Determination of fluoroquinolones in fish tissues, biological fluids and environmental waters

Pharmaceuticals
Fluoroquinolones: effects
Fluoroquinolones: method development

Lack of analytical methods
Objectives

1. Optimization LC-MS/MS analysis of FQs
2. Optimization of the extraction: fish tissue/biofluids + environmental water
3. Application to real samples
Experimental part

Focused Ultrasound Solid-Liquid Extraction (FUSLE)

Solid-Phase Extraction (SPE)

Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS)
Fluoroquinolones

Norfloxacin (NORF)
Ofloxacin (OFLO) and Levofloxacin (LEVO)
Lomefloxacin (LOME)
Enorfloxacin (ENO)
Ciprofloxacin (CIPRO)
Danofloxacin (DANO)
Pefloxacin (PEFLO)
Sparfloxacin (SPAR)
Environmental samples

Environmental waters

Fish tissues and biofluids
Results and Discussion

1. Optimization LC-MS/MS analysis of FQs
2. Optimization of the extraction: fish tissue/biofluids + environmental water
3. Application to real samples
Optimization of LC-MS/MS

- Column temperature: 35 °C
- Flow rate: 0.3 mL/min
- Gradient
- Max. injection volume: 3μL
- Capillary voltage: 3000 V
- Nebulizer pressure: 30 psi
- Drying gas flow: 8 L/h
- Drying gas temp.: 300 °C
- Fragmentor and collision energy

Chromatographic parameters

Electrospray ionization parameters

MS/MS parameters
## Fragmentor and collision energy

<table>
<thead>
<tr>
<th>Analyte</th>
<th>SRM transitions</th>
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<tbody>
<tr>
<td>NORF</td>
<td>320 (104 V) → 302/231/282 (17/41/29 eV)</td>
</tr>
<tr>
<td>ENO</td>
<td>321 (104 V) → 303/232/204 (17/37/45 eV)</td>
</tr>
<tr>
<td>PEFLO</td>
<td>334 (104 V) → 316/290/233 (17/13/25 eV)</td>
</tr>
<tr>
<td>OFLO/LEVO</td>
<td>362 (104 V) → 318/261/344 (17/25/17 eV)</td>
</tr>
<tr>
<td>CIPRO</td>
<td>332 (104 V) → 314/231/288 (17/41/13 eV)</td>
</tr>
<tr>
<td>DANO</td>
<td>358 (104 V) → 340/82/255 (21/45/41 eV)</td>
</tr>
<tr>
<td>LOME</td>
<td>352 (104 V) → 265/308/334 (21/13/17 eV)</td>
</tr>
<tr>
<td>ENRO</td>
<td>360 (104 V) → 342/316/286 (17/17/37 eV)</td>
</tr>
<tr>
<td>SPAR</td>
<td>393 (104 V) → 349/292/375 (17/21/17 eV)</td>
</tr>
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Results and Discussion

1. Optimization LC-MS/MS analysis of FQs
2. Optimization of the extraction: fish tissue/biofluids + environmental waters
3. Application to real samples
Optimization of the extraction

2.1 Fish tissues: liver and muscle

2.2 Fish biofluids: plasma and bile

2.3 Environmental waters: seawater, estuarine water and WWTP water
Optimization of the extraction

2.1 Fish tissues: liver and muscle

2.2 Fish biofluids: plasma and bile

2.3 Environmental waters: seawater, estuarine water and WWTP water
Fish tissues: FUSLE

- Extraction solvent
Fish tissues: FUSLE

- Extraction time
Fish tissues: FUSLE

- Extraction time

**Optimum**: 30 s x 2
Solid-Phase Extraction (SPE)

• Sorbent phase

![Bar chart showing normalized recovery for different drugs at various pH values.](image)
Liver: dirty extracts

Muscle

Liver

✓

✗
Liver: dirty extracts

- Positive matrix effect
Liver: extra step to remove lipids

• Clean-up strategies
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• Clean-up strategies
  1. Increase HLB phase
Liver: extra step to remove lipids

- Clean-up strategies
  1. Increase HLB phase
  2. Florisil-HLB
Liver: extra step to remove lipids

• Clean-up strategies
  1. Increase HLB phase
  2. Florisil-HLB
  3. Liquid-liquid extraction (LLE) prior to HLB
Optimization of the extraction

2.1 Fish tissues: liver and muscle

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Biofluids: SPE

• HLB: signal suppression
Biofluids: SPE

• HLB: signal suppression

• MIPs cartridges
Optimization of the extraction

2.1 Fish tissues: liver and muscle

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RP-SPE (Oasis HLB)

- Seawater
- Estuarine and effluent: dirty extracts
  - Signal suppression (> 50 %)
RP-SPE (Oasis HLB)

- Seawater
- Estuarine and effluent: dirty extracts
  - Signal suppression (> 50 %)

- Solution: WAX + HLB
- Cleaner extracts and less signal suppression (< 33 %)
Method validation

- Introduction
- Objectives
- Experimental part
- Results
- Conclusions

75 ng/g

200 ng/g

75 ng/L
Method validation

• Linearity
  • $R^2: 0.997-0.9997$

• Limits of detection
  • LOD < 2 ug/mL
  • LOQ < 5 ug/mL

• Precision
  • RSD < 15 %

• Accuracy
Method validation

- Apparent recoveries: 80 - 126 %

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<tr>
<td>NORF(^a)</td>
<td>107</td>
<td>96</td>
<td>93</td>
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<td>83</td>
<td>95</td>
</tr>
<tr>
<td>PEFLO(^b)</td>
<td>103</td>
<td>89</td>
<td>91</td>
</tr>
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<td>OFLO(^b)</td>
<td>96</td>
<td>94</td>
<td>110</td>
</tr>
<tr>
<td>CIPRO(^a)</td>
<td>92</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td>DANO(^b)</td>
<td>104</td>
<td>92</td>
<td>115</td>
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<tr>
<td>SPAR(^a)</td>
<td>14</td>
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\(^a\)Corrected with \([2H_8]-CIPRO\). \(^b\)Corrected with \([2H_5]-ENRO\).
Method validation

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Real Samples from Biscay Coast
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- **WWTP effluent**
  - 143 ng/L NORF
  - 160 ng/L CIPRO
  - 234 ng/L OFLO/LEVO

- **Estuarine water**
  - 44 ng/L NORF
  - 79 ng/L CIPRO
  - 278 ng/L OFLO/LEVO

- **Grey mullet liver**
  - 4 ng/g OFLO/LEVO

- **Seawater** < LOQ
Conclusions
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- Optimization of 10 FQs
  - Correction with labelled analogues
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• 7 matrices with different clean-up strategies:
  • Fish muscle and seawater: RP-SPE
  • Fish liver: LLE + RP-SPE
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• Real samples from the Biscay Coast:
  • CIPRO, NORF and OFLO/LEVO
  • Water and fish liver samples
Tussen takk!
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