Enantiomeric separation of chiral synthetic opioids and metabolites in DBS by UHPLC-HRMS

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New synthetic opioids

Years 2012 - 2019

Fentanyl derivatives

➢ Public health problems
➢ Social issues
➢ Increase in criminality

Enantiomers and their analysis

Same chemical structure but different pharmacological profiles

It is important to separate the two enantiomers in order to be able to study their peculiar characteristics


**Dried blood spot (DBS) microsampling**

**Capillary blood drop collection**

- Transfer onto a DBS card

**Drying after collection**


Advantages and issues in DBS microsampling

**Advantages**
- Increased analyte stability
- Feasible storage and transport
- Increased subject compliance

**Issues**
- Haematocrit influence
- Inaccurate sampling volume
- Low sample volume

Innovative technologies for “smart” DBS

Development of highly sensitive analytical methods for identification and quantitation of the analytes in DBS samples

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M. Protti, C. Marasca, M. Cirrincione, A. Cavalli, R. Mandrioli, L. Mercolini, Analyst 24 (2020) 5744-5753
Separation of chiral enantiomers is one of the main chromatography challenges.

Development of a chromatographic approach able to separate enantiomers of fentanyl derivatives

Highly selective method

The low sample volume and the low analyte concentration in the sample require to increase method sensitivity.

Design of an advanced UHPLC-HRMS methodology for identification and quantitation of analytes

Accurate and precise quantitation
The analytes

Fentanyl derivatives

\[
\begin{align*}
\beta\text{-hydroxythiofentanyl} & \quad \beta\text{-hydroxyfentanyl} \\
\end{align*}
\]

Fentanyl metabolites

\[
\begin{align*}
(\pm) \text{trans-3-methyl Norfentanyl} & \quad (\pm) \text{cis-3-methyl Norfentanyl} \\
\end{align*}
\]
UHPLC-HRMS instrumentation and parameters

**Ionisation:** ESI+

**Analysis mode:** Full scan TIC → XIC

UHPLC-QTOF, Waters Xevo G2-X2
Two different cellulose-based chiral stationary phases were selected for the separation of the enantiomers of the two fentanyl derivatives and the two metabolites. 

**β-hydroxyfentanyl and (±) trans-3-methyl Norfentanyl**

- **Stationary Phase:** Cellulose 2 (100x4.6mm; 5 µm)
- **Mobile Phase:** ACN/IPA 98/2 + 0.1% DEA + 0.1% FA

**β-hydroxythiofentanyl**

- **Stationary Phase:** Cellulose 2 (100x4.6mm; 5 µm)
- **Mobile Phase:** ACN/ETOH 95/5 + 0.1% FA + 0.1% DEA

**(±) cis-3-methyl Norfentanyl**

- **Stationary Phase:** Cellulose 4 (100x4.6mm; 5µm)
- **Mobile Phase:** ACN/IPA 98/2 + 0.1% FA + 0.1% DEA

**Flow rate:** 1 mL/min

**T:** 25°C

I. Varfaj, M. Protti, M. Cirrincione, A. Carotti, L. Mercolini, R. Sardella, J Chromatogr A 1643 (2021)
<table>
<thead>
<tr>
<th>Analyte</th>
<th>$t_{R1}$</th>
<th>$t_{R2}$</th>
<th>$k_1$</th>
<th>$k_2$</th>
<th>$\alpha$</th>
<th>$R_S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-hydroxythiofentanyl</td>
<td>7.33</td>
<td>8.47</td>
<td>8.77</td>
<td>10.29</td>
<td>1.32</td>
<td>2.31</td>
</tr>
<tr>
<td>$\beta$-hydroxyfentanyl</td>
<td>7.12</td>
<td>7.90</td>
<td>8.49</td>
<td>9.53</td>
<td>1.18</td>
<td>2.44</td>
</tr>
<tr>
<td>$(\pm)$ trans-3-methyl Norfentanyl</td>
<td>12.36</td>
<td>16.08</td>
<td>15.48</td>
<td>20.44</td>
<td>1.12</td>
<td>2.05</td>
</tr>
<tr>
<td>$(\pm)$ cis-3-methyl Norfentanyl</td>
<td>19.33</td>
<td>22.74</td>
<td>24.77</td>
<td>29.32</td>
<td>1.17</td>
<td>2.18</td>
</tr>
</tbody>
</table>

The DBS samples were pretreated by using an original procedure developed ad hoc:
The preliminary results proved:

- High selectivity of the chromatographic approach (all enantiomers have been identified)

- Good method sensitivity (analytes were identified even at low concentrations)

The next steps will be the complete validation of the methodology, applying it to real samples
The enantiomers of two fentanyl derivatives and two fentanyl metabolites were successfully chromatographically separated

A highly sensitive UHPLC-HRMS method for analyte identification has been developed

A miniaturised blood sampling and an original sample pretreatment were developed and optimised

The UHPLC-HRMS method has shown to be suitable also for the quantitation of the analytes in microsamples

This approach will be very useful for future studies on NPS in the clinical and forensic settings
Thanks to…

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