Chiral impurity analysis and enantiomeric excess determination with the Agilent 1260 Analytical SFC system

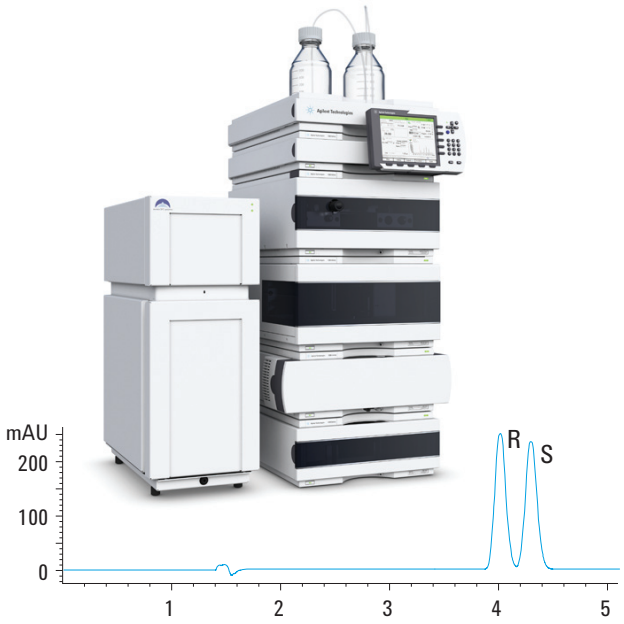
Application Note

Drug Development

Authors

Melissa N. Dunkle, Alberto dos Santos Pereira, Frank David, Pat Sandra
Research Institute for Chromatography
Kennedypark 26
8500 Kortrijk, Belgium

Martin Vollmer
Agilent Technologies
Waldbronn, Germany



Abstract

Chiral separation and enantiomeric excess (EE) determination were performed by Supercritical Fluid Chromatography using the Agilent 1260 Infinity Analytical SFC system. Three model chiral compounds: N-(3,5-dinitrobenzoyl)leucine, 2-(2,6-dioxopiperidin-3-yl)-1*H*-isoindole-1,3(2*H*)-dione (thalidomide), and 1,1'-bi-2-naphthol, were analyzed using a generic chiral SFC method. The low detector noise of the system due to the SFC pump technology resulted in excellent sensitivity for trace analyte detection of chiral impurities. When applying method optimization sufficient resolution was achieved for all compounds even under mass overload conditions. This allowed for the quantitation of impurities at 0.05 to 0.1% levels.

Agilent Technologies

Introduction

The enantiomeric excess (EE) of a substance is an indicator for the purity of a chiral chemical compound. The impurity is usually the undesired enantiomer that occurs frequently as a byproduct in chemical syntheses. EE is determined by the following equation:

$$EE = ((R-S)/(R+S)) \times 100$$

where R and S stand for the individual optical isomer in the mixture (and R + S = 1).

EE determinations are important in the pharmaceutical industry because undesired optical isomers of a drug can potentially alter pharmaceutical efficacy or result in toxicity.

Supercritical Fluid Chromatography (SFC) has shown high potential for chiral separations. Short analysis times and decreased equilibration time due to low mobile phase viscosity and superior diffusion characteristics are major benefits. In addition, significant decrease of operating costs can be achieved with the Agilent 1260 Infinity Analytical SFC since standard grade CO_2 can be used instead of expensive liquidified CO_2.

The Agilent 1260 Infinity Analytical SFC system is a modular system that consists of the Agilent 1260 Analytical SFC LC and the Aurora SFC Fusion™ A5 for pre- and post- conditioning of CO_2. The combination of both systems, results in virtual pulseless metering that leads to lowest baseline noise with high UV sensitivity and robustness, allowing the use of SFC in a routine environment. This opens up new application areas such as enantiomeric excess analysis (EE) illustrated in this study by the chiral separation of three selected model compounds.

The EE in this study was determined by injecting a high concentration of an optically "pure" enantiomer and determining the percentage of the contaminating enantiomer. Method optimization was performed in order to achieve sufficient resolution even under mass overloading conditions. In addition, high detector sensitivity and baseline stability (low noise and drift) was essential for the detection of trace compounds at levels below 0.1% of the main peak.

Experimental

Chemicals

The test compounds used are shown in Table 1. Stock solutions were prepared in methanol (5000 ppm). Working solutions were obtained by further dilution to either 50 ppm or 2.5 ppm of each enantiomer. For method validation, aliquots of the pure solutions were spiked with the corresponding enantiomers at 0.5%, 1% and 2%. Only the S-enantiomer was available for N-(3,5-dinitrobenzoyl)leucine.

Instrumentation

For this study the Agilent 1260 Infinity Analytical SFC system (G4309A) was used containing the Aurora SFC Fusion A5 and the modified Agilent RRLC binary system. Isocratic separation conditions were tuned so that resolutions of approximately 4.0, 2.0, and 1.5 were obtained for the three model chiral compounds. The initial separation conditions are given in Table 2.

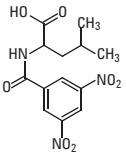
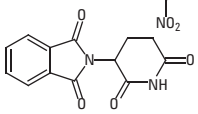
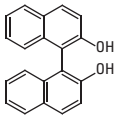
Abbreviation	Name	Structure
R,S-1	N-(3,5-dinitrobenzoyl)leucine	
R,S-2	Thalidomide	
R,S-3	1,1'-bi-2-naphthol	

Table 1
Selected test compounds.

Experimental chromatographic conditions	
Column	ChiralCel OD-H, 4.6 mm × 250 mm, 5 µm (Chiral Technologies)
Supercritical Fluid	CO_2
Modifier	MeOH w 0.1% TFA + 0.1% DEA
Outlet Pressure	150 bar
Flow Rate	2.0 mL/min
Isocratic Separation	% modifier
R,S 1	30%
R,S 2	17%
R,S 3	35%
Temperature	30 °C
Injection Volume	5 µL
Detection	DAD, 220 nm

Table 2
Initial SFC conditions.

Results and Discussion

Separation of racemic mixtures

All model compounds used were initially analyzed as a racemic mixture in a concentration of 50 ppm (Figure 1). Using the same generic method, different resolution values were achieved for the individual test components.

At a low concentration (2.5 ppm) comparable resolution was obtained (data not shown) and used to calculate sensitivity from the signal-to-noise ratios at 220 nm wavelength. Limit of detection (LOD, S/N > 3.0) was in all cases below 1 ppm. This value corresponds to 0.02% relative concentration if 5000 ppm is injected and indicated that EE determination is possible at 0.02% of the main peak.

Impurity analysis and method optimization

In order to determine potential impurities in "pure" formulations, the individual enantiomers were injected at very high concentrations (5000 ppm). None of the preparations revealed optical purity (Figures 2-4). For the "pure" enantiomers of all test compounds EE was between 90.2% and 99.6% (Table 3).

For compounds 1 and 2 the obtained resolution required no further method optimization to allow for quantitation even under mass overloading conditions. This decreased resolution in general by a factor of 10-50% (Figure 2 and Figure 3). Chromatograms displayed in Figure 4, however, indicated the necessity for further method development. Modifier concentration was decreased from 35% B to 25% B resulting in sufficient resolution between the enantiomers to quantify for EE (Table 3).

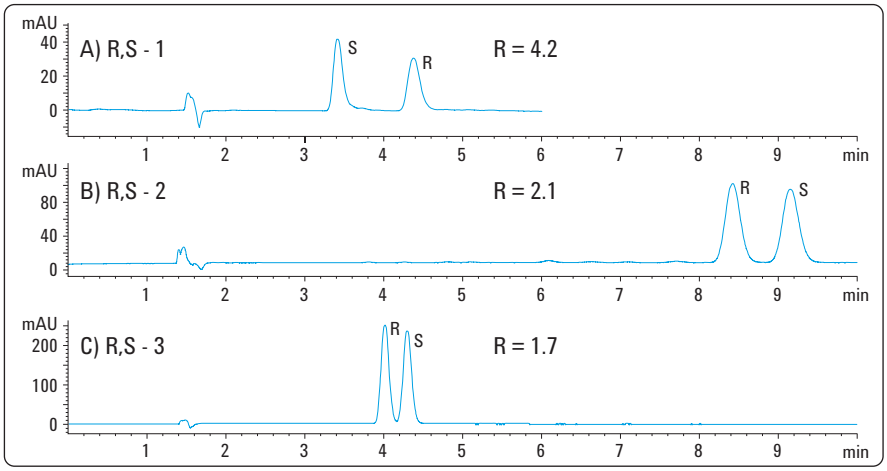


Figure 1
Isocratic separation of the racemic mixtures (50 ppm each isomer): A) R,S N-(3,5-dinitrobenzoyl)leucine, B) R,S thalidomide and C) R,S 1,1'-bi-2-naphthol

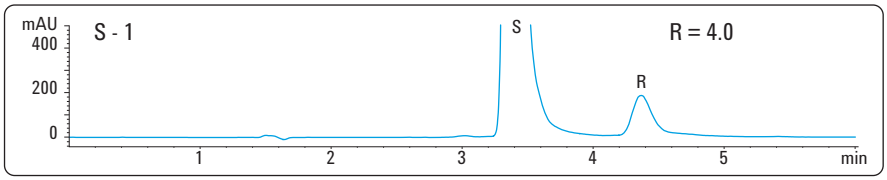


Figure 2
Chromatogram of the compound 1: enantiomer S – N-(3,5-dinitrobenzoyl)leucine at 5000 ppm.

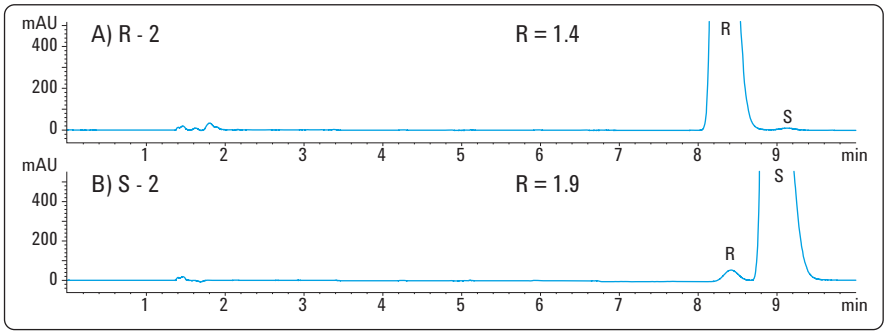


Figure 3
Chromatograms of compound 2: R – thalidomide (A) and S –thalidomide (B) at 5000 ppm. The separation conditions are the same as in Figure 1B.

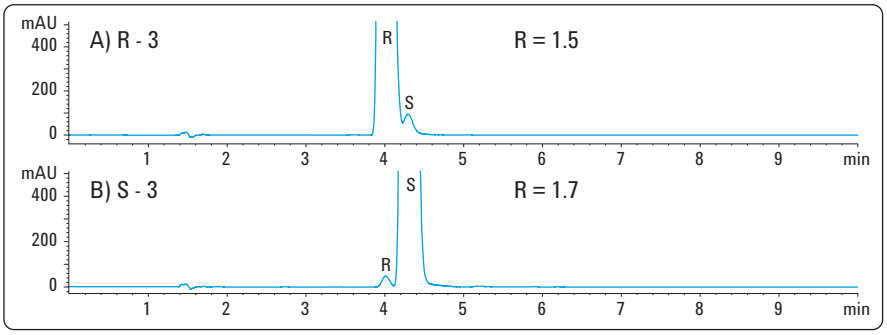


Figure 4
Chromatograms of compound 3: R – 1,1'-bi-2-naphthol (A) and S – 1,1'-bi-2-naphthol (B) at 5000 ppm.

Retention time and peak area repeatability

Retention time and peak area repeatability were measured at different concentration levels between 2.5 ppm and 100 ppm, corresponding to 0.05% and 2% relative to the main product. Typically, retention time repeatability was better than 0.1% (0.2% for thalidomide). Peak area repeatability was better than 5% at 0.1% or lower level, and in most cases better than 1% even at 0.5-2% impurity level. In all cases linearity was excellent over a wide concentration range. The concentrations and % EE were calculated for each "pure" compound by linear regression from the calibration curves (Table 4).

Conclusions

This Application Note demonstrates the determination of the enantiomeric excess (EE) in three model compounds on the Agilent 1260 Analytical Infinity SFC system.

Isocratic method development was successfully applied to achieve appropriate resolution for the separation of the racemic mixtures.

With the Agilent 1260 Infinity Analytical SFC system excellent repeatability and linearity was achieved. The low UV-detector noise of the Agilent 1260 Infinity Analytical SFC system enables the determination of enantiomeric excess at impurity levels below 0.05% and sets a new standard in analytical SFC performance.

Compound Name	Impurity (%)	EE (%)	Resolution
S – 1[(1)]	4.89	90.22%	3.97
R – 2	0.14%	99.72%	1.42
S – 2	0.86%	98.28%	1.94
R – 3	0.45%	99.10%	1.48
S – 3	0.20%	99.60%	1.70

Table 3
Impurity and resolution of enantiomeric test compounds at 5000 ppm.

Analyte		N-(3,5-dinitrobenzoyl)leucine		thalidomide		1,1′-bi-2-naphthol	
Isomer		**S-1**	**R-1**	**R-2**	**S-2**	**R-3**	**S-3**
Retention	**t_R (min)**	3.328	4.284	8.523	9.107	6.006	6.542
	s (min)	0.002	0.003	0.021	0.023	0.003	0.004
	% RSD	0.067	0.065	0.244	0.253	0.054	0.058
Level %							
0.05	RSD	5.09	3.62	3.48	3.18	0.61	0.92
0.10	RSD	1.47	1.63	2.16	2.02	0.43	0.74
0.20	RSD	1.55	1.03	1.20	1.16	0.21	0.31
0.50	RSD	0.28	0.54	0.57	0.58	0.17	0.14
1.00	RSD	0.44	0.30	0.34	0.34	0.15	1.33
2.00	RSD	0.42	0.43	0.25	0.20	0.12	0.14
Linearity	R2	0.999	0.999	0.999	0.999	0.998	0.999

Table 4
Validation data for enantiomeric excess determination by SFC

www.agilent.com/chem

July 1, 2010
Publication Number 5990-5969EN

Agilent Technologies