Measurement of Antibiotics in Environmental Waters using LC-QQQ and fully automated Online Enrichment
Trace Level Measurement – LC-QQQ
Measurement of Antibiotics in Environmental Waters using LC-QQQ and Online Enrichment

Antibiotics are widely used for the treatment of bacterial infections in humans. After excretion they ultimately enter waste water treatment plants (WwTP) with subsequent discharge into surface water and the aquatic environment.

Their potential impact on the environment and human health (e.g. antibiotic resistance) is of interest and therefore their presence needs to be closely monitored.

This flyer outlines a method to measure 23 antibiotics from nine different drug classes in environmental waters using an Agilent 1200/6400 LC-QQQ system incorporating 'front end' automated online solid phase enrichment.

**Linearity**
was observed within 0.005 - 50 ng (on column), except for amoxicillin (0.050 - 50 ng) and ornidazole as well as for chlortetracycline (0.005 to 30 ng).

**Accuracy and Precision** were within ± 20% of the nominal value and < 8.5%.

**Recovery and Precision** were > 65% (for most analytes) and < 15%.

The method was successfully applied to measure the antibiotics in two different waste water treatment plant inflows (hospital and domestic water), the WwTP influent and the WwTP effluent.

**Compounds**

23 compounds 9 drug families
wide mass and chemical properties range

<table>
<thead>
<tr>
<th>Fluoroquinolones</th>
<th>Quinolones</th>
<th>Tetracyclines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>Nalidixic acid</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Enoxacin</td>
<td>Oxolinic acid</td>
<td>Chlorotetracycline</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>Sulfoxide</td>
<td>ß-lactams</td>
</tr>
<tr>
<td>Flumequine</td>
<td>Sulfamethoxazole</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td></td>
<td>Cefotaxim</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td></td>
<td>Diaminopyrimidines</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td></td>
<td>Ormetoprim</td>
</tr>
<tr>
<td>Pipemidic Acid</td>
<td></td>
<td>Trimethoprim</td>
</tr>
<tr>
<td>Sarafloxacin</td>
<td></td>
<td>Glycopeptides</td>
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<td></td>
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<td>Vancomycin</td>
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</tbody>
</table>
**Materials and Methods**

**Analytical Column:** ZORBAX Eclipse Plus C18
(3.5 µm, 2.1 mm I.D. x 150 mm)

**Mobile Phase:** 
H₂O/ACN + 0.1% formic acid

**Flow rate:** 
0.5 mL/min (step gradient)

**Deuterated internal standards:** Norfloxacine-d₄, amoxicilline-d₄, sulfamethoxazole-d₄

**Optimization of the online enrichment for:**
- Cartridge Sorbent (C18)
- pH-value (fluorochino-lones pH 4, other compounds pH 7)
- Sample volume (1800 µL, 2 x 900 µL)
- Sample loading flow rate (1 mL/min)
- Sample elution (Backflush with LC gradient)

**Performance Examples**
• Qualifying and Quantifying 23 antibiotics in river water
• Fully automated SPE
• Easy to use and suited for routine analysis

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Drug class | Analyte | IDL (pg on column) | MDL (ng/L)
---|---|---|---
Macrolides | Tylosin 0.8 | 6.0
| Erythromycin 0.9 | 7.5
Tetracyclines | Chlorotetracycline 1.1 | 9.8
| Tetracycline 1.5 | 1.8
Beta-lactams | Amoxicillin 16 | 15
| Cefotaxime 3.5 | 3.6
Diaminopyrimidines | Trimethoprim 2.4 | 1.4
| Ormetoprim 1.6 | 1.9
Sulfonamides | Sulfadimidine 0.5 | 1.4
| Sulfamethoxazole 2.8 | 3.1
Quinolones | Oxolinic acid 2.2 | 1.7
| Nalidixic acid 2.1 | 3.3
Fluoroquinolones | Flumequine 2.3 | 2.1
| Pipemidic acid 4.6 | 15
| Enrofloxacin 2.6 | 3.3
| Enoxacin 3.3 | 4.6
| Lomefloxacin 2.6 | 3.3
| Sarafloxacin 0.6 | 1.1
| Norfloxacin 5.5 | 5.0
| Ciprofloxacin 2.6 | 4.3
| Ofloxacin 1.3 | 2.5
Imidazoles | Ornidazole 3.6 | 4.3
Glycopeptides | Vancomycin 2.2 | 5.0

Instrument (pg) and method detection limit (ng/L).

Drug Class | Analyte | Hospital Wastewater | Domestic Wastewater | WwTP influent | WwTP effluent
---|---|---|---|---|---
Macrolides | Tylosin 0.8 | < LOD | < LOD | < LOD | < LOD
| Erythromycin 0.9 | 1671 ± 45 | 5.2 ± 1.1 | 1440 ± 302 | 498 ± 35
Tetracyclines | Chlorotetracycline < LOD | < LOD | < LOD | < LOD
| Tetracycline < LOD | < LOD | < LOD | < LOD
Beta-lactams | Amoxicillin 151 ± 47 | < LOD | 20.2 ± 4.8 | 17.3 ± 2.1
| Cefotaxime < LOD | < LOD | < LOD | < LOD
Diaminopyrimidines | Trimethoprim 649 ± 33 | 1.9 ± 0.2 | 296 ± 72 | 401 ± 31
| Ormetoprin 14 ± 5 | 4.5 ± 0.3 | 15.0 ± 1.1 | 32.0 ± 1.9
Sulfonamides | Sulfadimidine < LOD | < LOD | < LOD | < LOD
| Sulfamethoxazole 1298 ± 150 | < LOD | 1015 ± 95 | 4084 ± 93
Quinolones | Oxolinic acid < LOD | < LOD | < LOD | < LOD
| Nalidixic acid < LOD | < LOD | < LOD | < LOD
Fluoroquinolones | Flumequine < LOD | < LOD | < LOD | < LOD
| Pipemidic acid < LOD | < LOD | < LOD | < LOD
| Enrofloxacin < LOD | < LOD | < LOD | < LOD
| Enoxacin 1425 ± 13 | 606 ± 36 | 842 ± 90 | 32 ± 9
| Lomefloxacin 1120 ± 90 | < LOD | 59 ± 4 | 8.8 ± 0.5
| Sarafloxacin < LOD | < LOD | < LOD | < LOD
| Norfloxacin 10898 ± 52 | 43 ± 8 | 6658 ± 146 | 217 ± 8
| Ciprofloxacin 11105 ± 37 | < LOD | 6730 ± 328 | 1101 ± 96
| Ofloxacin 13002 ± 527 | 79 ± 8 | 8115 ± 180 | 17086 ± 79
Imidazoles | Ornidazole < LOD | < LOD | < LOD | < LOD
Glycopeptides | Vancomycin 2204 ± 150 | < LOD | 1784 ± 116 | 1297 ± 29

Measured concentrations (ng/L) in hospital wastewater, domestic wastewater, WwTP influent and WwTP effluent.