Chiral Biotransformation Analysis in Rat Plasma by SFC

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Introduction

A compound developed in a pharmaceutical lead optimization project as a racemic mixture was separated into constituent enantiomers A and B using Supercritical Fluid Chromatography (SFC) with UV(230 nm) detection. The enantiomers were tested competitively in a rat efficacy model, and plasma was drawn from the animals for pharmacokinetic assays. In the course of the efficacy study, unexpected off-target effects were observed and the team suspected in vivo racemization. Unfortunately, insufficient compound remained for further in vivo work. In fact, only small residual samples in the vials of a mixture of the two enantiomers and of the active enantiomer A were available.

Given that an SFC method for rapid separation of the enantiomers had already been developed, a rapid SFC survey of the plasma samples for enantiomeric excess (ee) was the ideal way to answer the concern. However, detection by UV absorbance is problematic with plasma extracts. An added concern is extensive sample preparation which may reduce recovery of the drug from plasma beyond conventional SFC detection limits. The decision was to try SFC-MS detection on very crude extracts from the remaining rat plasma samples. We hoped ee determination, which is limited by the abundance of the minor isomer, would be possible.

Experimental

The residual samples of racemate and enantiomer A were used as standards, and plasma samples were treated only by deproteinization with acetonitrile prior to analysis.

Sample Preparation

150 µL plasma (4-8 mg/mL drug, by LCMSMS)
Add 450 µL acetonitrile
Sonicate 5 minutes, remove 550 mL supernatant
Dry samples in centrifuge dryer
Reconstitute in 500 µL acetonitrile
Analyze by SFC-MS

Standard Preparation

Standard samples of A+B mix and enant. A
~ 100 µg taken up in 1 mL acetonitrile
Analyze by SFC-MS

SFC Conditions

Column: RegisPack 5µm Kromasil, 4.6 x 100mm
Co-Solvent: MeOH:IPA (1:1) w/ 0.1% Isopropylamine
Composition: 60% CO_2, 40% Co-solvent isocratic
Flow rate: 4 mL/min
Makeup: MeOH:H2O (1:1) 0.5% Formic acid, 1 mL/min
*Split: 20:1 of SFC flow, then 5:1 of makeup flow into MS

* Note: the flow from the SFC system is split immediately post-backpressure regulator, and a makeup solvent that facilitates electrospray ionization is added post-split.

Injection Volume

Standards: 10 µL partial of a 100 µL loop
Samples: 50 µL partial of a 100 µL loop
Run Time: 5 minutes

MS Conditions

MS System:	Waters ZQ Mass Spectrometer
Ionization Mode:	ESI+
Capillary Voltage:	2500 V
Cone Voltage:	17 V
Desolvation Temp:	350 °C
Desolvation Gas:	400 L/Hr
Cone Gas:	50 L/Hr
Source Temp:	150 °C
Acquisition:	SIR, m/z = 330, 1 sec dwell

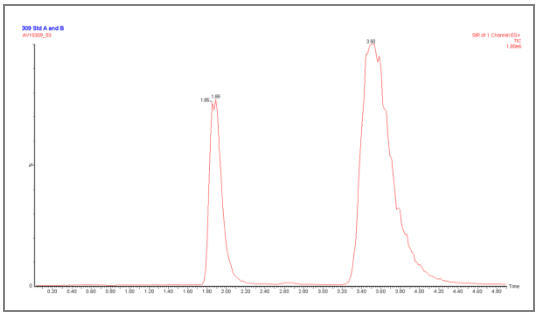

Figure 1: Analytical standard - mixture of enantiomer A and B SFC-MS, ESI+ with SIR m/z 330 detection

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Results and Discussion

Standard Injections

Standard samples of a mixture of A and B (Fig. 1) and of the active enantiomer A (Fig. 2) were analyzed using the previously developed resolution method. This work identified the enantiomer retention times and assured that they were detectable by MS. Sequential injections of the mixture and A alone indicated that carryover of enantiomer B (and thus presumably A) was negligible. Figure 3 shows that the compound in rat plasma is observed at 1.85 minutes only using MS detection and not by UV detection at λ_{max}.

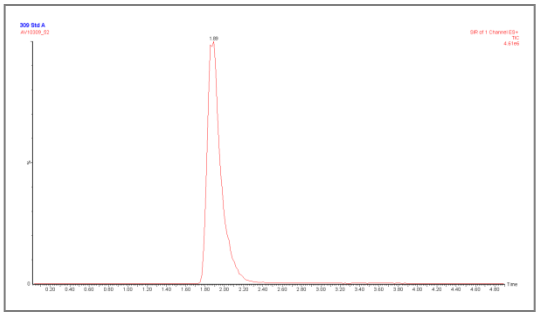

Figure 2: Analytical standard - enantiomer A
SFC-MS, ESI+ with SIR m/z 330 detection

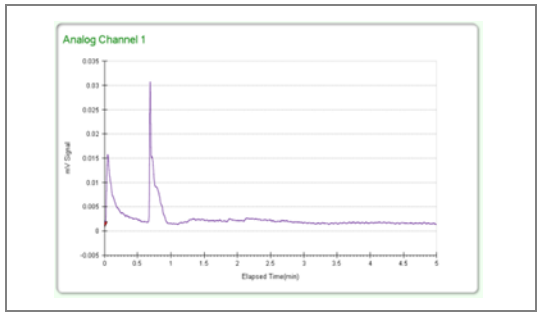

Figure 3: Enantiomer A Rat Plasma Sample
SFC without MS detection, UV 215nm

Sample Injections

Nine plasma samples were analyzed using this method and detection methodology. The Figures 4 and 5 below clearly indicate that the drug did not racemize to a detectable degree in the *in vivo* study.

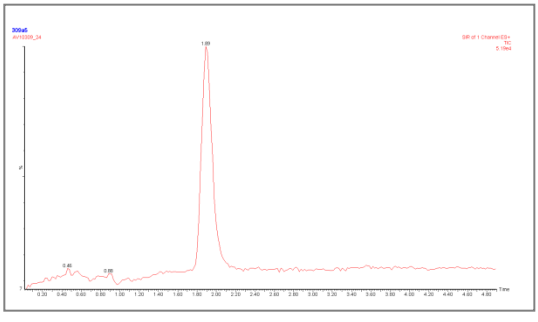

Figure 4: Enantiomer A Rat Plasma Sample
SFC-MS, ESI+ with SIR m/z 330 detection

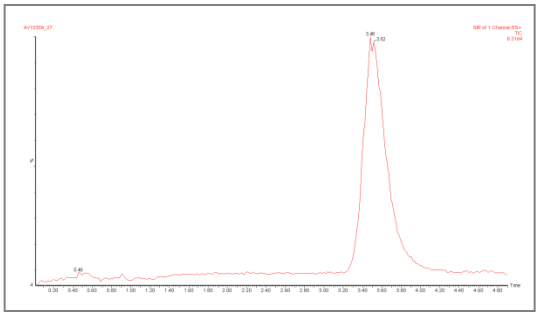

Figure 5: Enantiomer B Rat Plasma Sample
SFC-MS, ESI+ with SIR m/z 330 detection

Conclusions

SFC-MS, used with a chiral separation method identical to the one used to produce the tested enantiomers, is an appropriate and useful technology for studying their behavior in pharmacokinetic studies, and may prove useful if racemization *in vivo* is suspected.

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