Achiral Separation of Fluorofentanyl Derivatives on Chiral Stationary Phases in Varying Mobile Phase Modes

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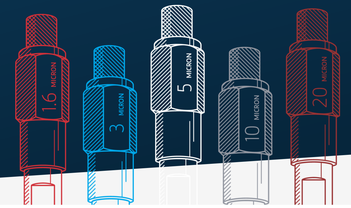

Introduction

Drug overdoses have consistently risen in the United States over the last two decades. The CDC estimates nearly 108,000 deaths in 2022 alone, with fentanyl and other synthetic opioids making up nearly 70% of those deaths. Derivatives of fentanyl, like fluorofentanyl, are appearing more frequently in seized samples when tested for composition. Para-fluorofentanyl is the most common regioisomer of fluorofentanyl, however it's possible for two other regioisomers, meta- and ortho-fluorofentanyl, to be present (Figure 1). While there are currently several methods published for the separation of these isomers, none are able to separate all 3 isomers in the same method.

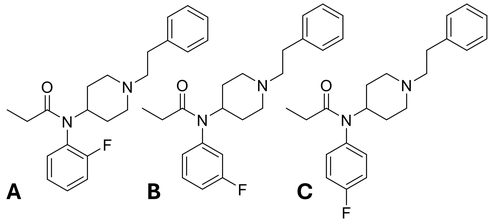

Figure 1: Ortho (A), Meta (B) and Para-fluorofentanyl (C)

This study is focused on establishing such a method by using Daicel polysaccharide-based chiral stationary phases (CSPs). Method development was conducted with both high-performance liquid chromatography (HPLC) mode with normal phase (NP) and reversed phase (RP) solvents, as well as with super-critical fluid chromatography (SFC) mode. Optimization of each mode as well as single isomer peak identification for each method are shared.

Experimental

The samples of ortho, meta, and para-fluorofentanyl were all purchased from Cayman Chemical. Diethylamine (DEA) was purchased from Sigma Aldrich. All solvents were purchased from Pharmco and were HPLC-grade. Specifically, the Hexanes (Hex) were 95% n-hexane. Carbon dioxide was purchased from Linde.

An Agilent 1200 HPLC configured with a quaternary mobile phase pump and a photodiode array detector was used, controlled by an Agilent ChemStation Version RevB.04.03[16].

A Waters UPC2 SFC configured with a quaternary mobile phase pump and a photodiode array detector was used, controlled by Empower 3.8.0.

The chiral columns used for screening included **CHIRALPAK® IA-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, IJ-3,** and **IK-3** and were 4.6 mm inner diameter (i.d.) by 150 mm length and a 3 µm particle size.

Results

A retention check was performed prior to the start of each screening to ensure reasonable elution was achieved. A single injection was made on IB N-3 with Hex/30% Ethanol (EtOH)/0.1% DEA or CO_2/20% methanol (MeOH)/0.2% DEA. For RP-HPLC mode, a gradient from 90% 20 mM ammonium bicarbonate pH = 9/10% acetonitrile to 10% 20 mM ammonium bicarbonate pH=9/90% acetonitrile over 20 minutes was used.

NP-HPLC MODE

Screening with both 30% EtOH and 30% isopropanol (IPA) produced several partial separations, and a near baseline separation on IK-3. Additional optimization was performed on a 250 mm length column and a weaker eluting mobile phase of 80-20-0.1 = Hex-IPA-DEA, to produce the separation seen in **Figure 2** (elution order shown).

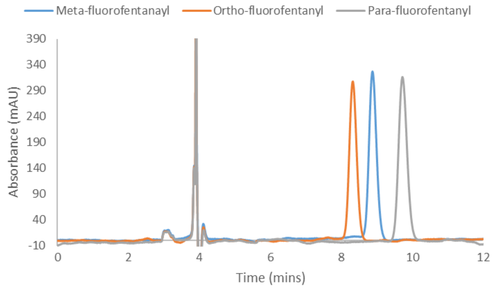

Figure 2: NP separation of Fluorofentanyl isomers on CHIRALPAK IK-3

RP-HPLC MODE

Screening on the above-described gradient showed only a partial 3 peaks separation on **IA-3**. For optimization, the column was switched to the coated equivalent**, CHIRALPAK® AD-3** to improve the selectivity, and the length increase to 250 mm. This gave the baseline separation shown in **Figure 3**.

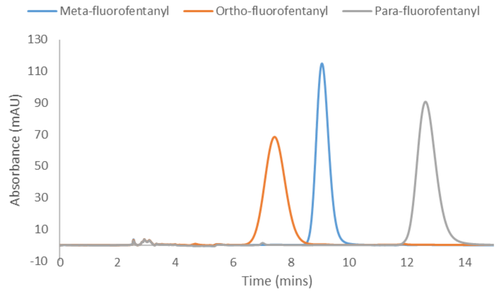

Figure 3: RP separation of Fluorofentanyl isomers on CHIRALPAK AD-3

SFC MODE

Screening with 10% MeOH, EtOH, and IPA modifiers showed a number of partial separations, but complete baseline separations on **IB N-3** with IPA (**Figure 4**).

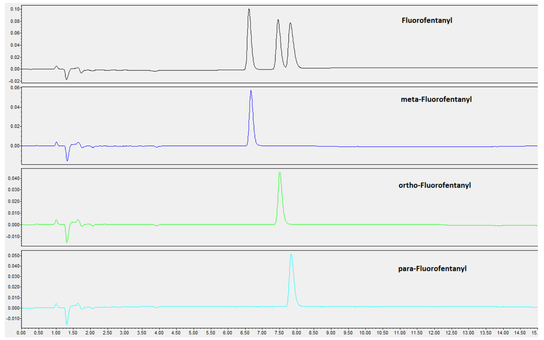

Figure 4: SFC separation of Fluorofentanyl isomers on CHIRALPAK IB N-3

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