

# Trace analysis of pharmaceuticals and organic contaminants in water

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## Key words

Environmental analysis, water analysis, drinking water, estrone, ibuprofen, nonylphenol, naproxen, trimethoprim, phenytoin, linuron, atenolol, PPCP, environmental monitoring, EQuan MAX Plus, TSQ Endura

## 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 to either 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).

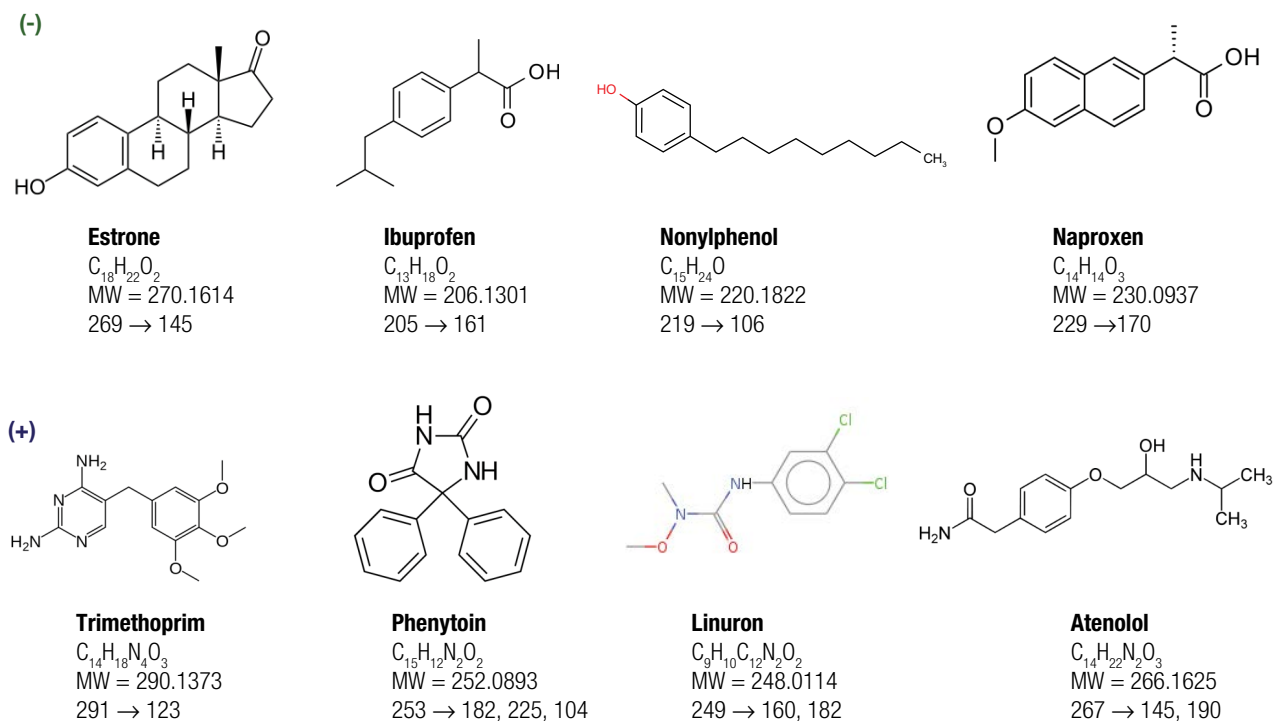


Figure 1. Target compounds.

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<br>B: 0.1% formic acid in methanol               |           |           |
|               | <i>Time</i>   | <i>%A</i> | <i>%B</i> |
|               | 0.00  | 100       | 0         |
|               | 1.00  | 100       | 0         |
| Gradient      | 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<br>B: 0.1% ammonium hydroxide in methanol |           |           |
|               | <i>Time</i>   | <i>%A</i> | <i>%B</i> |
|               | 0.00  | 90        | 10        |
|               | 1.00  | 90        | 10        |
| Gradient      | 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:

| <i>Parameter</i>              | <i>Setting</i>                       |
|-------------------------------|--------------------------------------|
| Spray voltage                 | Positive: 3000 V<br>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.

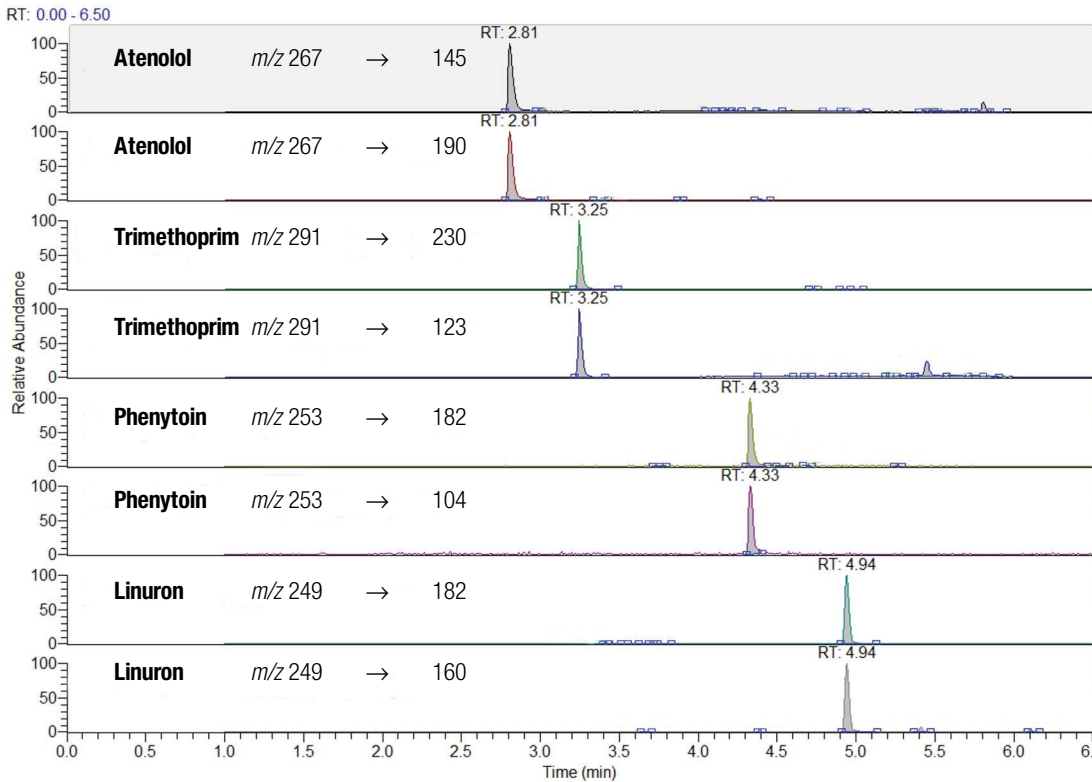


Figure 2. Positive ion SRM chromatograms (10× MEC).

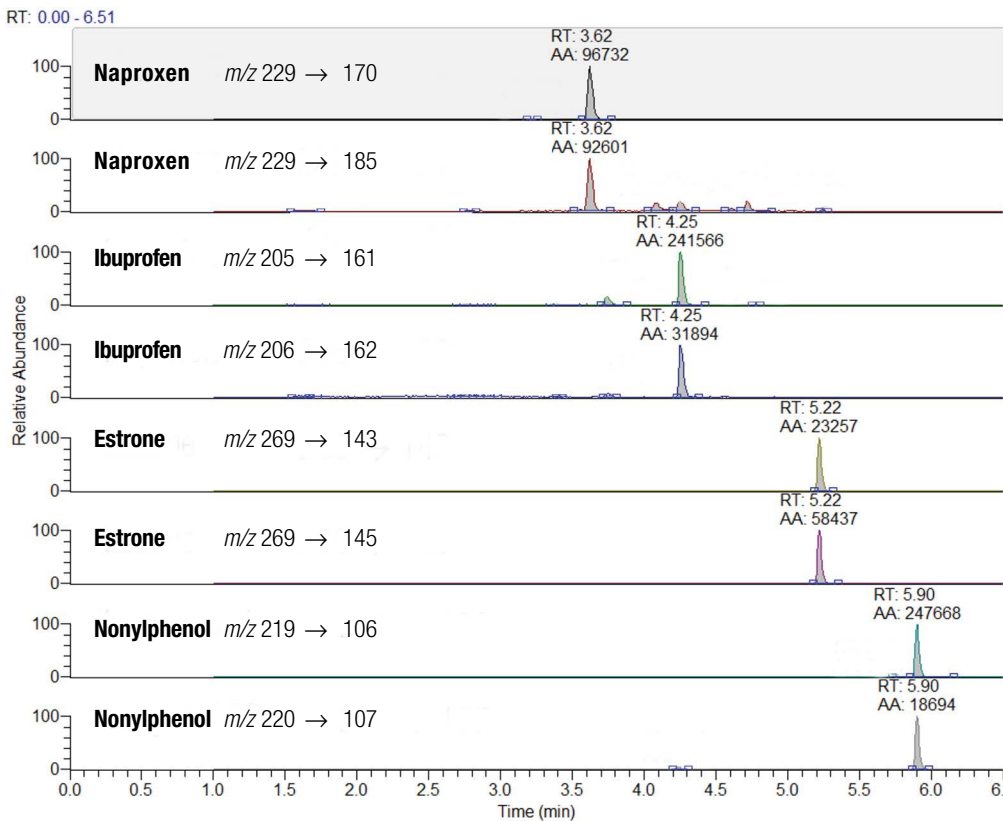


Figure 3. Negative ion SRM chromatograms (10× MEC).

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

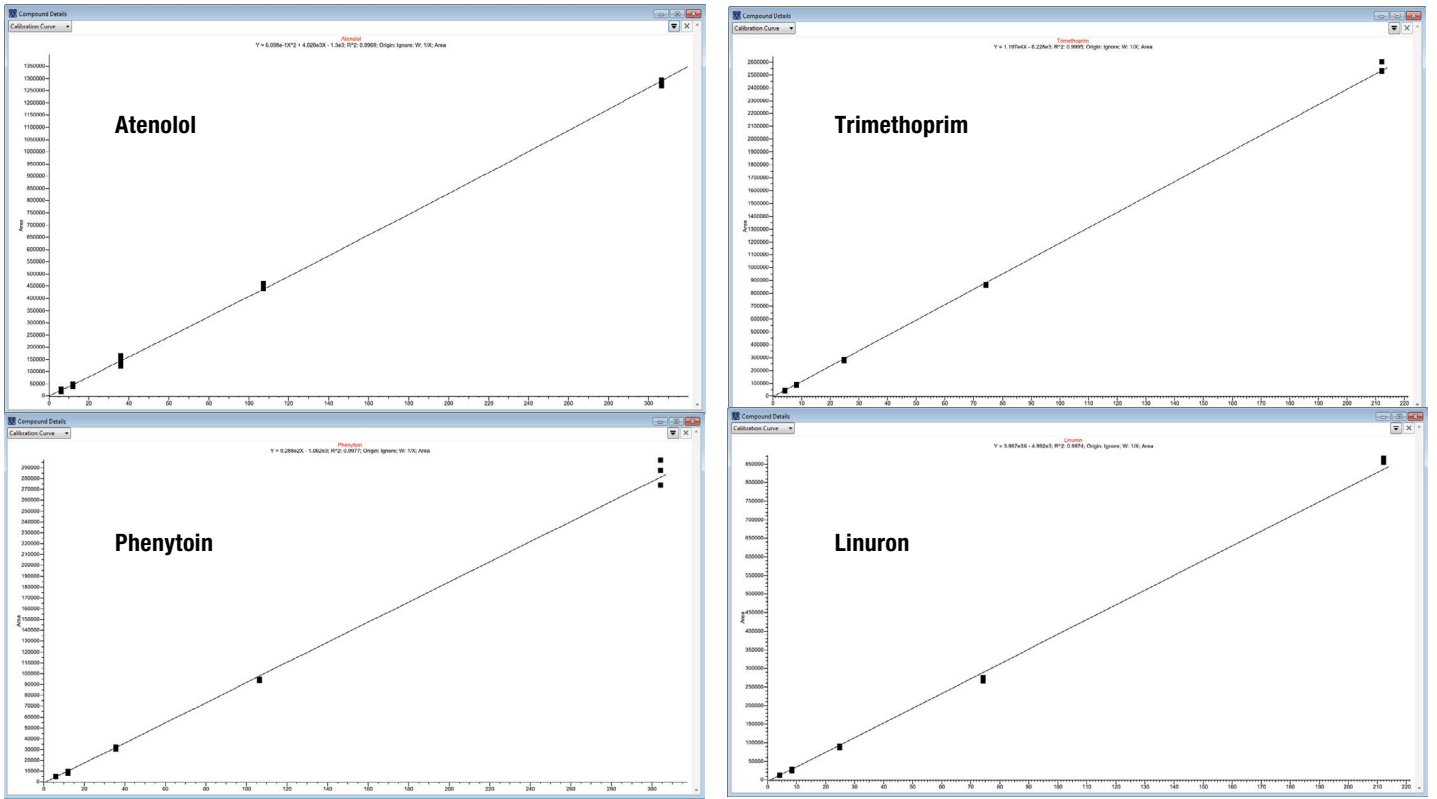


Figure 4. Standard calibration curves for positive ion compounds.

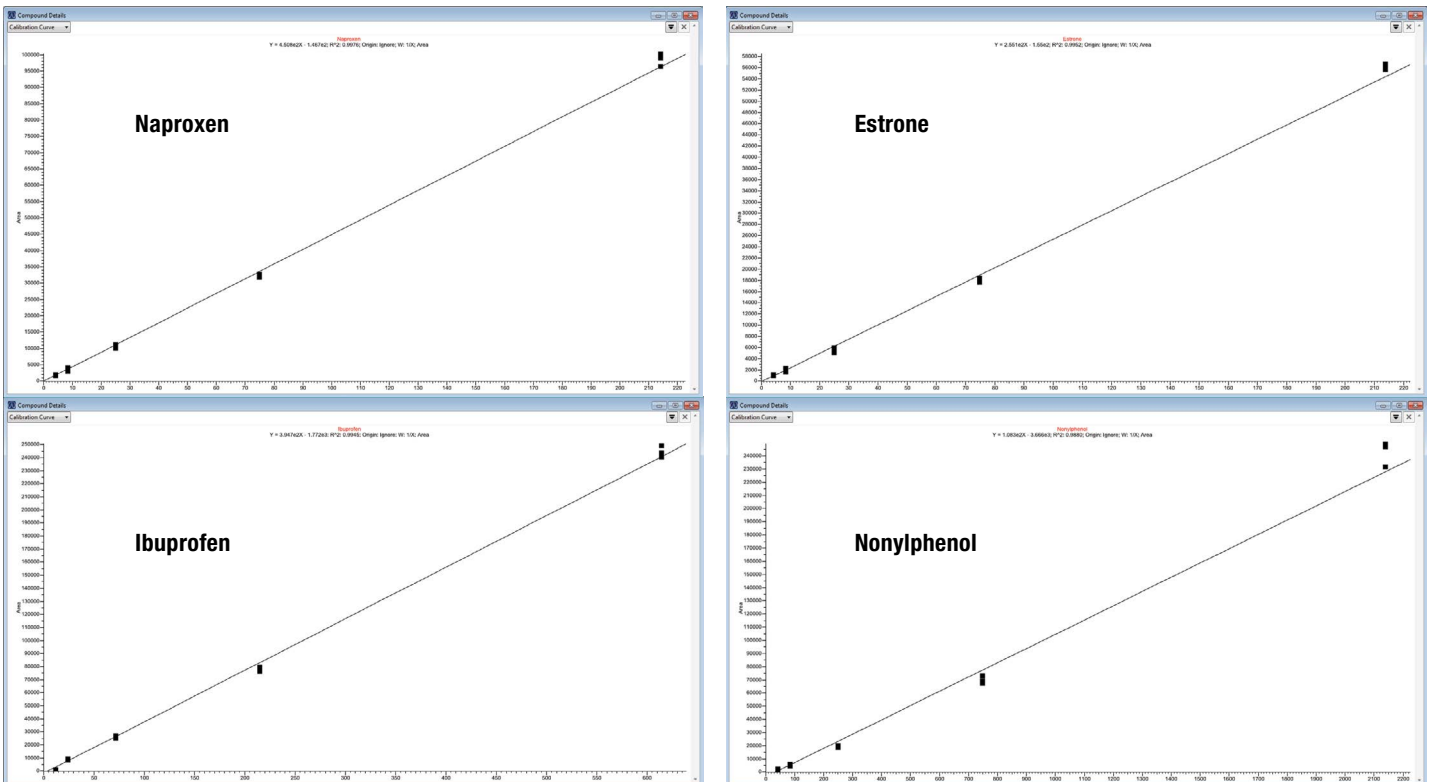


Figure 5. Standard calibration curves for negative ion compounds.

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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