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Fast Analysis of Chiral and
Structural Related Isomers
Using Supercritical Fluid
Chromatography Mass
Spectrometry

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Introduction

LC/MS is used extensively in drug discovery applications. However some limitations, including separation efficiency and speed of analysis for chiral and structural related isomers, have been noted. Supercritical fluid chromatography mass spectrometry (SFC/MS) offers an excellent alternative and/or orthogonal separation to LC/MS, mainly due to the high speed and unique selectivity of SFC for some compound types. Additionally, SFC has proven to be cost effective and can be considered a “green” technique by reducing organic solvent consumption.

This work describes the utility of the Agilent SFC/MS for fast analysis of a number of chiral and structurally related isomers. The analytical performance in terms of the separation efficiency, speed of analysis, reproducibility, and quantitative aspect is demonstrated.

Experimental

Methods

Several chiral and structurally related isomers were selected for the evaluation. The structures and molecular information of these compounds are shown in **Figure 1**. Samples of individual test compounds were prepared in methanol. Analyses were performed using the Agilent 1260 Infinity Analytical SFC System coupled to the Agilent 6130 Single Quadrupole mass spectrometer. Chromatographic separation was optimized using the chiral Lux Cellulose-1 column and the normal phase Rx-SIL column respectively with the mobile phase containing CO₂ and methanol with 20 mM ammonium formate. Full MS and/or SIM scans in the positive ion mode were used for qualitative and quantitative analysis of the analyte of interest.

Instrumentation

SFC/MS system consists of the Agilent 1260 Infinity SFC control model, Agilent 1260 Infinity SFC binary pump, Agilent 1260 Infinity SFC Autosampler, Agilent 1290 Infinity TCC, Agilent 1260 Infinity Hip Degasser, Agilent 1260 Infinity isocratic pump, Agilent 1260 Infinity DAD VL⁺, and Agilent 6130 Single Quadrupole mass spectrometer. The instrument configuration is shown in **Figure 2**. In this study, the UV detector was bypassed and the TCC was used as a heating device. As previously described¹, the heating device prevents freezing of the lines caused by the expansion of the CO₂ upon entering the MS ion source. Additionally, a make-up flow was added prior to the backpressure regulator (BPR) through an Agilent zero dead volume T-piece. This configuration provides the best retention time and peak area reproducibility and thus it is recommended for qualitative and quantitative analysis.

The SFC/MS experimental conditions are summarized in **Table 1**.

Experimental

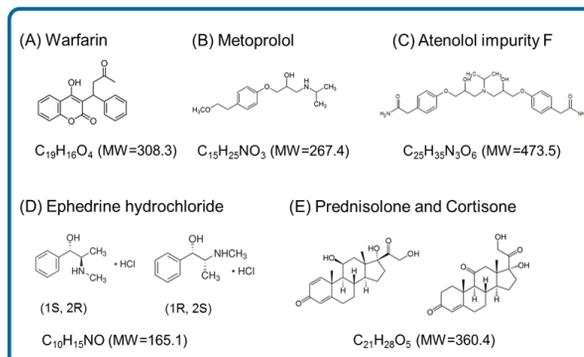


Figure 1. Structures of test compounds

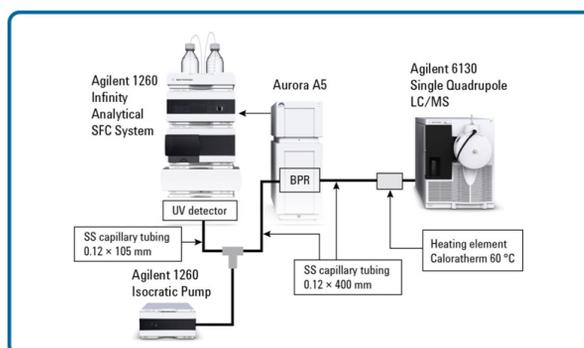


Figure 2. Agilent 1260 Infinity Analytical SFC/6130 Single Quadrupole mass spectrometer configuration

| Table 1: SFC/MS conditions | |
|---|--|
| Column | (1) Agilent ZORBAX Rx-SIL, 4.6 x 100 mm, 1.8 μm (PN: 828975-901) (2) Phenomenex Lux Cellulose-1, 4.6 x 150 mm, 3 μm |
| Column temperature | 40 °C |
| Injection volume | 5 μL |
| BPR pressure | 140 bar |
| Temperature of heating device | 60 °C |
| Make-up flow | 0.15 mL/min @100% B |
| Supercritical fluid | CO ₂ |
| Modifier | B ₁ : Methanol with 20 mM ammonium formate B ₂ : Methanol with 0.1% formic acid |
| SFC flow and isocratic condition | (1) 4 mL/min @ 20% B for Warfarin (2) 2 mL/min @ 35% B for Metoprolol (3) 4.5 mL/min @ 40% B for impurity F (Cellulose-1 column) (4) 4.5 mL/min @ 40% B for impurity F (Qual., Rx-SIL column) (5) 4.5 mL/min @ 30% B for impurity F (Quant., Rx-SIL column) (6) 4 mL/min @ 20% B for prednisolone and cortisone |
| SFC flow and gradient (for Ephedrine mixture) | 5% B (initial), 5-10% B (0-7 min), 10-40% B (7-7.5 min), hold 40% B for 0.5 min, 40-5% B (8-8.5 min) |
| ESI | - Ion mode: positive - Capillary voltage: 3000 v - Drying gas: 12 L/min @ 350°C - Nebulizer: 50 psi - MS Scan (200-450) with 20% cycle time for SIM (warfarin, metoprolol, and the mixture of prednisolone and cortisone) - MS Scan (100-250) with 20% cycle time for SIM (Ephedrine) - SIM (ion 474.3) (quantitative analysis for impurity F) |

Results and Discussion

Separation Efficiency

SFC/MS provides several advantages over LC/MS, including high separation efficiency, fast speed of analysis, and reduced solvent consumption. **Figure 3** shows the chromatographic separation of three representative chiral and structure related isomers using the Lux Cellulose-1 or the ZORBAX Rx-SIL column by SFC/MS. All three pairs of isomers are well separated in less than 3.5 minutes.

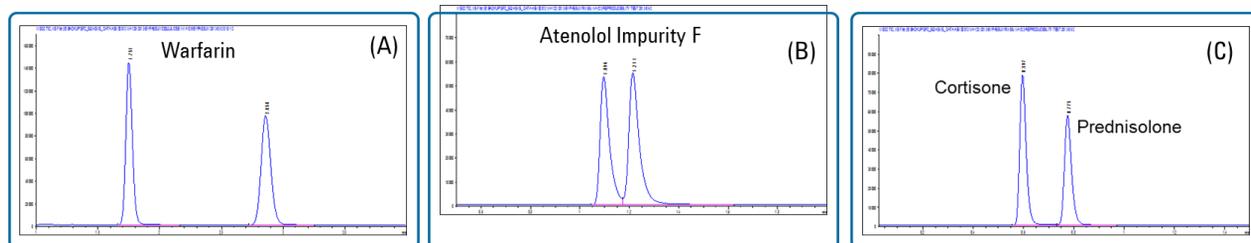


Figure 3. SIM chromatograms of (A) 500 ng/mL of warfarin using the Lux Cellulose-1 column at 20% B₁, (B) 200 ng/mL of atenolol impurity F using the Agilent ZORBAX Rx-SIL column at 40% B₁, and (C) 200 ng/mL of cortisone and prednisolone mixture using the Agilent ZORBAX Rx-SIL column at 20% B₁.

Effect of Modifiers on Analytical Performance

Chiral discrimination is a very complex phenomenon. It is almost impossible to predict which chiral stationary phase and modifier combination will provide the best separation. Optimal conditions can vary greatly and are compound specific. In this study, two types of modifiers (B₁ and B₂) were selected for the evaluation. **Figures 4** shows separation profiles of three representative chiral molecules using the Lux Cellulose-1 column and the mobile phase CO₂ in combination with either modifier B₁ (methanol containing 20 mM ammonium formate) or B₂ (methanol containing 0.1% formic acid). It is clear that a modifier has a significant effect on both chromatographic separation and MS signal response. Overall modifier B₁ provides higher MS signals than modifier B₂ for all the isomers studied here. However the effect on the chromatographic separation is compound dependent. For example, modifier B₂ significantly reduced separation efficiency of metoprolol (Figure 4A), atenolol impurity F (Figure 4B), and a mixture of (1R,2S) and (1S, 2R)-ephedrine isomers (data not shown), while similar chromatographic separation of warfarin was obtained using the modifier B₂ compared with the modifier B₁ (Figure 4C). This modifier effect was also observed for atenolol impurity F using the ZORBAX Rx-SIL column (data not shown).

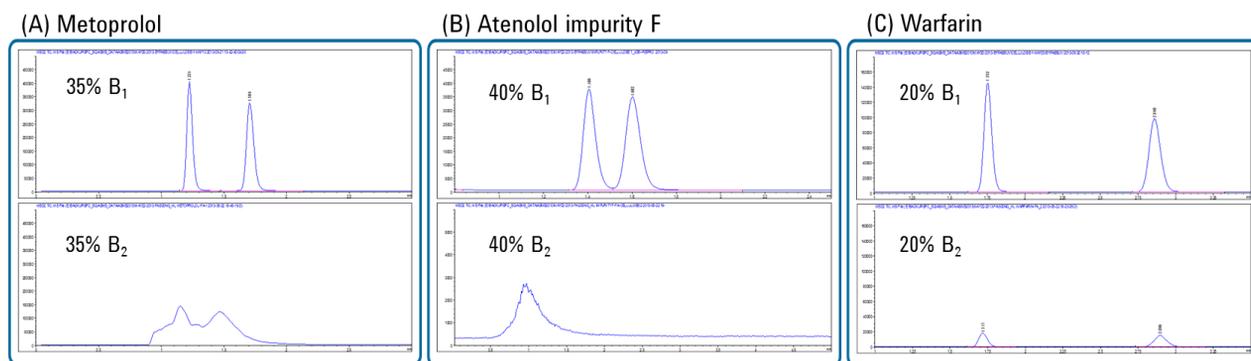


Figure 4. SIM chromatographic separation profiles of (A) metoprolol, (B) atenolol impurity F, and (C) warfarin using the Lux Cellulose-1 and modifier B₁ (top panel) and B₂ (bottom panel)

Reproducibility

Reproducibility is critical for quantitation. **Figure 5** shows the overlaid SIM chromatograms of 6 consecutive injections of warfarin (5A), metoprolol (5B), atenolol impurity F (5C), and a mixture of (1R, 2S) and (1S, 2R) ephedrine isomers (5D). Excellent reproducibility was obtained with RSDs of less than 0.1% for the retention time and less than 5% for the peak area.

Results and Discussion

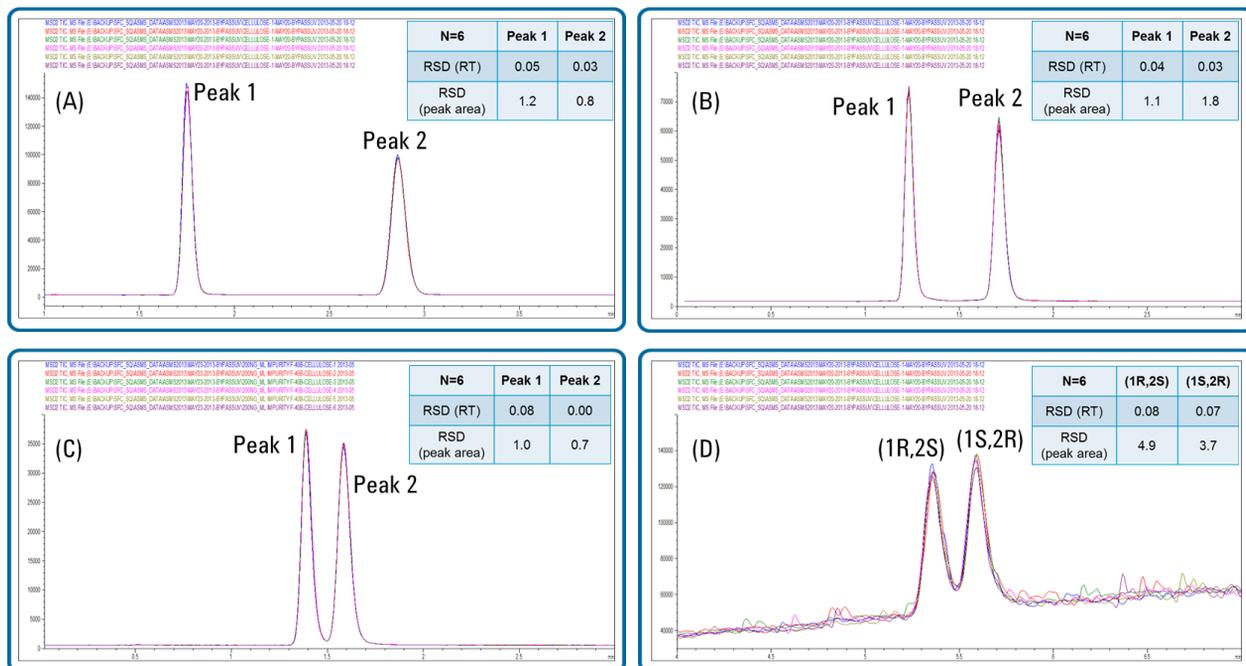


Figure 5. Overlay of the SIM chromatograms from 6 replicate injections of (A) 500 ng/mL of warfarin, (B) 100 ng/mL of metoprolol, (C) 200 ng/mL of atenolol impurity F, and (D) 200 ng/mL of (1R,2S) and (1S,2R) ephedrine mixture using the SFC/MS. The separation was performed using the Lux-Cellulose-1 column and the mobile phase CO₂ in combination with the modifier B₁.

Quantitative Performance

ZORBAX Rx-SIL column provides similar separation efficiency for atenolol impurity F as the Lux Cellulose-1 column. Since the silica column offers a cost benefit compared to the chiral column, quantitative performance was further evaluated for atenolol impurity F using the Rx-SIL column and the SFC/MS at the SIM mode only. As shown in **Figure 6**, the lower limit of quantitation (LLOQ) is 1.0 ng/mL with a linear dynamic range 1.0 - 250 ng/mL.

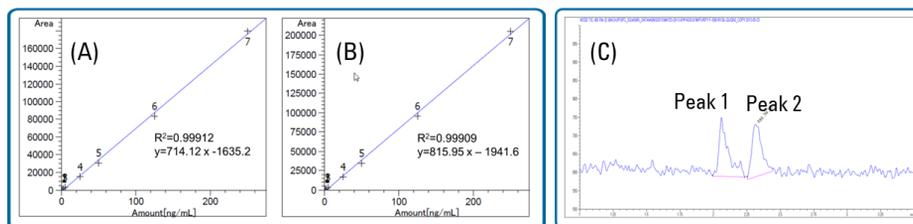


Figure 6. Quantitative analysis of atenolol impurity F using the ZORBAX Rx-SIL column and 30% B₁ at 4.5 mL/min. (A) calibration curve of the peak 1, (B) calibration curve of the peak 2, (C) SIM chromatogram of the impurity F at 1.0 ng/mL (LLOQ).

Conclusions

The Agilent 1260 Infinity Analytical SFC System coupled to the Agilent 6130 Single Quadrupole System provides excellent analytical performance for chiral and structure related isomers. The resulting data show the good sensitivity, high separation efficiency, excellent retention time and peak area reproducibility, and reliable quantitation. Optimization of modifier composition is important and methanol containing 20 mM ammonium formate proved to enable the successful SFC/MS analyses of all isomers in this study. Furthermore, the Agilent SFC/MS system allows to use of standard grade CO₂ instead of liquid SFC grade C, which results an additional benefit of 10-15x lower operating costs.

Reference: M. Dunkel, G. Vanhoenacker, F. David, P. Sandra, M. Vollmer, The Agilent 1260 Infinity SFC/MS Solution, Agilent Technologies Publication Number 5990-7972EN, 2011