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

Application Note

Authors

Yuqin Dai and Lisa Zang
Agilent Technologies, Inc.
Santa Clara, CA, USA

Abstract

This Application Note demonstrates the use of Agilent 1260 Infinity Analytical Supercritical Fluid Chromatography coupled to an Agilent 6130B Single Quadrupole mass spectrometer (SFC/MS) to study chiral and structurally related isomers with high analysis speed and excellent separation efficiency. The quantitative performance and analytical reproducibility were evaluated and proved to be highly reliable. This study also illustrates that modifier composition has a great effect on both chromatographic separation and MS signal response in ESI positive ion mode, thus optimization of modifier is crucial for successful SFC/MS analysis.

Agilent Technologies

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. SFC/MS offers an excellent alternative or orthogonal separation to LC/MS. Advantages of SFC include very low system back pressure with liquid CO_2 and organic modifiers, which allow high throughput analysis with high flow rate and unique selectivity of SFC for some compound types. Additionally, SFC has proven to be cost effective and is considered a green technique by reducing organic solvent consumption.

This Application Note describes the utility of SFC/MS for fast analysis of a number of chiral and structurally related isomers. The performance of an Agilent 1260 Infinity SFC coupled to an Agilent 6130B Single Quadrupole MS, in terms of the separation efficiency, speed of analysis, reproducibility, and quantitative aspect is demonstrated.

Experimental

Chemicals and method

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. Chromatographic separation was optimized using the chiral Lux Cellulose-1 column and an Agilent ZORBAX Rx-SIL column respectively. Research grade CO_2 gas (from Airgas) was used as the supply for mobile phase A and methanol with 20 mM ammonium formate (mobile phase B) was used as the modifier of supercritical liquid CO_2. Full MS or SIM scans in positive ion mode were used for qualitative and quantitative analyses of the analytes of interest.

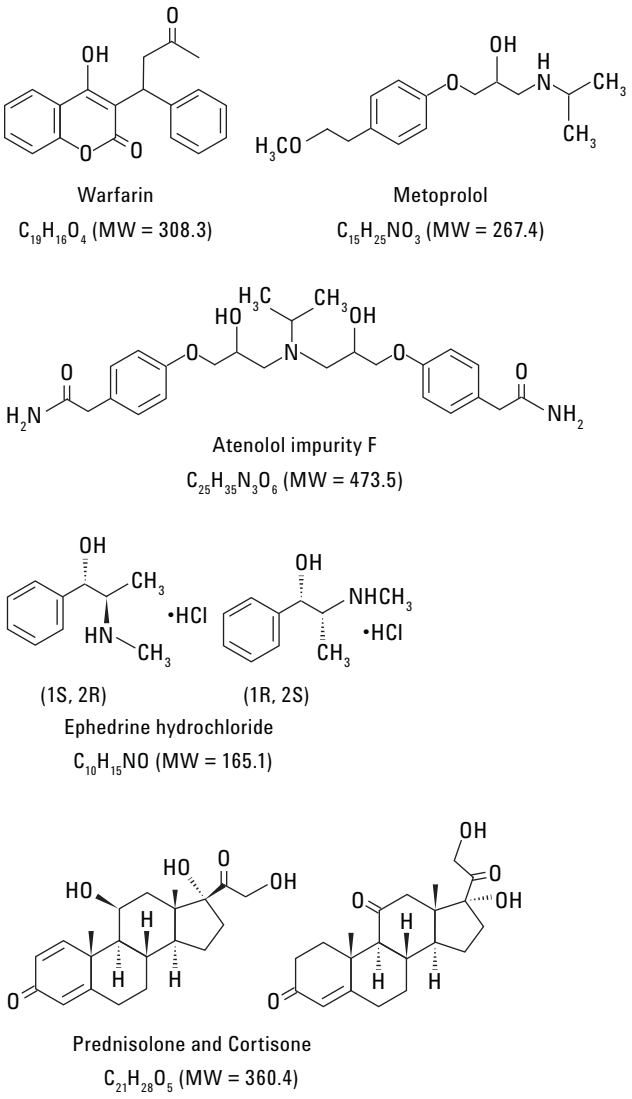

Figure 1. Structures of test compounds.

Instrumentation

Analyses were performed using the Agilent 1260 Infinity Analytical SFC system coupled to the Agilent 6130 Single Quadrupole mass spectrometer. The SFC/MS system consists of the following modules:

- Agilent 1260 Infinity SFC control model (G4301A)
- Agilent 1260 Infinity SFC binary pump (G3402A)
- Agilent 1260 Infinity High Performance Degasser (G4225A)
- Agilent 1260 Infinity SFC Autosampler (G4303A)
- Agilent 1290 Infinity TCC (G1316C) with a 2-column selection valve
- Agilent 1260 Infinity isocratic pump,
- Agilent 1260 Infinity DAD (G1315C) with high pressure SFC flow cell
- Agilent 6130B Single Quadrupole Mass Spectrometer with ESI source.

The instrument configuration is illustrated in Figure 2. Agilent OpenLab ChemStation Edition software version C.01.05 was used to control the SFC/MS instrument. In this study, the UV detector was bypassed. The pre-MS heating was achieved by flow through an additional TCC's 6-µL preheating block with temperature set at 60 °C. As previously described[1], the heating device prevents freezing of the lines caused by the expansion of CO_2 upon exiting backpressure regulator (BPR). Additionally, a make-up flow was introduced prior to the BPR through an Agilent zero dead volume T-connector. This configuration provides the best retention time and peak area reproducibility, thus it is used for qualitative and quantitative analysis.

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

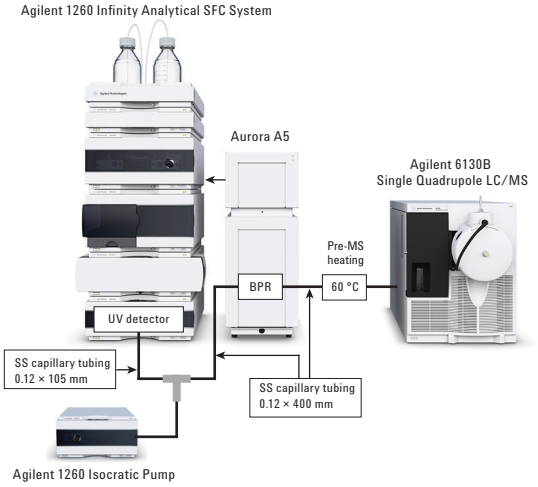

Figure 2. Agilent 1260 Infinity Analytical SFC/6130B Single Quadrupole mass spectrometer configuration.

Table 1. SFC/MS conditions.

SFC	
Columns	1) Agilent ZORBAX Rx-SIL, 4.6 × 100 mm, 1.8 µm (p/n 828975-901) 2) Phenomenex Lux Cellulose-1, 4.6 × 150 mm, 3 µm
Column temperature	40 °C
Injection volume (full loop)	5 µL
BPR pressure	140 bar
BPR temperature	60 °C
Pre-MSD heating	60 °C
Make-up flow	0.15 mL/min at 100 % B
Supercritical fluid (A)	CO_2
Modifier (B)	B_1) Methanol with 20 mM ammonium formate B_2) Methanol with 0.1 % formic acid
SFC flow and isocratic conditions	1) 4 mL/min at 20 % B for warfarin 2) 2 mL/min at 35 % B for metoprolol 3) 4.5 mL/min at 40 % B for impurity F (Cellulose-1 column) 4) 4.5 mL/min at 40 % B for impurity F (Qual., Rx-SIL column) 5) 4.5 mL/min at 30 % B for impurity F (Quant., Rx-SIL column) 6) 3 mL/min at 20 % B for prednisolone and cortisone
SFC flow and gradient (for ephedrine mixture)	5 % B (initial), 5–10 % B (0-7 minutes), 10–40 % B (7–7.5 minutes), hold 40 % B for 0.5 minutes, 40–5 % B (8–8.5 minutes)
MS	
Ion mode	positive
Capillary voltage	3,000 V
Drying gas	12 L/min at 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 only (ion 474.3) (quantitative analysis for impurity F)
Peak width	0.03 minutes
Fragmental voltage	120

Results and Discussion

Separation efficiency

SFC/MS provides several advantages over LC/MS, including high separation efficiency, shorter analysis time, and reduced organic solvent consumption. Figure 3 shows the isocratic chromatographic separation of three representative chiral and structurally related isomers using either Lux Cellulose-1 or ZORBAX Rx-SIL columns by SFC/MS. All three pairs of isomers were well separated in less than 3.5 minutes.

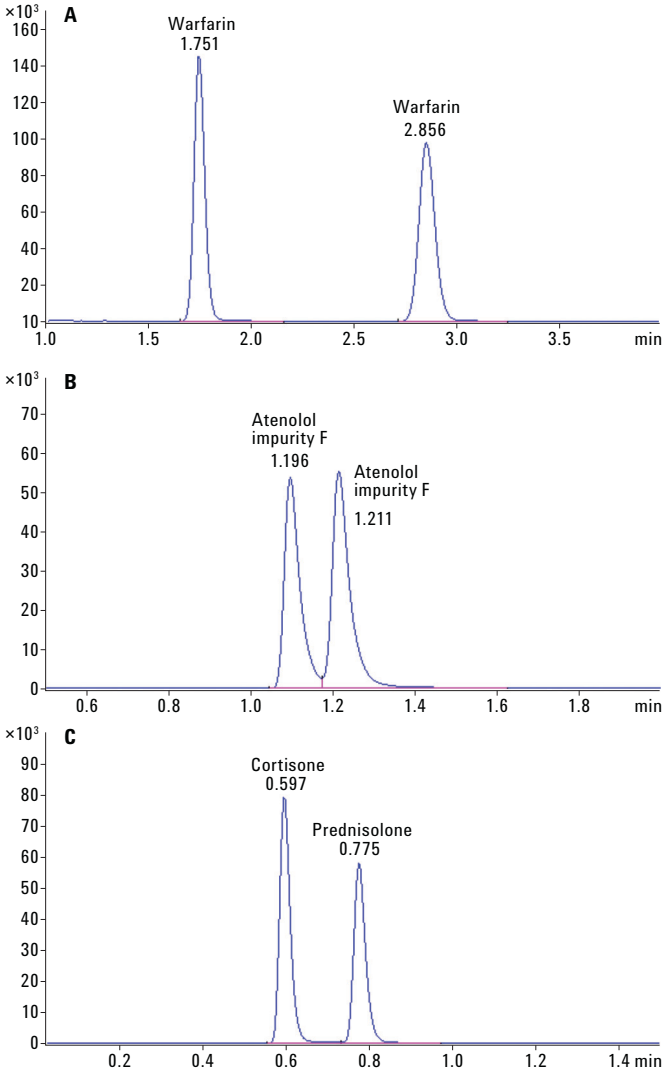

Figure 3. SIM chromatograms of (A) 500 ng/mL of warfarin using the Lux Cellulose-1 column at 20 % B_1, (B) 200 ng/mL of atenolol impurity F using the Agilent ZORBAX Rx-SIL column at 40 % B_1, and (C) 200 ng/mL of cortisone and prednisolone mixture using the Agilent ZORBAX Rx-SIL column at 20 % B_1.

Effect of Additive in Methanol

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 (B_1 and B_2) were selected for the evaluation. Figure 4 shows separation profiles of three representative chiral molecules using Lux Cellulose-1 column and mobile phase CO_2 in combination with either modifier B_1 (methanol containing 20 mM ammonium formate) or B_2 (methanol containing 0.1 % formic acid). It is clear that a modifier had a significant effect on both chromatographic separation and MS signal response. Overall, modifier B_1 provided higher MS signals than modifier B_2 for all the isomers studied. However, the effect on the chromatographic separation was compound dependent. For example, modifier B_2 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 modifier B_2 compared with modifier B_1 (Figure 4C). This modifier effect was also observed for atenolol impurity F using ZORBAX Rx-SIL column (data not shown). It has been reported that the SFC elution strength of MeOH can be adjusted by acid-salt additives and their concentrations in separation of acidic enantiomer compounds[2]. Our finding showed a similar trend, in that the acidic additive (formic acid) did not help in chiral separation of the compounds studied. We found ammonium formate additive provided better separations.

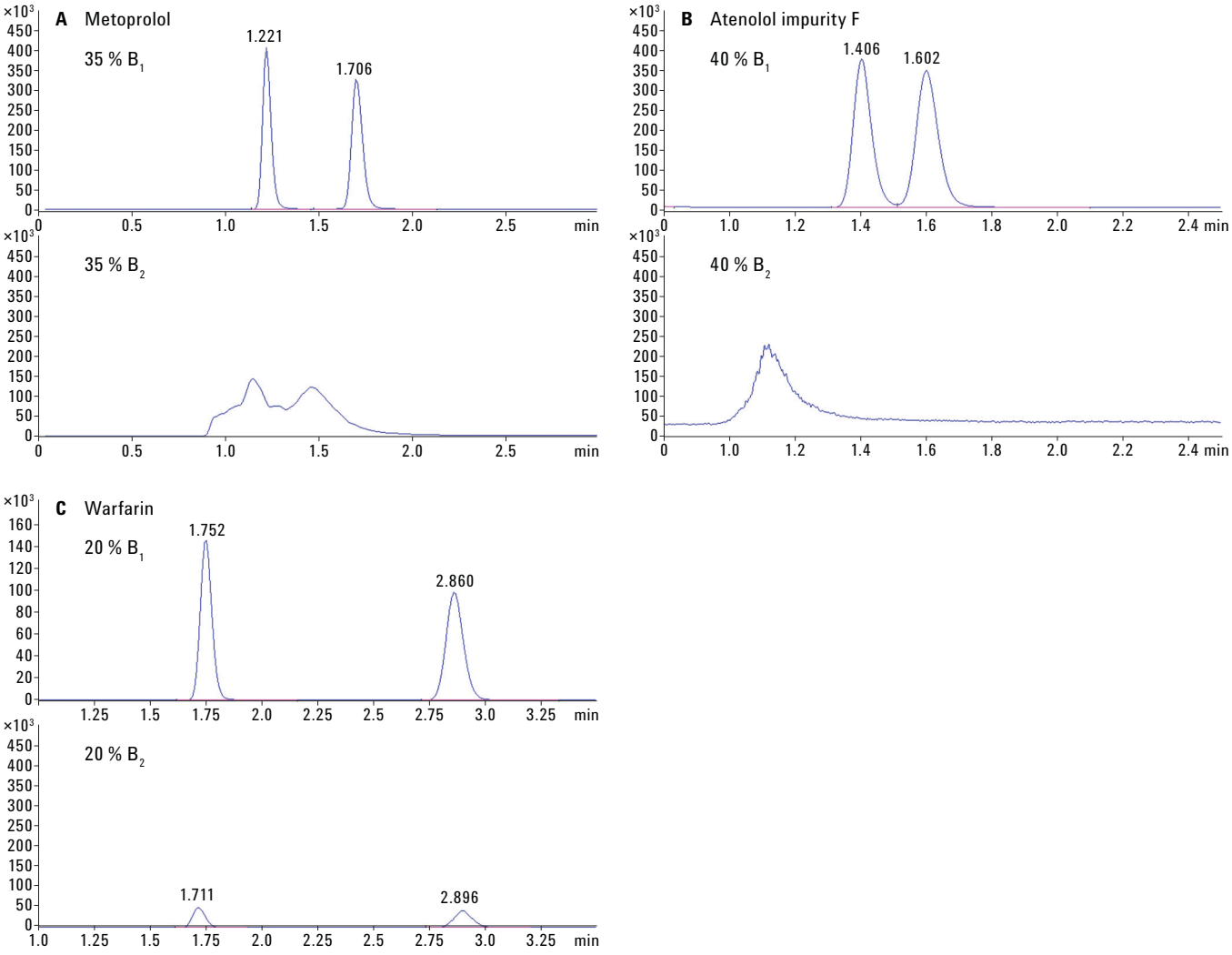

Figure 4. SIM chromatographic separation profiles of (A) metoprolol, (B) atenolol impurity F, and (C) warfarin using the Lux Cellulose-1, modifier B_1 (top panel) and B_2 (bottom panel)

Reproducibility

Reproducibility is critical for quantitation. Figure 5 shows the overlaid SIM chromatograms of six 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 retention time and less than 5 % for peak area.

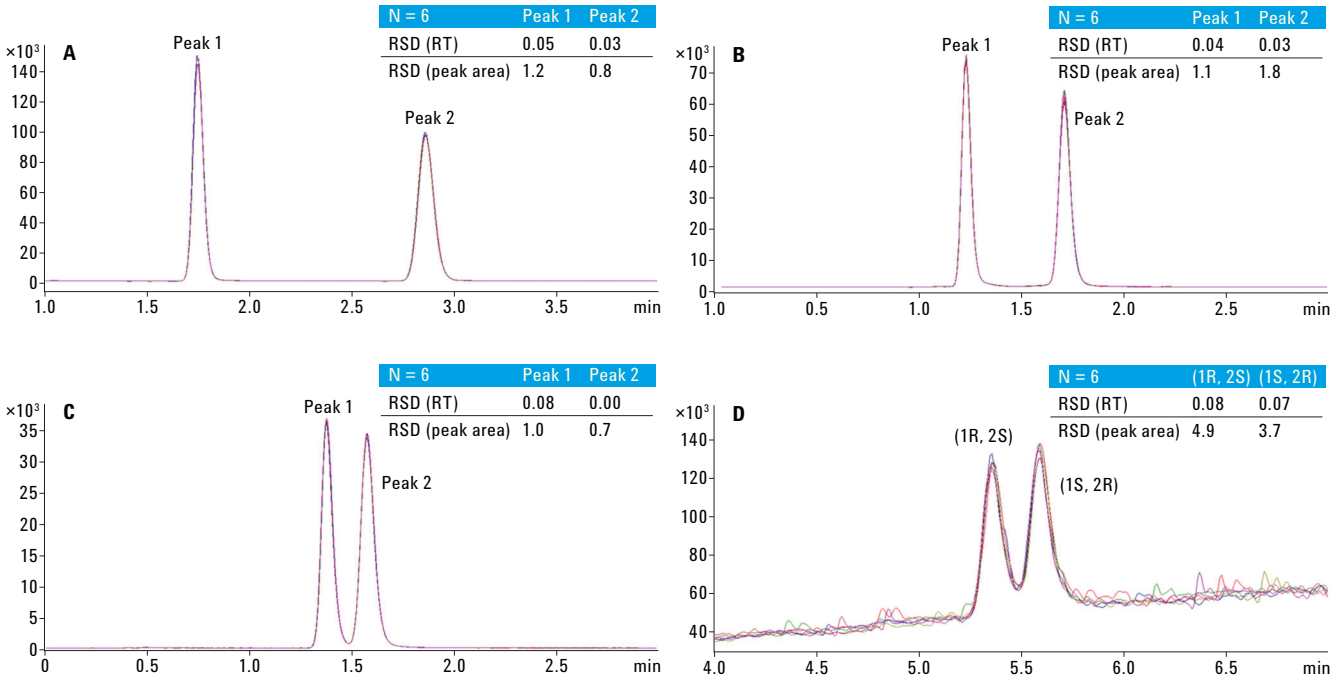

Figure 5. Overlay of the SIM chromatograms from six replicate injections of (A) 500 ng/mL of warfarin, (B) 100 ng/mL of metoprolol, (C) 200 ng/mL of atenolol impurity F, and (D) 200 ng/mL of (1R, 2S) and (1S, 2R) ephedrine mixture using the SFC/MS. The separation was performed using the Lux-Cellulose-1 column and the mobile phase CO_2 in combination with the modifier B_1.

Quantitative performance

The ZORBAX Rx-SIL column provided similar separation efficiency for atenolol impurity F as 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 ZORBAX Rx-SIL column and the SFC/MS in SIM mode only. As shown in Figure 6, the lower limit of quantitation (LLOQ) was 1.0 ng/mL with a linear dynamic range of 1.0–250 ng/mL.

Conclusions

The Agilent 1260 Infinity Analytical SFC System coupled to the Agilent 6130B Single Quadrupole System provided excellent analytical performance for analyzing chiral and structurally related isomers. The resulting data showed good sensitivity, high separation efficiency, excellent retention time and peak area reproducibility, and reliable quantitation. Optimization of modifier composition was 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 uses standard grade CO_2 gas instead of liquid SFC grade C CO_2 gas, which results in 10–15x lower operating costs.

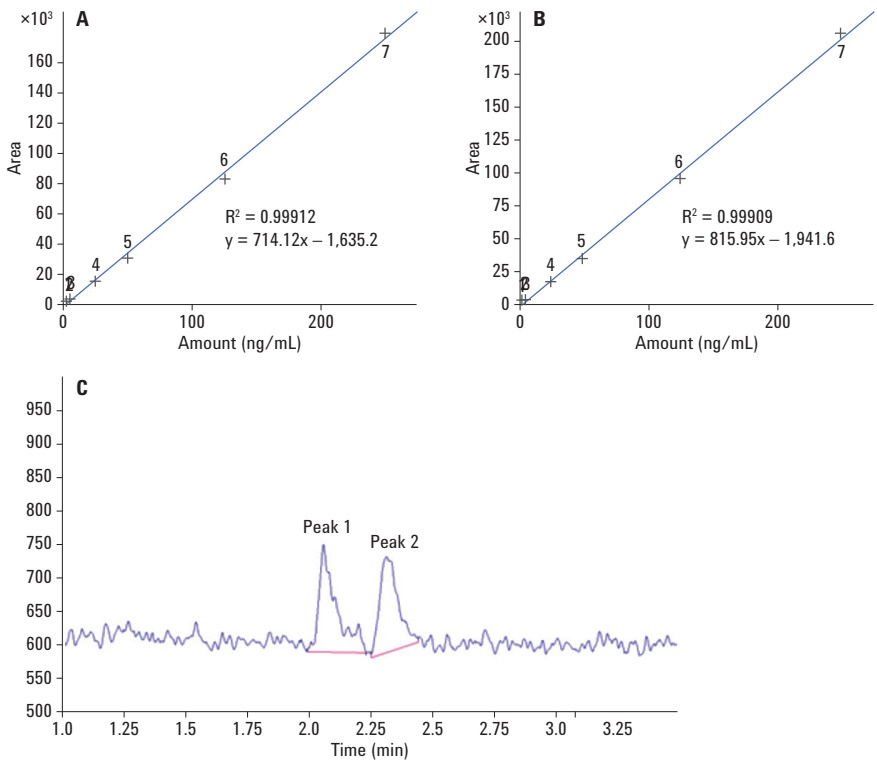

Figure 6. Quantitative analysis of atenolol impurity F using the Agilent ZORBAX Rx-SIL column and 30 % B_1 at 4.5 mL/min. (A) calibration curve of Peak 1, (B) calibration curve of Peak 2, (C) SIM chromatogram of impurity F at 1.0 ng/mL (LLOQ).

References

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

2. T. Zhang, D. Nguyen, P. Franco (Chiral Technologies, Europe IIIkirch, France), M. Vollmer (Agilent Technologies, Waldbronn, Germany) “Enantiomer Separation of Acidic Compounds” Agilent Technology Publication Number 5990-9594EN, **2012**.

www.agilent.com/chem

Published in the USA, February 3, 2014
5991-3859EN

Agilent Technologies