. SRM transitions for negative ions.

Compound	Start Time (min)	End Time (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Ibuprofen	1	6.5	Negative	205.12	161.13	8	53
Ibuprofen	1	6.5	Negative	206.14	162.14	8	53
Nonylphenol	1	6.5	Negative	219.18	106.04	22	140
Nonylphenol	1	6.5	Negative	220.18	107.05	22	140
Naproxen	1	6.5	Negative	229.09	170.07	15	56
Naproxen	1	6.5	Negative	229.09	185.1	8	56
Estrone	1	6.5	Negative	269.15	143.05	58	225
Estrone	1	6.5	Negative	269.15	145.07	39	225

## Results and discussion

Example SRM chromatograms for Group A (positive ion) and Group B (negative ion) compounds at 10× MEC are shown in Figure 2 and Figure 3, respectively. For ibuprofen

and nonylphenol, a second product ion was not observed. Instead for demonstration purposes, the A+1 isotope was fragmented and its product ion was used as the confirming ion.

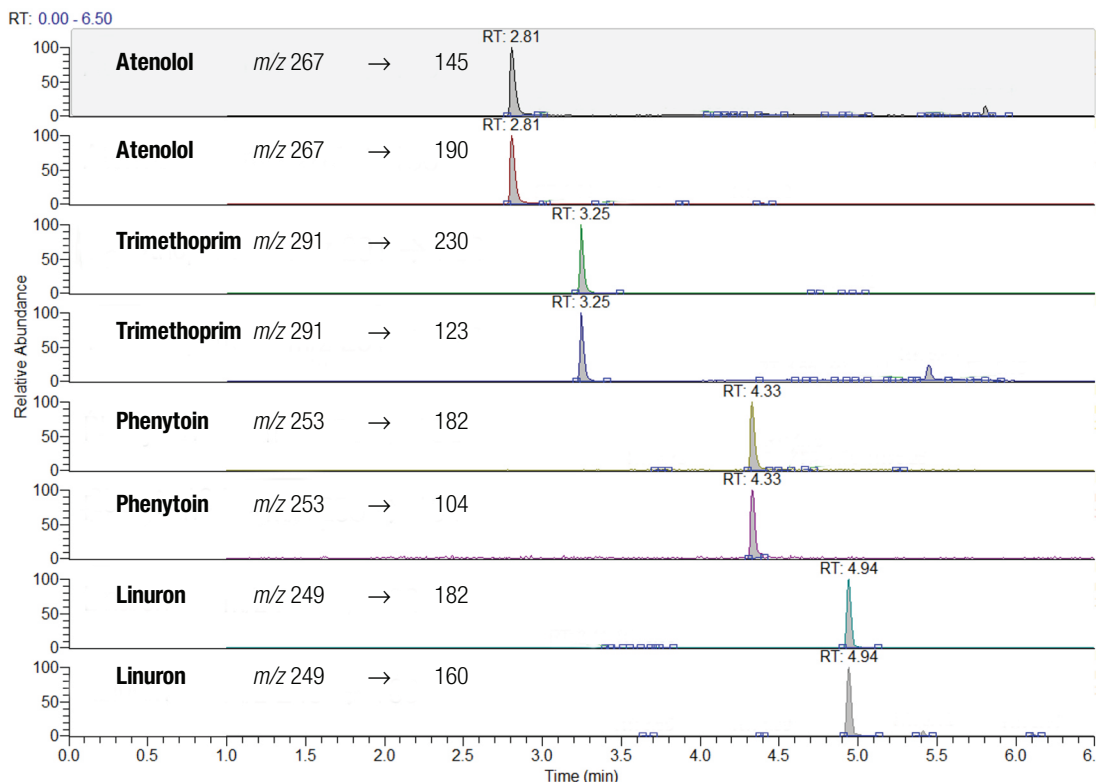


Figure 2. Positive ion SRM chromatograms (10× MEC).

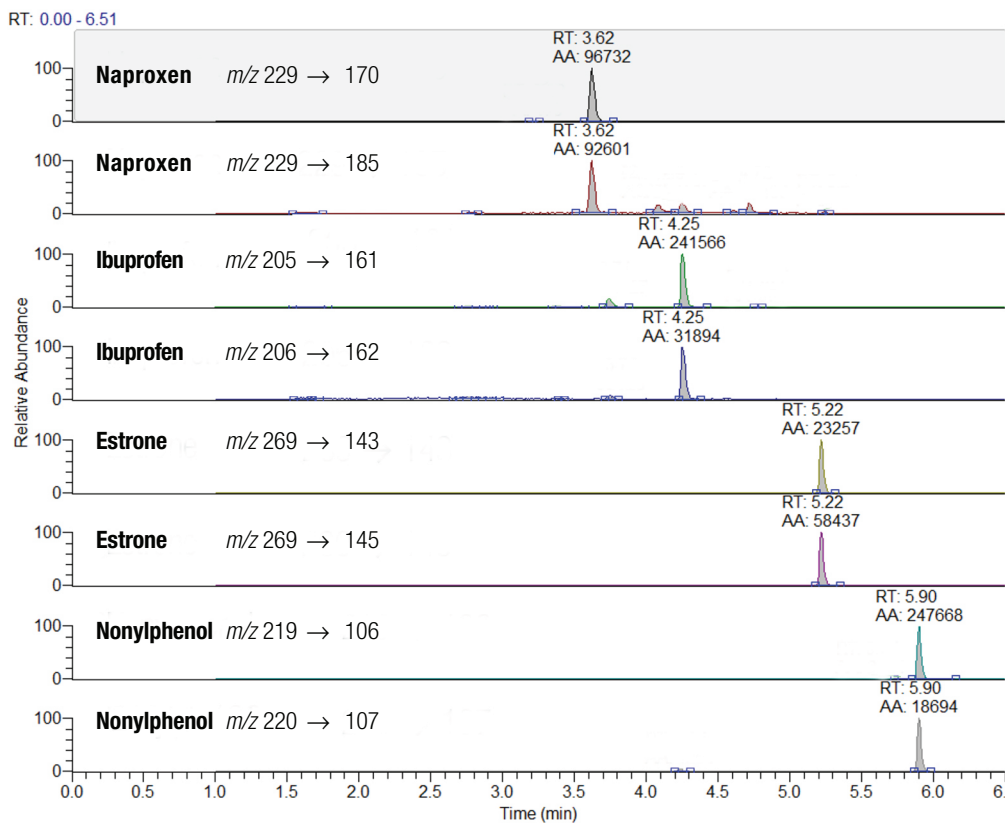


Figure 3. Negative ion SRM chromatograms (10× MEC).

Calibration curves for target organic contaminants in tap water are shown in Figures 4 and 5, which demonstrate performance to levels below MEC.

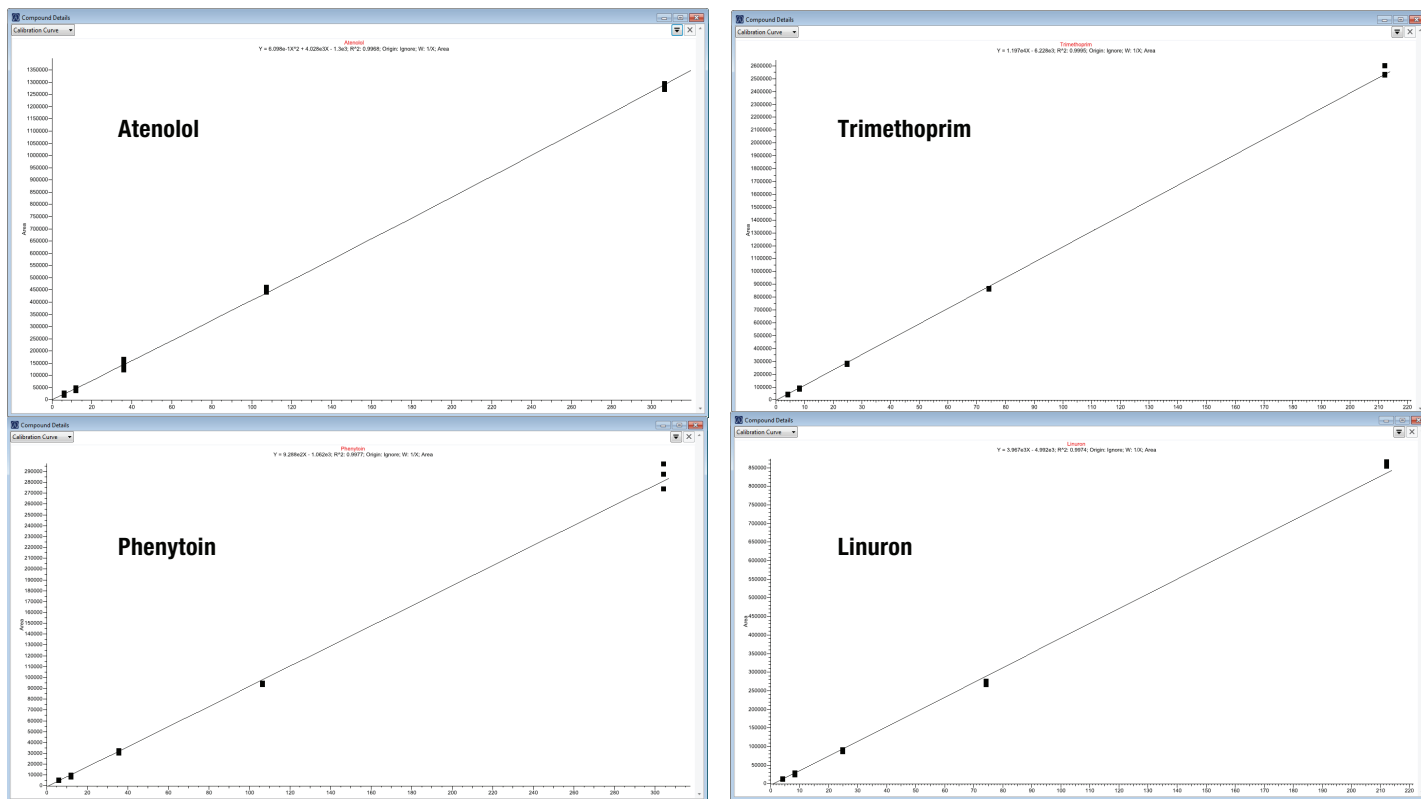


Figure 4. Standard calibration curves for positive ion compounds.

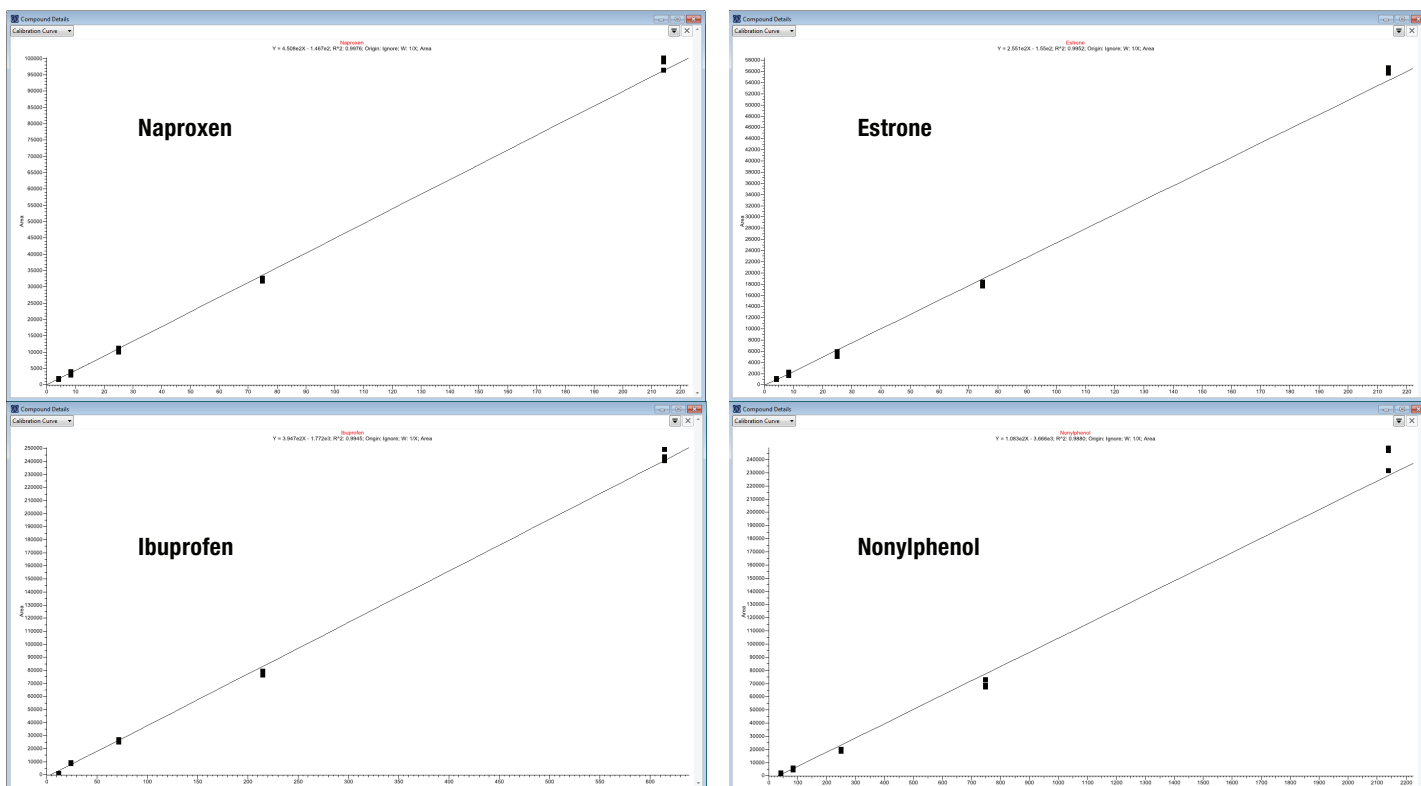


Figure 5. Standard calibration curves for negative ion compounds.

Tables 8 and 9 show system reproducibility as %RSDs for N=7 replicate injections for spiked tap water and customer-submitted samples, respectively. The EQuan MAX Plus LC-MS system demonstrated excellent reproducibility for the target compounds in water at 0.37× MEC in spiked tap water using 1 mL injections. Several compounds showed a significantly lower response in the customer water samples

versus the spiked tap water at a similar concentration (for example, >90% loss for trimethoprim and ibuprofen, and nonylphenol yielding erratic results barely distinguishable from the matrix blanks). This may be due to sample degradation and/or sample adsorption losses. It is clear that these samples need to be analyzed fresh, and not stored.

**Table 8. Reproducibility for freshly prepared San Jose tap water samples.**

Conc	Atenolol	Linuron	Phenytoin	Trimethoprim	Conc	Naproxen	Estrone	Ibuprofen	Nonylphenol
0.37× MEC	38822	28437	10100	44582	0.37× MEC	3574	1736	8707	5340
0.37× MEC	44001	28137	8129	47123	0.37× MEC	3864	1865	9115	6011
0.37× MEC	46701	27867	10171	47820	0.37× MEC	3636	2089	9140	5321
0.37× MEC	49224	28763	9764	49229	0.37× MEC	4099	1817	9654	5650
0.37× MEC	45401	25168	9220	47522	0.37× MEC	3600	2316	9134	6155
0.37× MEC	47108	26598	9577	50476	0.37× MEC	3795	2341	9423	5306
0.37× MEC	41807	29374	9499	48323	0.37× MEC	3595	2061	8863	5854
<b>Average</b>	<b>44723.4</b>	<b>27763.4</b>	<b>9494.3</b>	<b>47867.9</b>	<b>Average</b>	<b>3737.6</b>	<b>2032.1</b>	<b>9148.0</b>	<b>5662.4</b>
<b>RSD</b>	<b>7.86%</b>	<b>5.16%</b>	<b>7.25%</b>	<b>3.85%</b>	<b>RSD</b>	<b>5.20%</b>	<b>11.75%</b>	<b>3.48%</b>	<b>6.24%</b>
Conc	Atenolol	Linuron	Phenytoin	Trimethoprim	Conc	Naproxen	Estrone	Ibuprofen	Nonylphenol
1.11× MEC	135637	92213	30762	148796	1.11× MEC	10435	5884	25247	20106
1.11× MEC	165827	90151	31562	148943	1.11× MEC	11296	5902	25967	19574
1.11× MEC	134388	88078	30732	146316	1.11× MEC	10764	5447	25933	20118
1.11× MEC	128029	88202	30999	143493	1.11× MEC	11206	5579	27224	20216
1.11× MEC	149999	87276	31073	147697	1.11× MEC	10431	5835	26132	19773
1.11× MEC	139983	87589	30450	147524	1.11× MEC	10374	6015	26701	19520
1.11× MEC	124696	88837	32708	149571	1.11× MEC	10069	5079	27037	20207
<b>Average</b>	<b>139794.1</b>	<b>88906.6</b>	<b>31183.7</b>	<b>147477.1</b>	<b>Average</b>	<b>10653.6</b>	<b>5677.3</b>	<b>26320.1</b>	<b>19930.6</b>
<b>RSD</b>	<b>10.09%</b>	<b>1.95%</b>	<b>2.43%</b>	<b>1.40%</b>	<b>RSD</b>	<b>4.28%</b>	<b>5.81%</b>	<b>2.66%</b>	<b>1.51%</b>



Table 9. Reproducibility for customer-supplied water samples.

Conc	Atenolol	Linuron	Phenytoin	Trimethoprim	Conc	Naproxen	Estrone	Ibuprofen	Nonylphenol
0.5× MEC	34248	20349	8696	1303	0.5× MEC	1647	236	148	6416
0.5× MEC	32075	21716	8235	1546	0.5× MEC	1331	236	192	4494
0.5× MEC	31781	17293	7549	1223	0.5× MEC	1730	256	325	4133
0.5× MEC	28063	19953	8268	1338	0.5× MEC	1351	227	236	3273
0.5× MEC	21982	19352	8452	1645	0.5× MEC	1371	258	295	3575
0.5× MEC	22017	19292	7535	1805	0.5× MEC	1474	327	2395	3095
0.5× MEC	34238	20793	7810	2377	0.5× MEC	942	175	1421	3045
<b>Average</b>	<b>29200.6</b>	<b>19821.1</b>	<b>8077.9</b>	<b>1605.3</b>	<b>Average</b>	<b>1406.6</b>	<b>245.0</b>	<b>716.0</b>	<b>4004.4</b>
<b>RSD</b>	<b>18.27%</b>	<b>7.05%</b>	<b>5.60%</b>	<b>24.78%</b>	<b>RSD</b>	<b>18.19%</b>	<b>18.56%</b>	<b>120.60%</b>	<b>29.79%</b>
Conc	Atenolol	Linuron	Phenytoin	Trimethoprim	Conc	Naproxen	Estrone	Ibuprofen	Nonylphenol
2.5× MEC	174508	113980	40225	12788	2.5× MEC	7032	776	2056	8434
2.5× MEC	178278	114151	42507	13232	2.5× MEC	4873	677	2012	9585
2.5× MEC	182584	115500	43456	13444	2.5× MEC	7510	835	2440	1147
2.5× MEC	188987	115823	43815	13195	2.5× MEC	6072	710	1726	7226
2.5× MEC	182180	118057	44245	13136	2.5× MEC	5321	631	1146	7150
2.5× MEC	187777	113413	44513	10081	2.5× MEC	4748	761	1190	7958
2.5× MEC	183085	114405	38815	10571	2.5× MEC	5302	701	1939	8690
<b>Average</b>	<b>182485.6</b>	<b>115047.0</b>	<b>42510.9</b>	<b>12349.6</b>	<b>Average</b>	<b>5836.9</b>	<b>727.3</b>	<b>1787.0</b>	<b>7170.0</b>
<b>RSD</b>	<b>2.76%</b>	<b>1.37%</b>	<b>5.13%</b>	<b>11.36%</b>	<b>RSD</b>	<b>18.44%</b>	<b>9.38%</b>	<b>26.48%</b>	<b>38.89%</b>

## Conclusion

- The TSQ Endura triple quadrupole mass spectrometer in concert with the EQUAN MAX Plus online pre-concentration liquid chromatography system proves to be a reliable and accurate system for the quantitative analysis of contaminants at the pg/mL level in drinking water.
- Samples prepared freshly from reference standard stock solutions show better performance than those prepared and stored for significant periods.
- Excellent reproducibility was shown for the target compounds in tap water using 1 mL injections at 0.37× MEC.
- Using timed-SRM, where target compounds are measured only during a specific time window, the reproducibility (%RSDs) near the LOD would be improved.

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