Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 23, No. 1, 97-101, 2010

단 신

1Determination of optical purity of *N*-acetyl-1-naphthylethylamine by chiral chromatography and NMR spectroscopy

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(Received October 1, 2009; Accepted January 9, 2010)

키랄 크로마토그래피와 NMR 분광법에 의한 *N*-acetyl-1-naphthylethylamine의 광학순도 측정

정영한[1] · 류재정[2,★]

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(2009. 10. 1. 접수, 2010. 1. 9. 승인)

Abstract: (R)-*N*-3,5-dinitrobenzoyl (DNB) phenylglycinol derived chiral selector was used as a HPLC chiral stationary phase (CSP) for the resolution of racemic *N*-acylnaphthylalkylamines. In this study, determination of optical purity was performed by both chiral chromatography and NMR spectroscopy by using the (R)-phenylglycinol derived chiral selector. The data of accuracy and precision of each optical purity value are calculated from the results of NMR and HPLC experiments by comparing with true value. Average error of the NMR method was +2.2% with average RSD of 4.54%, while that of HPLC method was -3.5% with average RSD of 3.23%.

요 약: (R)-*N*-3,5-dinitrobenzoyl (DNB) phenylglycinol로 부터 만들어진 키랄 선택제가 라세미 *N*-acylnaphthylalkylamines의 분리에 HPLC 키랄 정지상으로 이용된 바 있다. 본 연구에서는 (R)-phenylglycinol 유도체 키랄 선택제를 이용하여 키랄 크로마토그래피와 NMR 분광법에 의한 광학순도를 측정하였다. NMR과 HPLC 실험결과를 참값과 비교하여 각 광학순도 측정값의 정확도와 정밀도를 계산하였다. NMR 방법의 오차는 +2.2%, 평균 RSD는 4.54% 이었고, HPLC 방법의 오차는 -3.5%, 평균 상대표준편차는 3.23% 이었다.

Key words: chiral selector, HPLC, *N*-acylnaphthylalkylamines, optical purity, NMR

1. Introduction

The study of optically active chiral compounds became more active after three organic chemists won the Nobel Prize for their successful development of asymmetric catalysts in 2001.[1-3] Accordingly, an effort

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to establish the accurate measurement of the optical purity for newly synthesized or separated chiral compounds produced by asymmetric organic synthesis or chiral separation became important. Many optical purity measurement methods including liquid chromatography,[4] gas chromatography,[5] capillary electrophoresis,[6] NMR spectroscopy,[7] and mass spectrometry[8] have been reported. Among them, the most common and popularly used method is chiral high performance liquid chromatography with ultraviolet detection. However, performances of these optical purity measurement methods have not systematically compared to each other in terms of accuracy and precision. In this study, a liquid chromatography and 1H NMR spectroscopy methods for determining the optical purity of *N*-acetyl-1-naphthylethylamine were compared to each other by using same chiral selector.

Because racemic *N*-acyl-1-naphthylaminoalkanes were resolved well on *N*-(3,5-dinitrobenzoyl)-(R)-phenylglycinol derived **CSP 1**,[9] it was hypothesized that the **Sel 1** which is the precursor and selector of **CSP 1** could be used as a NMR chiral solvating agent for *N*-acetyl-1-naphthylethylamine. Therefore, the **Sel 1**[9] and the mixture of (R) - and (S) - isomer of an *N*-acetyl-1-naphthylethylamine, compound **1a** with ratio of 2:1, 1:1, 1:2 were prepared for checking the accuracy and precision of each optical purity measurement method.

2. Experimental

2.1. Instruments and Materials

1H-NMR spectra were recorded on Bruker, Avance Digital 400. All chiral selectors and analytes used in this study were prepared via the known procedure.[9] HPLC was performed on a JASCO HPLC system, consisting a JASCO PU-2080 Plus Intelligent Pump, a Rheodyne Model 7125 injector with a 20 μL sample loop, and a JASCO UV-2075 Plus Intelligent UV/VIS detector from JASCO (Tokyo, Japan). All HPLC solvents were supplied by Merck Korea (Seoul, Korea).

2.2. Determination of optical purity by NMR

Samples were prepared by mixing (R)-**1a** and (S)-**1a** and **Sel 1** with the molar ratio of 1:1:1, 1:2:1, 2:1:1 as listed in *Table* 1. The 1H-NMR experiments for each sample were performed 15 times. (same day: 5 times, different day: 3 times)

Table 1. Amount of each compound dissolved in 50.0 ml $CDCl_3$ solution

Sample Number	Ratio of R:S:**Sel 1**[1]	Amount of chemicals		
		(R)-**1a**	(S)-**1a**	**Sel 1**
1	1:1:1	383 mg	383 mg	864 mg
2	1:2:1	383 mg	766 mg	864 mg
3	2:1:1	766 mg	383 mg	864 mg

[1]R:S:**Sel 1**=1:1:1=30 mM (R)-enantiomer: 30 mM (S)-enantiomer: 30 mM **Sel 1**.

2.3. Determination of optical purity by HPLC

The NMR samples in *Table* 1 are diluted three times with methanol and the diluted solutions are directly used for HPLC experiment. The chiral HPLC was performed 21 times (same day; 7 times, different day 3 times) with **CSP 1**. Optical purity is calculated by peak area of each chromatographic peak. HPLC eluent was 20% IPA(isopropyl alcolol) in hexane and injection volume was 5.0 μL. Flow rate was 1.5 mL/min and detection wavelength was 254 nm UV.

2.4. Comparison of the two optical purity determination methods

The integrated area of NMR peaks or the area of HPLC peaks for three different ratios of (R)- and (S)-enantiomer mixtures of compound **1a** was calculated. The true values (theoretical value) in *Table* 1 and results of the two independent methods were compared on the point of accuracy (differences between true values and average values obtained from above two methods) or reproducibility (comparison on the RSD values obtained from above two methods).

3. Result and Discussion

The samples **1**(**R-1a:S-1a:Sel 1**=1:1:1), **2**(**R-1a:S-1a:Sel 1**=1:2:1) and **3**(**R-1a:S-1a:Sel 1**=2:1:1) were prepared by mixing (R)-**1a** and (S)-**1a** and **Sel 1** with

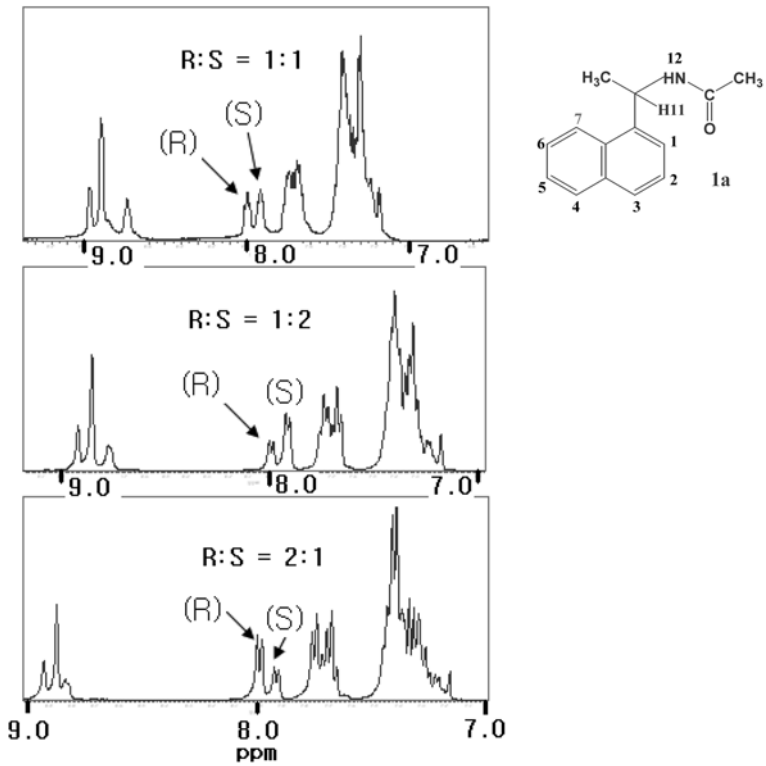

Fig. 1. NMR spectra of H7 proton on different ratio of (R)- and (S)- *N*-acetyl-1-naphthylethylamide(**1a**) in the presence of **Sel 1.**

Table 2. Ratio of (S)-**1a** to (R)-**1a** calculated from 1H NMR integration area of H7 proton[1]

Sample	Day 1		Day 2		Day 3		Total
	Average	RSD(SD)	Average	RSD(SD)	Average	RSD(SD)	
1	1.03 (S/R=1.03/1.00)	4.31% (0.044)	0.99 (S/R=1.00/1.01)	2.57% (0.026)	0.99 (S/R=1.00/1.01)	5.26% (0.053)	Average; 1.02 Error; +2.0% RSD 4.05%
2	2.01	4.63% (0.093)	1.99	3.81% (0.076)	2.02	3.61% (0.073)	Average; 2.01 Error; +0.50% RSD 3.93%
3	0.52	5.58% (0.029)	0.53	6.25% (0.033)	0.51	5.10% (0.026)	Average; 0.52 Error; +4.0% RSD 5.64%

[1]Average error; +2.2%, Average RSD; 4.54.

the molarity ratios of 30 mM:30 mM:30 mM, 30 mM:60 mM:30 mM, 60 mM:30 mM:30 mM in 50.0 mL $CDCl_3$. In the course of making the samples **1**, **2**, **3**, the reagents were weighed very carefully to reduce weighing error and make exact molar ratio of the samples. Respective 1H NMR spectra of each sample **1**(R:S=1:1), **2**(R:S=1:2), and **3**(R:S=2:1) were shown in *Fig.* 1.

As shown in *Fig.* 1, proton 7 (H7) of (R)-**1a** appeared at about 8.0 ppm while that of (S)-**1a** was shown at 7.9 ppm. The integrations of each peak were automatically calculated by NMR system and the ratio of (S)-**1a** to (R)-**1a** calculated from 1H NMR integration area of H7 are shown in *Table* 2.

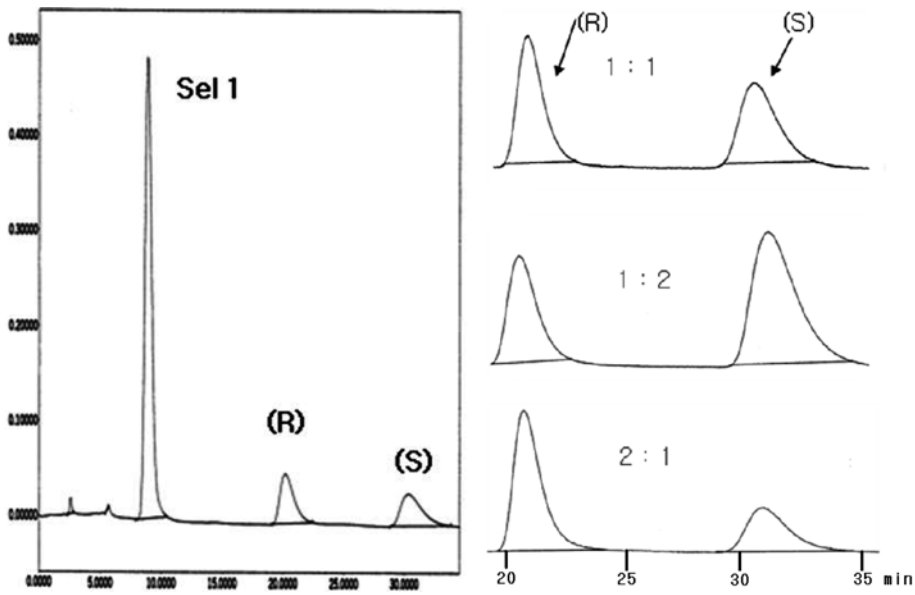

Fig. 2. Chromatograms of (R)- and (S)-**1a** mixed samples in the presence of **Sel 1** on **CSP 1.**

Table 3. Ratio of (S)-**1a** to (R)-**1a** calculated from HPLC experiment[1]

Sample	Day 1		Day 2		Day 3		Total
	Average	RSD(SD)	Average	RSD(SD)	Average	RSD(SD)	
1	0.96	2.66% (0.025)	0.95	2.84% (0.027)	0.95	3.05% (0.028)	Average; 0.95 Error; -5.0% RSD 2.85%
2	1.92	2.50% (0.048)	1.95	3.15% (0.061)	1.91	4.56% (0.087)	Average; 1.93 Error; -3.5% RSD 3.40%
3	0.49	4.47% (0.022)	0.49	3.53% (0.017)	0.49	2.35% (0.012)	Average; 0.49 Error; -2.0% RSD 3.45%

[1]The ratio are calculated from the peak area of each chromatographic peak of (R)- and (S)-**1a**. Average error; -3.5%, Average RSD; 3.23%.

As shown in *Table* 2, the integration average of H7 peak area in (S)-**1a** was larger by about 2.2% than that of in (R)-**1a** and average relative standard deviation (RSD) of the data is 4.54%. Therefore, the accuracy and reproducibility of this method is considerably good for determining the optical purity of this compound.

By using diluted NMR samples, chiral HPLC was performed to check the accuracy and reproducibility in determining the optical purity by HPLC method. Three main peaks appeared in the chromatogram of sample **1** on **CSP 1.** (*Fig.* 2) **Sel 1** was first eluted for nearly 10 min and (R)-**1a** was at 20 min, and (S)-**1a** could be found at 30 min.

The areas of each peak were automatically calculated by HPLC system and the ratio of (S)-**1a** to (R)-**1a** calculated from peak area are shown in *Table* 3.

As shown in *Table* 3, the integration of peak area in (R)-**1a** was slightly larger (about 3.5%) than that of in (S)-**1a** and average relative standard deviation (RSD) of these data is 3.23%. Therefore, accuracy and reproducibility of this method are good to check

optical purity of this compound.

In a comparison between chiral HPLC and NMR spectroscopy results of this study in determination of optical purity, the accuracy of NMR method is slightly better than that of the HPLC method, however, the precision is slightly better in HPLC method. In the case of other experimental data for optical purity of (R)-terbutaline,[10] both accuracy and reproducibility of the chiral HPLC method appeared better than the NMR (500 MHz) method. In general, resolution depends on the quality of each instrument and skill of the researcher, and the resolution may influence the reproducibility of optical purity severely. Therefore, it is impossible to conclude which method is better than the others at this time.

4. Conclusion

Determination of optical purity by chiral HPLC and NMR spectroscopy was performed well without any technical skill. The accuracy of NMR method is slightly better than that of HPLC method, however, precision is slightly better in the HPLC method. Even though this research is not a representative example for comparison of HPLC and NMR method in determination of optical purity, this data is useful to determine the optical purity of various chiral compounds. In addition, this study provides a possibility that a precursor of CSP can be used as a good chiral NMR solvating agent for a sample which is resolved on the CSP.

Acknowledgment

This work was supported by Korean Research Foundation Grant (KRF-2006-J02402).

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