Determinación de sinthéticos musk fragancias en muestras de pez utilizando SPME Arrow-GC-IT-MS/MS

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Master’s in Applied Chromatographic Techniques
INTRODUCTION

OBJECTIVES

EXPERIMENTAL PART

RESULTS AND DISCUSSION

CONCLUSIONS
INTRODUCTION

EMERGING ORGANIC COMPOUNDS (EOCs)
- Pesticides
- Industrial additives
- Detergents
- Pharmaceuticals
  - “Life-style compounds”
- Personal Care Products
  - Ibuprofen
  - Caffeine

PERSONAL CARE PRODUCTS (PCPs)
- Disinfectants
- Synthetic Musk fragrances
- UV filters
- Preservatives
- Stabilizers
  - Octocrylene
  - Oxybenzone

SYNTHETIC MUSK FRAGRANCES
- Nitro musks
- Polycyclic musks
- Macrocyclic musks
- Alicyclic musks
  - AHTN
  - Exaltone
INTRODUCTION

Cashmeran, DPMI
Celestolide, ADBI
Phantolide, AHMI
Traseolide, ATTI
Galaxolide, HHCB
Tonalide, AHTN
Musk xylene, MX
Musk ketone, MK
HHCB-lactone

Polycyclic musks
Nitro musks
Degradation product
INTRODUCTION

PCPs

Excretion

WWTP effluent without musks elimination

Bioaccumulation in fish

Diet exposure

Direct exposure
INTRODUCTION

Novel extraction technique

SPME Arrow
INTRODUCTION

Solid Phase Micro Extraction (SPME)

- Fully automatable
- Limited robustness
- Small sorption volumes

Stir Bar Sorptive Extraction (SBSE)

- High sorption volumes
- Limited commercial coatings
- Specific desorption port

SPME Arrow

- Fully automatable
- High sorption volumes
- Wide range of coatings
- No need of a specific desorption port
OBJECTIVES

- Development of an analytical method for the determination of musk fragrances based on gas chromatography coupled to ion trap tandem mass spectrometry (GC-IT-MS/MS) using a novel micro extraction technique such as PAL SPME Arrow.
- Comparison of the effectiveness of using the PAL SPME Arrows compared to the conventional SPME fibres. Analysis of the main advantages and drawbacks of this novel technique.
- Application of the developed method to commercially available fish samples.
EXPERIMENTAL PART

Sample treatment

SPME Arrow extraction

GC-IT-MS/MS
1. Sample treatment

- Fresh fish samples
- Fillet dissection
- Fish lyophilization
- Sieve through a 500 µm mesh
- Store until analysis
2. SPME Arrow Extraction

0.25 g sample

Incubation 100 °C, 1 min

PDMS Arrow needle penetration

Extraction 100 °C, 45 min 750 rpm

Arrow retraction

Desorption 250 °C, 3 min
3. GC-IT-MS/MS conditions

<table>
<thead>
<tr>
<th>Chromatographic conditions</th>
<th>MS conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Injector temperature</strong></td>
<td><strong>Ionization Mode</strong></td>
</tr>
<tr>
<td><strong>Oven temperature program</strong></td>
<td>Trap</td>
</tr>
<tr>
<td><strong>Flow rate</strong></td>
<td>Manifold</td>
</tr>
<tr>
<td><strong>Column</strong></td>
<td>Transfer line</td>
</tr>
</tbody>
</table>

250°C

70 °C for 5 min
50 °C/min to 200 °C
5 °C/min to 290 °C
290 °C for 3.4 min

1 mL/min He

Zebron ZB-50 (50% phenyl + 50% PDMS)
(30 m x 0.25 mm i.d., 0.25 µm)
### 3. GC-IT-MS/MS conditions

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Precursor Ion (m/z)</th>
<th>Product Iona (m/z) CID Amplitude (V)</th>
<th>m/z Range</th>
<th>Scan time (s/scans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPMI</td>
<td>7.5</td>
<td>191</td>
<td>107 (29), 135 (78), 173 (100)</td>
<td>0.83</td>
<td>94 - 201</td>
</tr>
<tr>
<td>ADBI</td>
<td>8.6</td>
<td>229</td>
<td>131 (8), 173 (100), 187 (17)</td>
<td>0.92</td>
<td>110 - 239</td>
</tr>
<tr>
<td>AHMI</td>
<td>9.1</td>
<td>229</td>
<td>131 (6), 145 (7), 187 (100)</td>
<td>0.93</td>
<td>110 - 239</td>
</tr>
<tr>
<td>ATTI</td>
<td>9.7</td>
<td>215</td>
<td>131 (3), 171 (32), 173 (100)</td>
<td>0.88</td>
<td>104 - 225</td>
</tr>
<tr>
<td>HHCB⁵</td>
<td>9.9</td>
<td>243</td>
<td>171 (3), 213 (100)</td>
<td>0.96</td>
<td>132 - 253</td>
</tr>
<tr>
<td>AHTN⁵</td>
<td>9.9</td>
<td>243</td>
<td>145 (24), 159 (16), 187 (100)</td>
<td>0.96</td>
<td>113 - 253</td>
</tr>
<tr>
<td>MX</td>
<td>10.6</td>
<td>282</td>
<td>265 (100), 266 (12), 280 (33)</td>
<td>1.08</td>
<td>134 - 292</td>
</tr>
<tr>
<td>MK</td>
<td>12.5</td>
<td>279</td>
<td>191 (100), 247 (3), 280 (10)</td>
<td>1.07</td>
<td>132 - 289</td>
</tr>
<tr>
<td>HHCB-Lactone</td>
<td>15.1</td>
<td>257</td>
<td>183 (14), 201 (89), 239 (100)</td>
<td>1.00</td>
<td>123 - 267</td>
</tr>
</tbody>
</table>

⁵ Quantification ions (m/z) are shown in bold type. Qualifier/quantifier ratio is in % in brackets.

Compounds were separated using Multiple Reaction Monitoring mode.

Extracted ion chromatogram (XIC) of the quantifier ions of the 9 musk fragrances at a concentration level of 1 mg L⁻¹.
RESULTS AND DISCUSSION

SPME Arrow optimization

Conventional SPME optimization

Comparison of SPME Arrow and conventional SPME

Method quality parameters

Application of the method
RESULTS AND DISCUSSION

SPME Arrow optimization

1. Coating selection

- **COD**
  - 0.25 g
  - Extraction $\rightarrow$ $T = 80 \, ^\circ C$, $t = 45$ min, 750 rpm
  - 1 mg kg$^{-1}$
  - Desorption $\rightarrow$ $T = 250 \, ^\circ C$, $t = 3$ min

- **Analyte properties**
  - Low Polarity
  - High Polarity
  - Low Volatility
  - High Volatility

- **Carboxen**
- **PDMS 100\,\mu m**
- **PDMS 30\,\mu m**
- **PDMS 7\,\mu m**
- **PDMS-DVB**

Area (log$_{10}$ counts)

<table>
<thead>
<tr>
<th></th>
<th>DPMI</th>
<th>ADBI</th>
<th>AIMI</th>
<th>ATTI</th>
<th>HHCB</th>
<th>AHTN</th>
<th>MX</th>
<th>MK</th>
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<tr>
<td>PA</td>
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<td></td>
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<tr>
<td>PDMS</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PDMS/DVB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

SPME Arrow optimization

2. Extraction temperature

✓ PDMS Arrow
✓ T = 100 °C
RESULTS AND DISCUSSION

SPME Arrow optimization

3. Extraction time

- Extraction time

✓ PDMS Arrow
✓ $T = 100 \, ^\circ C$
✓ $t = 45 \, \text{min}$
RESULTS AND DISCUSSION

SPME Arrow optimization

4. Water addition

- PDMS Arrow
- T = 100 °C
- t = 45 min
- No water

Heating 100 °C

↑ HS humidity

Water condensed in the sorbent

0
50000
100000
150000
200000
250000
300000
350000
400000
450000
Area (counts)

No Water addition 0,5 mL Water 1 mL Water
RESULTS AND DISCUSSION

Comparison of SPME Arrow and conventional SPME

Method quality parameters

Application of the method
Conventional SPME optimization

1. Extraction temperature

\[ T = 80 \, ^\circ\text{C} \]

2. Extraction time

\[ t = 60 \, \text{min} \]

COD
0.25 g
Extraction \( t = 45 \, \text{min}, 750 \, \text{rpm} \)

1 mg kg\(^{-1}\)
Desorption \( T = 250 \, ^\circ\text{C}, t = 3 \, \text{min} \)
RESULTS AND DISCUSSION

SPME Arrow optimization
Conventional SPME optimization
Comparison of SPME Arrow and conventional SPME
Method quality parameters
Application of the method

Vs.
Comparison of SPME Arrow and conventional SPME

**SPME Arrow**
- PDMS Arrow
- T = 100 °C
- t = 45 min
- No water

**Conventional SPME**
- PDMS Fibre
- T = 80 °C
- t = 60 min
- No water

Desorption → 250 °C, 3 min

Analysis of 0.25 g COD 1 mg kg⁻¹

SPME Arrow = ~ 10 x conventional SPME

ENHANCED SENSITIVITY
RESULTS AND DISCUSSION

SPME Arrow optimization vs. Conventional SPME optimization

Comparison of SPME Arrow and conventional SPME

Method quality parameters

Application of the method
RESULTS AND DISCUSSION

Method quality parameters

- Linear range:
  - low: 2.5 – 75 ng g⁻¹ (d.w.)
  - high: 75 – 500 ng g⁻¹ (d.w.)
- MQLs → 2.5 – 5 ng g⁻¹ (d.w.)
- MDLs → 0.5 – 2.5 ng g⁻¹ (d.w.)
- Repeatability %RSD < 15%
- Reproducibility %RSD < 15%

<table>
<thead>
<tr>
<th>Compound</th>
<th>MDL (ng g⁻¹)</th>
<th>MQL (ng g⁻¹)</th>
<th>Linear range (ng g⁻¹)</th>
<th>( r^2 )</th>
<th>Repeatability¹</th>
<th>Reproducibility¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPMI</td>
<td>0.5</td>
<td>5</td>
<td>5 - 250</td>
<td>0.9920</td>
<td>4</td>
<td>11</td>
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<tr>
<td>ADBI</td>
<td>0.5</td>
<td>2.5</td>
<td>2.5 - 75</td>
<td>0.9996</td>
<td>4</td>
<td>12</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>75-500</td>
<td>0.9990</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHMI</td>
<td>0.5</td>
<td>5</td>
<td>5 - 250</td>
<td>0.9998</td>
<td>5</td>
<td>10</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ATTI</td>
<td>1</td>
<td>5</td>
<td>5-75</td>
<td>0.9977</td>
<td>5</td>
<td>13</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>75-500</td>
<td>0.9993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHCB</td>
<td>1</td>
<td>5</td>
<td>5 - 75</td>
<td>0.9997</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>75-500</td>
<td>0.9950</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHTN</td>
<td>0.5</td>
<td>2.5</td>
<td>2.5 - 75</td>
<td>0.9995</td>
<td>2</td>
<td>9</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>75-500</td>
<td>0.9990</td>
<td></td>
<td></td>
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<tr>
<td>MX</td>
<td>2.5</td>
<td>5</td>
<td>5-50</td>
<td>0.9889</td>
<td>15</td>
<td>23</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>50-500</td>
<td>0.9991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK</td>
<td>0.5</td>
<td>2.5</td>
<td>2.5 - 25</td>
<td>0.9992</td>
<td>6</td>
<td>12</td>
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<tr>
<td></td>
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<td></td>
<td>25-500</td>
<td>0.9991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHCB-Lactone</td>
<td>1</td>
<td>2.5</td>
<td>2.5 - 100</td>
<td>0.9999</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100-500</td>
<td>0.9996</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Expressed as Relative Standard Deviation (%RSD); n=5.
RESULTS AND DISCUSSION

SPME Arrow optimization

Conventional SPME optimization

Comparison of SPME Arrow and conventional SPME

Method quality parameters

Application of the method
RESULTS AND DISCUSSION

Application of the method

• 3 fish species: cod (*Gadus morhua*), sole (*Solea solea*) and hake (*Merluccius merluccius*).

• Acceptance criteria:

  1. Retention time.
  2. Presence of quantifier and qualifier ions.

<table>
<thead>
<tr>
<th>Relative intensity (% of base peak)</th>
<th>EI-GC-MS (relative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 50 %</td>
<td>± 10 %</td>
</tr>
<tr>
<td>&gt; 20 % to 50 %</td>
<td>± 15 %</td>
</tr>
<tr>
<td>&gt; 10 % to 20 %</td>
<td>± 20 %</td>
</tr>
<tr>
<td>≤ 10 %</td>
<td>± 50 %</td>
</tr>
</tbody>
</table>

Extracted Ion Chromatogram (XIC) of a sole sample.
## RESULTS AND DISCUSSION

### Application of the method

<table>
<thead>
<tr>
<th>Compound</th>
<th>COD (Gadus morhua)</th>
<th>SOLE (Solea solea)</th>
<th>HAKE (Merluccius merluccius)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Product ions (m/z)</td>
<td>ng g⁻¹</td>
<td>Product ions (m/z)</td>
</tr>
<tr>
<td>DPMI</td>
<td>-</td>
<td>n.d.</td>
<td>107 (32), 135 (82), 173 (100)</td>
</tr>
<tr>
<td>ATTI</td>
<td>131 (3), 171 (29), 173 (100)</td>
<td>&lt;MQL</td>
<td>-</td>
</tr>
<tr>
<td>HHCB</td>
<td>171 (4), 213 (100)</td>
<td>17.5</td>
<td>171 (4), 213 (100)</td>
</tr>
<tr>
<td>AHTN</td>
<td>145 (24), 159 (18), 187 (100)</td>
<td>5.1</td>
<td>145 (22), 159 (18), 187 (100)</td>
</tr>
<tr>
<td>HHCB-lactone</td>
<td>-</td>
<td>n.d.</td>
<td>183 (14), 201 (86), 239 (100)</td>
</tr>
</tbody>
</table>

* MQL: method quantification limit; n.d.: not detected.

* Quantifier and qualifier ions found in the sample and qualifier/quantifier ratio in %

**HHCB and AHTN**

- Most used polycyclic musks
- Reduced AHTN content due to European Directive 2008/42/EC, 2008

**Restricted by European Directive 2002/34/EC**
1. A novel SPME Arrow extraction technique was tested for the determination of synthetic musk fragrances in fish samples. The developed method allowed the determination of the fragrances with MDLs ranging between 0.5 ng g\(^{-1}\) (d.w.) and 2.5 ng g\(^{-1}\) (d.w.) and MQLs ranged from 2.5 ng g\(^{-1}\) (d.w.) to 5 ng g\(^{-1}\) (d.w.) with only a lyophilization of the sample step as sample handling.

2. SPME Arrow demonstrated being able of extracting the compounds from the fish samples with an efficiency up to 10 times higher than conventional SPME fibres, providing better sensitivity and robustness.

3. Trace levels of these compounds were determined in different commercial fish samples with concentration levels of low ng g\(^{-1}\) of HHCB and AHTN.
Master's Final Thesis

DETERMINATION OF SYNTHETIC MUSK FRAGRANCES IN FISH SAMPLES USING SPME ARROW-GC-IT-MS/MS

Óscar Castro Serrano

June 2018
Master's in Applied Chromatographic Techniques