# Direct Aqueous Analysis of Pharmaceuticals in Water at ppt Levels by LC/MS/MS with the Agilent 6490 Triple Quadrupole LC/MS System with Ion Funnel Technology

Application Note

Environmental


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## Abstract

Pharmaceutically active compounds, including drugs and their active metabolites, are an important, if not dominant, water quality issue for both the scientific community and the lay public. Pharmaceutical residues in water may have an adverse impact on humans, wildlife, and fish. Therefore, sensitive and reliable analytical methods are necessary to detect these compounds at trace levels. This study illustrates the direct analysis of pharmaceutical and personal care products (PPCPs) in surface water at the ng/L concentration level using an Agilent 1290 Infinity LC System and an Agilent 6490 Triple Quadrupole LC/MS with Agilent Jet Stream and Dual Ion Funnel Technology. No sample preconcentration is required with this instrument to measure a suite of 20 PPCPs at limits of detection that vary from 1 to 500 ng/L depending on the analyte's chemical structure and ionization efficiency. The elimination of sample preconcentration steps dramatically reduces sample preparation time, ease and cost of analysis, while offsetting potential matrix effects common to SPE methods.


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## Experimental Methods

### Sample Preparation

Pharmaceutical analytical standards were purchased from Sigma-Aldrich, (St. Louis, MO). Individual pharmaceutical stock solutions were prepared at ~1 mg/ml in pure acetonitrile or methanol depending on the solubility of each individual compound, and stored at -18 ºC. From these solutions, working standard solutions were prepared by dilution with acetonitrile and water.

Wastewater samples were collected from an effluent site in Boulder Creek (Boulder, CO) and surface water samples were taken from different rivers and lakes in Colorado.

**LC Conditions for Agilent 1290 Infinity LC**

| | |
|---|---|
| Column | Agilent ZORBAX Eclipse Plus C-18 RRHT, 2.1 x 50 mm, 1.8 µm (p/n 959741-902) |
| Column temp | 25 °C |
| Mobile phase | 10% ACN and 90% $H_2O$ with 0.1% $CH_3COOH$ |
| Flow-rate | 0.4 mL/min |
| Gradient | $t_0$ = 10% ACN<br>$t_{1.7}$ = 10% ACN<br>$t_{10}$ = 100% ACN<br>$t_{10.3}$ = 100% ACN |
| Injection volume | 40 µL |

**Agilent 6460 Triple Quadrupole LC/MS Spraychamber Conditions for Positive Ion Mode**

| | |
|---|---|
| Drying gas temperature | 250 ºC |
| Drying gas flow | 10 L/min |
| Nebulizer pressure | 45 psi |
| Sheath gas temperature | 375 ºC |
| Sheath gas flow | 11 L/min |
| Capillary | 4000 V |
| Nozzle voltage | 0 V |
| Delta EMV | 400 V |

**Agilent 6460 Triple Quadrupole LC/MS Spraychamber Conditions for Negative Ion Mode**

| | |
|---|---|
| Drying gas temperature | 250 ºC |
| Drying gas flow | 10 L/min |
| Nebulizer pressure | 45 psi |
| Sheath gas temperature | 300 ºC |
| Sheath gas flow | 11 L/min |
| Capillary | 3500 V |
| Nozzle voltage | 1500 V |
| Delta EMV | 400 V |

**Agilent 6490 Triple Quadrupole LC/MS Spraychamber Conditions for Positive Ion Mode**

| | |
|---|---|
| Drying gas temperature | 250 ºC |
| Drying gas flow | 15 L/min |
| Nebulizer pressure | 45 psi |
| Sheath gas temperature | 350 ºC |
| Sheath gas flow | 11 L/min |
| Capillary | 4000 V |
| Nozzle voltage | 0 V |
| Delta EMV | 400 V |

**Agilent 6490 Triple Quadrupole LC/MS Spraychamber Conditions for Negative Ion Mode**

| | |
|---|---|
| Drying gas temperature | 250 ºC |
| Drying gas flow | 15 L/min |
| Nebulizer pressure | 45 psi |
| Sheath gas temperature | 300 ºC |
| Sheath gas flow | 11 L/min |
| Capillary | 3000 V |
| Nozzle voltage | 1500 |
| Delta EMV | 400 V |

## Results and Discussion

Table 1 shows the MRM transitions and MS operating parameters chosen for the PCPPs analyzed in this study. Twenty compounds were selected from a list of PPCPs that are part of EPA Method 1694. These 20 compounds represent commonly occurring PPCPs in water and wastewater, which have been reported in the literature. Two transitions were obtained for all compounds, both a quantitative ion and a qualifier ion. Detection limits are based on the presence of both ions. Both positive and negative electrospray was used for the ionization method.

*Table 1. MRM Transitions and MS Operating Parameters Selected for the Analysis of PPCP Compounds In Positive and Negative Ion Mode Electrospray. Compounds Detected in Negative Ion Mode are Shown in Bold.*

| Compound | Fragmentor voltage | MRM transitions (m/z) | Collision energy (eV) |
|---|---|---|---|
| Acetaminophen | 90 | 152 > 110 | 15 |
| | | 152 > 65 | 35 |
| Albuterol | 90 | 240 > 148 | 15 |
| | | 240 > 166 | 5 |
| Atenolol | 130 | 267 > 145 | 20 |
| | | 267 > 190 | 15 |
| Caffeine | 110 | 195 > 138 | 15 |
| | | 195 > 110 | 25 |
| Carbamazepine | 120 | 237 > 194 | 15 |
| | | 237 > 179 | 35 |
| Cotinine | 90 | 177 > 98 | 25 |
| | | 177 > 80 | 25 |
| DEET | 110 | 192 > 119 | 15 |
| | | 192 > 91 | 30 |
| Dehydronifedipine | 130 | 345 > 284 | 25 |
| | | 345 > 268 | 25 |
| **Diclofenac** | **70** | **294 > 250** | **5** |
| | | **294 > 214** | **10** |
| Diltiazem | 130 | 415 > 178 | 25 |
| | | 415 > 150 | 25 |
| Diphenhydramine | 70 | 256 >167 | 15 |
| | | 256 > 152 | 35 |
| **Gemfibrozil** | **70** | **249 > 121** | **35** |
| **Ibuprofen** | **50** | **205 > 161** | **0** |
| Metoprolol | 135 | 268 > 116 | 15 |
| | | 268 > 56 | 30 |
| **Naproxen** | **50** | **229 > 170** | **5** |
| | | **229 > 169** | **25** |
| Sulfadimethoxine | 80 | 311 > 156 | 20 |
| | | 311 > 92 | 35 |
| Sulfamethoxazole | 80 | 254 > 156 | 10 |
| | | 254 > 92 | 30 |
| **Triclocarban** | **90** | **313 > 160** | **5** |
| | | **313 > 126** | **15** |
| **Triclosan** | **75** | **287 > 35** | **5** |
| | | **289 > 37** | **5** |
| Trimethoprim | 75 | 291 > 230 | 25 |
| | | 291 > 261 | 25 |

Table 2 shows the limits of detection obtained by direct aqueous injection of a 40-µL water sample and a comparison between the Agilent 6460 Triple Quadrupole LC/MS (Jet Stream only) and the Agilent 6490 Triple Quadrupole (Jet Stream with Dual Ion Funnel Technology). Generally, the increase in sensitivity with the Agilent 6490 Triple Quadrupole shows an increase of three to five times in both positive and negative ion electrospray, but does vary from compound to compound. The limit of detection for the Agilent 6490 Triple Quadrupole varied from 1 to 500 ng/L with the median limit of detection being 10 ng/L.

*Table 2. Limits of Detection (LOD) are Shown for PCPPs Analyzed on Two Agilent Triple Quads: Agilent 6460 Triple Quadrupole LC/MS with Agilent Jet Stream Technology and on Agilent 6490 Triple Quadrupole LC/MS with Agilent Jet Stream and Dual Ion Funnel Technology.*

| Compound | LOD 6460 (ng/L) | LOD 6490 ( (ng/L) | Increase in LOD (times) |
|---|---|---|---|
| Acetaminophen | 75 | 25 | 3 |
| Albuterol | 10 | 5 | 2 |
| Atenolol | 50 | 10 | 5 |
| Caffeine | 500 | 50 | 10 |
| Carbamazepine | 25 | 5 | 5 |
| Cotinine | 50 | 10 | 5 |
| DEET | 10 | 1 | 10 |
| Dehydronifedipine | 10 | 1 | 10 |
| Diclofenac | 500 | 100 | 5 |
| Diltiazem | 30 | 10 | 3 |
| Diphenhydramine | 10 | 10 | 1 |
| Gemfibrozil | 500 | 25 | 20 |
| Ibuprofen | 1000 | 500 | 2 |
| Metoprolol | 25 | 5 | 5 |
| Naproxen | 500 | 500 | 1 |
| Sulfadimethoxine | 50 | 10 | 5 |
| Sulfamethoxazole | 75 | 50 | 1.5 |
| Triclocarban | 75 | 25 | 3 |
| Triclosan | 500 | 50 | 10 |
| Trimethoprim | 75 | 25 | 3 |

Figure 1 shows an example standard curve for atenolol in water using the Agilent 6490 Triple Quadrupole LC/MS for analysis. In general, all compounds gave linear results with excellent sensitivity over three orders of magnitude, with $r^2$ values of 0.99 or greater.

Figure 2 shows the limits of detection and ion ratios obtained for the Agilent 6490 Triple Quadrupole LC/MS analysis of dehydronifedipine, a common anti-anginal pharmaceutical. This figure demonstrates that 40 femtograms of this compound on-column can be detected with both MRM transitions present for identification.

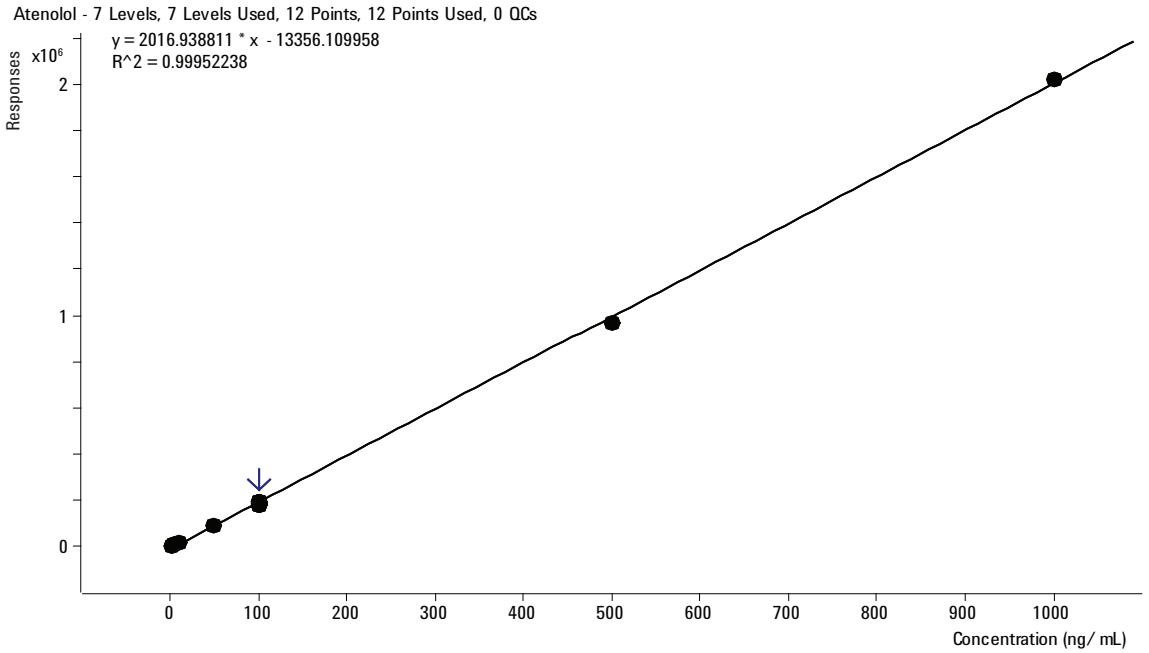


*Figure 1. Calibration curve for atenolol (from 1 ng/L to 1000 ng/L).*

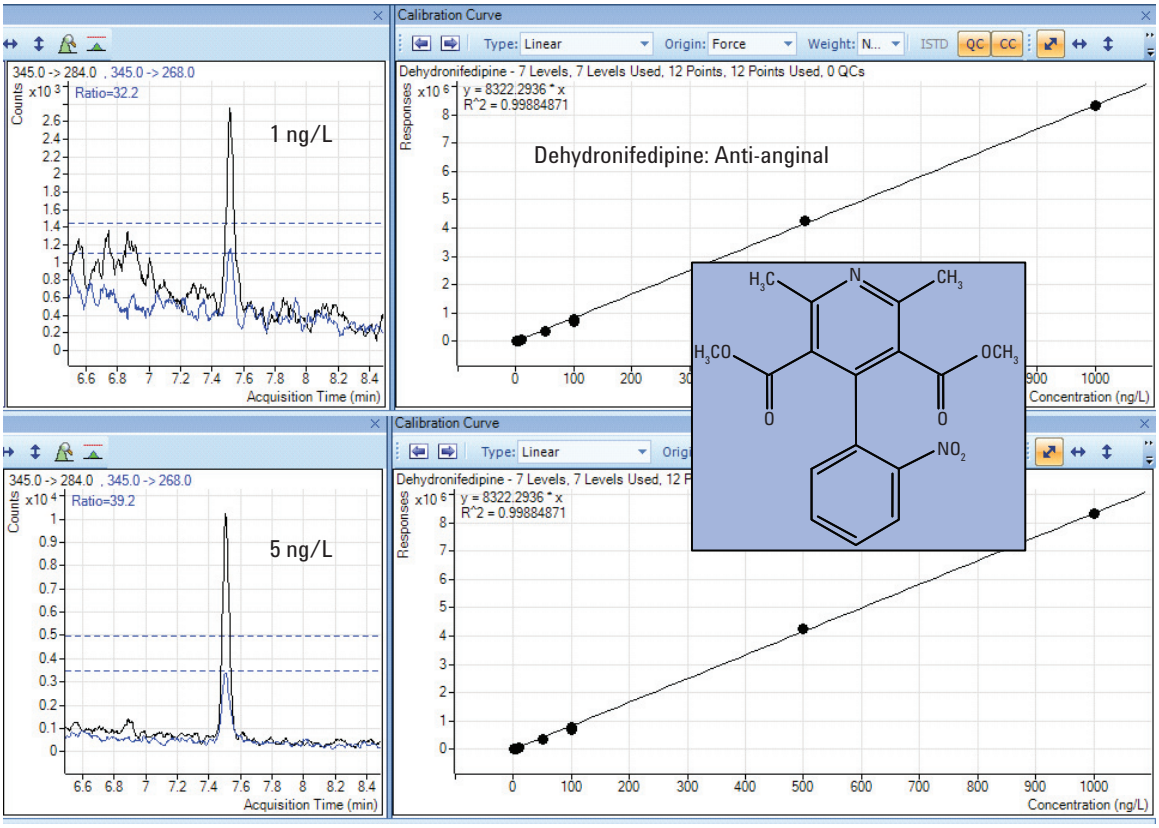


*Figure 2. Ion ratios showing both transitions identified and calibration curve for dehydronifedipine.*

Finally, wastewater and surface water samples were analyzed with the Agilent 6490 Triple Quadrupole LC/MS by direct aqueous injection and the presence of several PCPPs was confirmed. Figure 3 shows the qualifying ion abundance ratios for two of these compounds, diltiazem and sulfamethoxazole, identified in a surface water sample. As shown in Figure 3 in the two ion profiles, both pharmaceuticals were readily identified in this complex matrix due to the selectivity of the MRM transitions and instrument sensitivity.

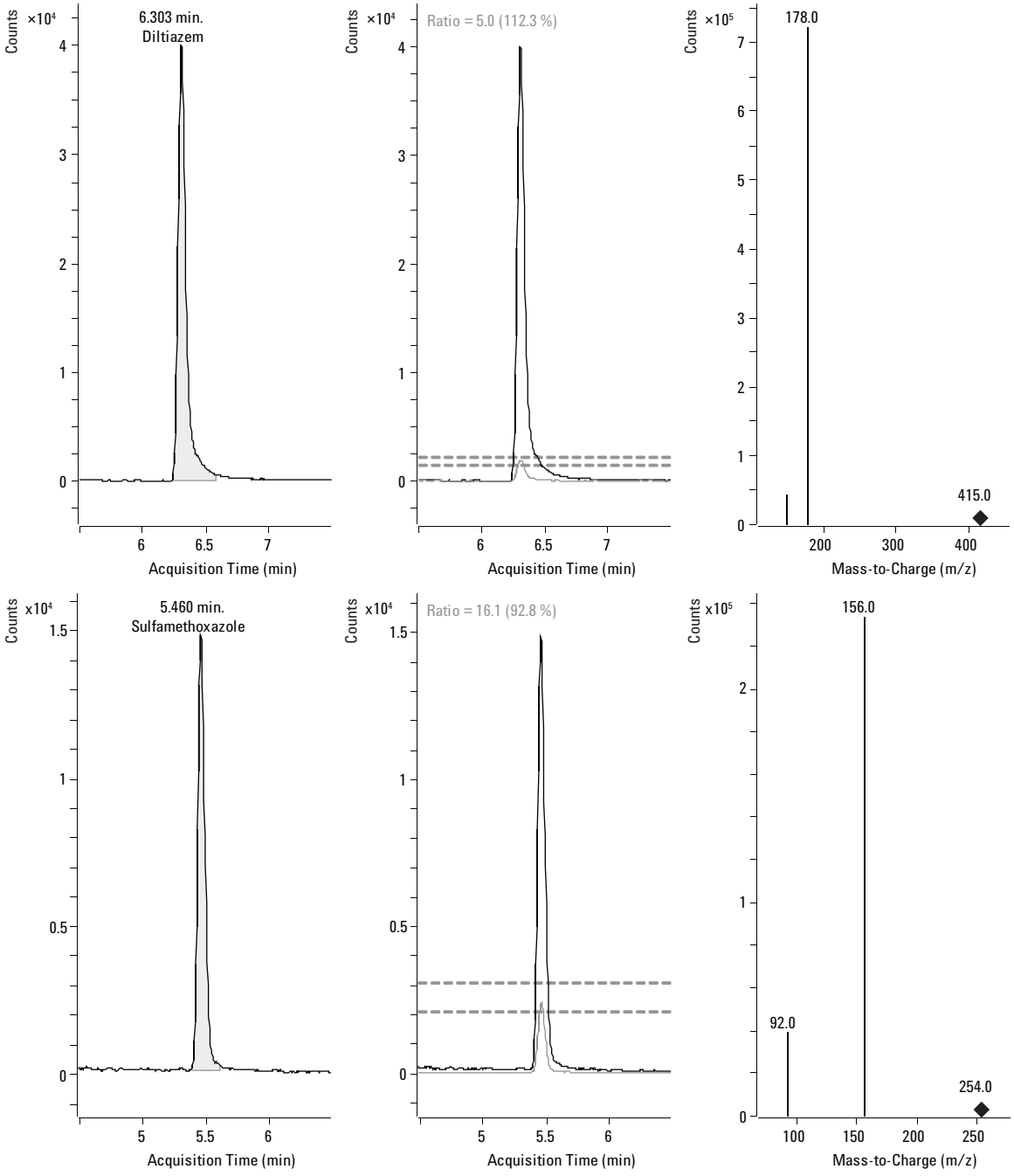


*Figure 3. Ion chromatograms showing positive findings for (a) diltiazem and (b) sulfamethoxazole in a surface water sample collected near Denver, Colorado. Ion ratios are shown, as well as corresponding spectra using the Agilent 6490 Triple Quadrupole LC/MS.*

## Conclusions

The Agilent 6490 Triple Quadrupole LC/MS system with Agilent Jet Stream and Dual Ion Funnel Technologies was compared to the Agilent 6460 Triple Quadrupole LC/MS without the ion funnel and found to be approximately three to five times more sensitive for the majority of compounds tested. This sensitivity enhancement was mainly due to the new hexabore capillary inlet and dual ion funnel technology. The excellent sensitivity in combination with the selectivity of the triple quadrupole makes this an ideal instrument for the direct determination of pharmaceuticals and personal care products in water samples such as the surface water example given here. Both the 6460 and 6490 instruments were well suited for the analysis of PPCPs in environmental water samples with excellent limits of detection.

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