APPLICATION NOTE

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

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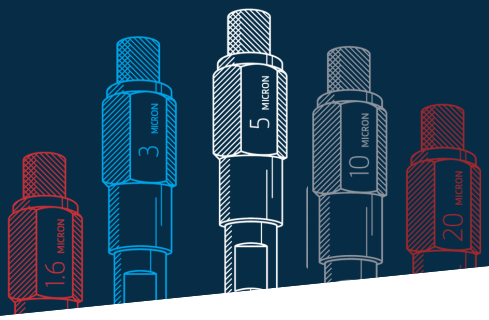

DAICEL Chiral Technologies

INTRODUCTION

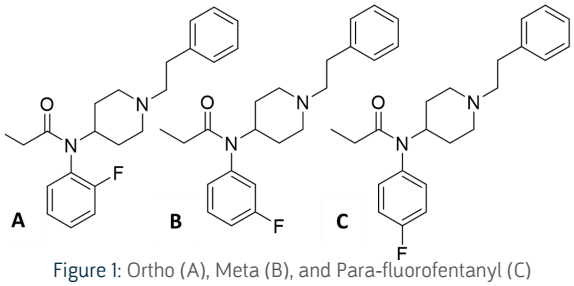

Figure 1: Ortho (A), Meta (B), and Para-fluorofentanyl (C)

Drug overdoses have consistently risen in the United States over the last two decades.[1] The CDC estimates nearly 108,000 deaths in 2022 alone, with fentanyl and other synthetic opioids making up nearly 70% of those deaths.[2] 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[3] (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.

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

Chromatographic Conditions for the Separation of Fluorofentanyl Isomers

Column	CHIRALPAK® IK-3 (250 mm x 4.6 mm i.d.) Part #: 91525	CHIRALPAK® AD-3 (250 mm x 4.6 mm i.d.) Part #: 19525	CHIRALPAK® IB N-3 (250 mm x 4.6 mm i.d.) Part #: 89525
Mobile Phase	Hex-EtOH-DEA = 80-20-0.1	20mM Amm. Bicarb. pH=9-ACN = 20-80	CO_2-(IPA-0.2% DEA) = 90-10
Flow Rate	1.0 ml/min		3.0 ml/min
Detection	UV 210 nm ref. 450 nm		
Temperature	25°C		40°C
Sample	Standards - 1.0 mg/ml in MeOH Mixture – 1:1:1 of Standard solutions		
Injection Volume	10 µl		

Ortho, meta, and para-fluorofentanyl were purchased from Cayman Chemical. The solvents used were all purchased from Scientific Equipment Company (SECO), were HPLC-grade, and were used as-is. Specifically the ethanol (EtOH) was reagent alcohol, which contains 90% EtOH, 5% methanol (MeOH), and 5% isopropanol (IPA). Diethylamine (DEA) and Ammonium Bicarbonate (Amm. Bicarb.) were reagent grade and purchased from Sigma Aldrich. Carbon Dioxide (CO2) was instrument grade K with educator tube purchased from Linde US.

Screening and optimization for HPLC mode was performed on an Agilent 1200 configured with high-pressure mixing quaternary mobile phase delivery system, vacuum degasser, autosampler, temperature controlled column compartment, and photodiode array UV detector. The instrument was controlled by an Agilent ChemStation Version RevB.04.03. Screening and optimization for SFC mode was performed on a Waters UPC2 configured with a quaternary mobile phase pump and a photodiode array UV detector, 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.

DISCUSSION

NP-HPLC MODE

A retention check was performed prior to the start of the screening to ensure reasonable elution was achieved before committing to a full screen. A single injection was made on IB N-3 with Hex-EtOH-DEA = 70-30-0.1. It was observed that this produced reasonable retention and was thus used for screening.

Several partial separations were observed with both EtOH and IPA mobile phases on IA-3, IB N-3, IC-3, IF-3, and IG-3, and a near baseline separation with IPA on IK-3. To improve the separation, the column length was increased to 250 mm length, and a weaker eluting mobile phase of 80-20-0.1 = Hex-IPA-DEA was used. This produced the separation seen in Figure 2.

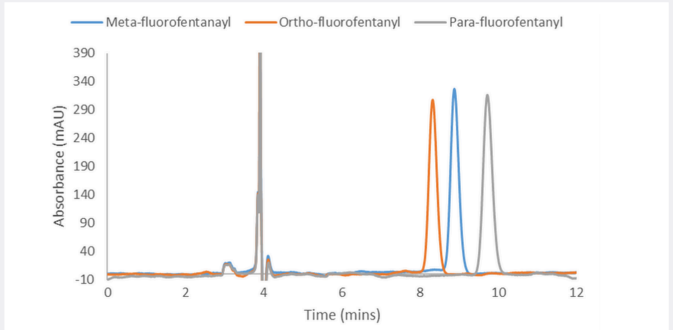

Figure 2: NP-HPLC separation of fluorofentanyl isomers on CHIRALPAK IK-3

RP-HPLC

Rather than perform a retention check like for NP-HPLC, a gradient of 20 mM Amm. Bicarb. pH = 9-ACN from 90-10 to 10-90 acetonitrile over 20 minutes was used for screening. It's often more preferable to use a gradient for screening in RP mode as it reduces the amount of time required for the initial screening setup in isocratic mode.

Partial two peak separations were observed on IC-3, ID-3, IE-3, IF-3, IG-3, and IH-3, and a partial three peak separation observed on IA-3. Based on the time of elution in the gradient, it was determined that the first isomer began eluting between 70 and 80% ACN. So to begin optimization, the gradient was converted to an isocratic method of 20 mM Amm. Bicarb. pH = 9-ACN = 20-80.

It is well established that while the immobilization process increases the robustness and solvent compatibility of immobilized CSPs, the chiral polymer's movement becomes somewhat restricted from this process compared to the coated equivalents. This very often leads to a slight decrease in the selectivity when a direct comparison is made, but is often overcome and improved when using alternative solvents. So for this reason, instead of using IA-3 for the optimization, the coated version, CHIRALPAK AD-3, was chosen. A longer 250 mm length column was also used, which produced the separation seen in Figure 3. It was observed that the elution order was conserved from NP-HPLC mode.

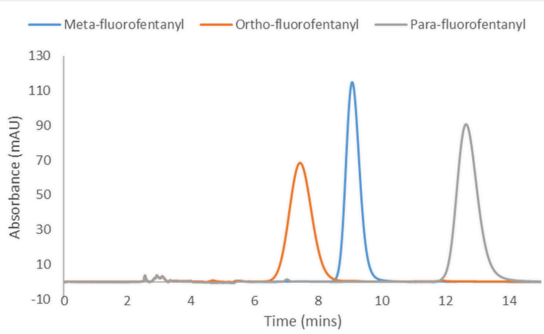

Figure 3: RP-HPLC separation of fluorofentanyl isomers on CHIRALPAK AD-3

SFC MODE

Similar to NP-HPLC mode, a retention check was performed on IB N-3 with CO2-(MeOH + 0.2% DEA) = 80-20 before committing to a full screening. It was observed that this produced reasonable retention and was thus used for the full screening.

Several partial separations were observed with MeOH on IB N-3, ID-3, and IF-3, with EtOH on IB N-3, IC-3, ID-3, and IF-3, and with IPA on IB N-3, ID-3, and IF-3. Near or complete baseline separations were observed with all modifiers on IB N-3 and ID-3, with EtOH on ID-3 and IPA with IB N-3 being the best in terms of resolution of all three peaks.

To optimize the separation on ID-3, a longer 250 mm length column was chosen, and a slightly weaker mobile phase of CO2-(EtOH + 0.2% DEA) = 90-10 used. This produced the separation in Figure 4.

To optimize the separation on IB N-3, a longer 250 mm length column was chosen, and a slightly weaker mobile phase of CO2-(IPA + 0.2% DEA) = 90-10 used. This produced the separation in Figure 5. In both ID-3 and IB N-3 cases, we see different elution order compared to LC mode, and within SFC mode by varying the mobile phase and selector. This is a fairly common occurrence and can be beneficial for the optimization process, where putting priority or low-level impurities first can provide a lower level-of-detection (LOD) or level-of-quantification (LOQ).

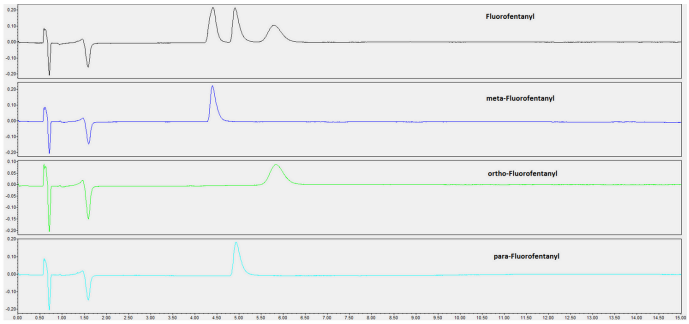

Figure 4: SFC separation of fluorofentanyl isomers on CHIRALPAK ID-3

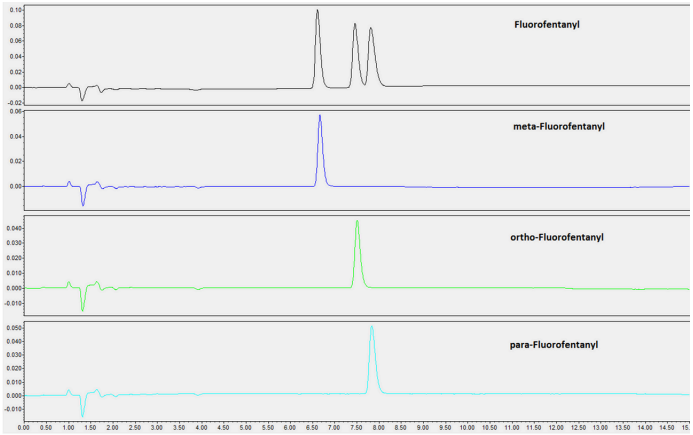

Figure 5: SFC separation of fluorofentanyl isomers on CHIRALPAK IB N-3

CONCLUSIONS

The establishment of suitable conditions to separate the three isomers of fluorofentanyl has been achieved in the four methods detailed above. Using several polysaccharide-based CSPs, baseline separation was demonstrated in three different elution modes. This provides flexibility for the implementation of said methods in a range of scenarios, including mass spec compatibility in the case of the RP-HPLC method.

This application further highlights the importance of compound screening - it's impossible to predict what combination of CSP and mobile phase will produce a separation, as demonstrated here where four different chemistries achieved baseline separation in three different mobile phases. Moreover it's impossible to predict what changing CSP and mobile phase will do to the elution order. This is something that can't be achieved with traditional achiral column chemistries and makes CSPs a unique and versatile tool for all separations.

REFERENCES

1. https://www.cdc.gov/nchs/data/databriefs/db491.pdf
2. https://nida.nih.gov/research-topics/trends-statistics/overdose-death-rates#Fig2
3. The Center for Forensic Science Research and Education. Fluorofentanyl Identified in Forensic Casework as Wave of Fentanyl-Related Substances Appears in the United States; 2020. Available from: https://www.cfsre.org/images/content/reports/public_alerts/2020.12.03.public-alert_fluorofentanyl_nps-discovery_120320.pdf [Last accessed on 2024 Oct 29].
4. Aderorho, Ralph, and Christopher D. Chouinard. "Improved separation of fentanyl isomers using metal cation adducts and high-resolution ion mobility-mass spectrometry." Drug Testing and Analysis, vol. 16, no. 4, 25 July 2023, pp. 369–379, https://doi.org/10.1002/dta.3550.