HPLC Determination of Enantiomeric 2-Azabicyclo[2.2.1] hept-5-en-3-one on Chiral Stationary Phase

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Abstract

A simple high-performance liquid chromatography method has been developed and validated for determination of enantiomeric purity of 2-azabicyclo[2.2.1]hept-5-en-3-one with a run time of less than 10 min. Complete separation of enantiomers has been achieved on a Chiralcel OD-H analytical column (250 × 4.6 mm) using *n*-hexane–isopropanol (80:20, v/v) as the mobile phase at a flow rate of 1.0 mL/min under UV and optical rotation detection. The effect of the mobile phase and temperature on enantioselectivity for the enantiomers were further evaluated. The method was validated with respect to precision, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness. The recoveries were between 99.1% and 102.2%, with percentage relative standard deviation less than 1.14%. The LOD and LOQ for the (+)-2-azabicyclo[2.2.1]hept-5-en-3-one were 1.2 and 4.3 µg/mL and for the (–)-2-azabicyclo[2.2.1]hept-5-en-3-one were 1.3 and 4.4 µg/mL, respectively. This method is expected to be helpful for the determination of the enantiomeric purity of 2-azabicyclo[2.2.1]hept-5-en-3-one in bulk samples.

Introduction

2-Azabicyclo[2.2.1]hept-5-en-3-one (γ-lactam, Figure 1) is a bicyclic lactam in which there are two asymmetric centers on the rings. But due to the rigidity of the structure, it has only one pair of enantiomers. The γ-lactam was found to be a versatile synthon for the preparation of carbocyclic nucleosides (1–3). Nucleosides of the body are chiral, so the search of new therapeutic carbocyclic nucleosides agents drives the need for the synthesis of new enantiomerically pure compounds (4). The resolution of this synthetic γ-lactam is an important step in the synthesis of a group of chiral carbocyclic nucleosides drugs (4). At present, the main resolution method of the (rac)-γ-lactam is lipase-catalyzed resolution by lactamase (4–8). Moreover, an optical resolution method of γ-lactam by complexation with brucine has been reported (9).

The enantiomers of γ-lactam are widely used as the precursor of the carbocyclic nucleosides drugs (5). Chiral γ-lactam can be used for synthesis of (–)-carbovir (10) and (+)-cyclaradine (11). The approach from chiral γ-lactam proves to be a highly efficient route to carbocyclic analogs of ribavirin (12). The single enantiomers of γ-lactam are also used as chiral auxiliary for asymmetric synthesis (13). It is very important to determine the enantiomeric purity of the γ-lactam in pharmaceutical preparations. However, very few reports of separation for the γ-lactam have been reported. Resolution of the γ-lactam was always done with super-subcritical fluid chromatography (14) that needs expensive equipment and has a higher operation cost. A pentaproline based chiral stationary phase was used for resolution of γ-lactam (15), but its resolution (R_s) was not sufficient for the baseline separation ($R_s < 0.7$). Thus an efficient and economic method for precisely determining value of enantiomeric excess of the γ-lactam enantiomers is necessary to develop. Among the available methods for the separation of enantiomers, the high-performance liquid chromatography (HPLC) method with chiral stationary phases (CSPs) is more rapid and efficient in terms of resolution (16–18). So in this study, the γ-lactam enantiomers were separated using a chiral HPLC column.

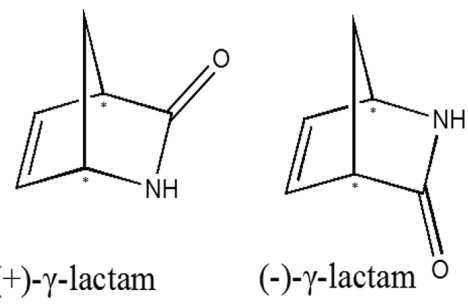

Figure 1. Chemical structure of the γ-lactam.

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Experimental

Chemicals and reagents

(rac)-γ-Lactam (98%) and (–)-γ-lactam (98%) were purchased from J&K Chemical Co., Ltd., (Beijing, China). *n*-Hexane, ethanol, and isopropanol of HPLC grade were supplied by Hangjia Chemical Co., Ltd., (Chengdu, China).

Equipment

Analysis was carried out on a Waters 2487 series liquid chromatography system (Waters, Milford, MA), equipped with CBL Model 515 HPLC pump, Waters 2487 Dual λ absorbance detector, a model 100 column heater (Photoelectron Technology, Taiwan) and JASCO Model OR-2090 optical rotation detector (JASCO, Tokyo, Japan). Chromatographic parameters such as peak areas, retention times, theoretical plates, etc. were calculated using the Allchrom Plus Client/Service workstation (Multilink Services Co., Ltd., Union, NJ).

Table I. Resolution Obtained Using Various Columns and Mobile Phases*

Column	Mobile phase	k_1	k_2	α	R_s
1	A	1.13	1.83	1.6	2.0
	B	1.10	1.51	1.3	1.4
2	A	1.99	1.99	1.0	0.0
	B	2.24	2.24	1.0	0.0
3	A	2.35	2.35	1.0	0.0
	B	1.78	1.78	1.0	0.0
4	A	2.45	2.73	1.2	0.7
	B	2.12	2.34	1.1	0.4
5	A	3.54	3.54	1.0	0.0
	B	3.26	3.26	1.0	0.0

* Columns 1–5: Chiralcel OD-H, DNB-Leucine, DNB-PG, Whelk-O1, and CHI-DMB, successively. Mobile phase A: n-hexane–isopropanol (85:15, v/v); mobile phase B: n-hexane–ethanol (85:15, v/v); k1: capacity factor of the (+)-γ-lactam; k2: capacity factor of the (–)-γ-lactam; α: separation factor; Rs: resolution; flow rate: 1.0 mL/min; column temperature: 30°C.

Table II. Effect of Isopropanol and Ethanol on Selectivity and Resolution of the γ-Lactam Enantiomers*

Mobile phase	k_1	k_2	t_1 (min)	t_2 (min)	α	R_s
n-hex–iso† (95:5)	3.517	5.873	14.021	21.333	1.669	3.047
n-hex–iso† (90:10)	1.949	3.189	9.115	13.003	1.635	2.523
n-hex–iso† (85:15)	1.130	1.835	6.613	8.801	1.623	2.010
n-hex–iso† (80:20)	0.852	1.376	5.749	7.375	1.615	1.929
n-hex–iso† (75:25)	0.659	1.058	5.151	6.390	1.605	1.817
n-hex–iso† (70:30)	0.546	0.869	4.798	5.803	1.593	1.682
n-hex–eth‡ (90:10)	1.732	2.377	8.483	10.485	1.372	2.023
n-hex–eth‡ (85:15)	1.106	1.508	6.539	7.787	1.363	1.468
n-hex–eth‡ (80:20)	0.615	0.841	5.013	5.717	1.359	1.241
n-hex–eth‡ (70:30)	0.380	0.508	4.285	4.683	1.337	1.143

* k_1: capacity factor of the (+)-γ-lactam; k_2: capacity factor of the (–)-γ-lactam; t_1: retention time of the (+)-γ-lactam; t_2: retention time of the (–)-γ-lactam; α: separation factor; R_s: resolution; flow rate: 1.0 mL/min; column temperature: 30°C.
† *n*-hexane–isopropanol = *n*-hex–iso.
‡ *n*-hexane–ethanol = *n*-hex–eth.

Results and Discussion

Chromatographic conditions

Chiralcel OD-H (250 × 4.6 mm; particle size 5 µm) (Daicel, Osaka, Japan), DNB-Leucine (250 × 4.6 mm; particle size 5µm), DNB-PG (250 × 4.6 mm; particle size 5 µm), Whelk-O1 (250 × 4.6 mm; particle size 5 µm) (Regis Technologies, Morton Grove, IL), and Kromasil CHI-DMB (250 × 4.6 mm; particle size 5 µm) (Akzo Nobel, Bohus, Sweden) were used for the separation. The mobile phase consisted of *n*-hexane–isopropanol or ethanol (85:15, or other v/v) and the column temperature was 30°C. The flow rate was 1.0 mL/min, and the detection wavelength was kept at 254 nm. Void times were determined using ethanol as a marker. The injection volume was approximately 5 µL. The sample solution was prepared by dissolving the sample in *n*-hexane at 80 µg/mL.

Choice of the chiral stationary phases

The racemic sample of γ-lactam was used in the method development, and five different chiral stationary phases were employed as follows: Chiralcel OD-H, DNB-Leucine, DNB-PG, Whelk-O1, and Kromasil CHI-DMB. Different trials were made and details are presented in Table I. The results indicated that the γ-lactam enantiomers could only be well separated on Chiralcel OD-H with *n*-hexane–isopropanol–ethanol as mobile phases. Therefore, the separation, retention, and elution order of enantiomers on Chiralcel OD-H should be further investigated.

Effect of organic modifiers

The types and concentrations of organic modifiers were found to influence the retention and resolution of the γ-lactam enantiomers dramatically. The selectivity and resolution of the enantiomers on Chiralcel OD-H column were investigated when isopropanol and ethanol were used as modifiers (shown in Table II). Both organic modifiers showed good selectivity for the enantiomers. However, isopropanol has showed better selectivity compared with ethanol. On decreasing the concentration of organic modifier, the capacity factors as well as resolutions were increased. In isopropanol case, sharp peaks with higher resolu-

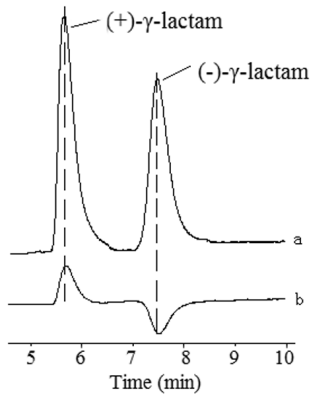

Figure 2. Chromatograms obtained from the (rac)-γ-lactam enantiomers on Chiralcel OD-H. Conditions: mobile phase, *n*-hexane–isopropanol (80:20, v/v); flow rate, 1.0 mL/min; column temperature, 30°C; detection wavelength, 254 nm; UV detection (A); and optical rotation detection (B).

tion and higher sensitivity (higher detections limits) were obtained. Thus, isopropanol was chosen as an organic modifier. As a compromise between resolution and retention time, 20% of isopropanol in *n*-hexane was found to be an optimum mobile phase for analysis purpose. The chromatograms of the (rac)-γ-lactam using isopropanol as organic modifier are shown in Figure 2. It was found that the (+)-γ-lactam was eluted first. Obviously, the two enantiomers were satisfactorily separated under the conditions compared with literature (14,15).

Effect of temperature

Temperature is an important factor in controlling enantiomeric recognition processes (19,20). The effects of column temperature on selectivity and resolution of the γ-lactam enantiomers were studied in the range 288–328 K (15–55°C). When the temperature was increased, the retentions were decreased. These results could be attributed to the fact that the analytes on molecular level have lower adsorption as temperature increased and therefore migrates quickly through the column (21). According to the Van't Hoff equation (21–24):

$$\text{Ln}\, k = -\frac{\Delta H^{\theta}}{RT} + \frac{\Delta S^{\theta}}{R} \qquad \text{Eq. 1}$$

where k is the retention factor, R is the gas constant, and T is the absolute temperature; Van't Hoff's plots were drawn for logarithm of retention factor (ln k) versus inverted temperature ($1/T$) for the two enantiomers, which yielded straight lines (Figure 3). ΔH^{o} and ΔS^{o} for the two enantiomers were obtained from the slope and intercept of the straight lines, respectively. The change in free energy ($\Delta\Delta G^{o}$) accompanying the separation of two enantiomers was given by

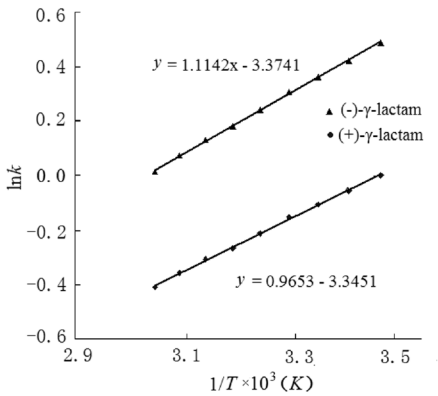

Figure 3. Plot of ln k versus 1/T. Conditions: Mobile phase, *n*-hexane–isopropanol (80:20, v/v); flow rate, 1.0 mL/min; UV detection wavelength, 254 nm.

Table III. Thermodynamic Data Calculated from the Van't Hoff Plots of the γ-Lactam Enantiomers

Enantioner	ΔH^{θ} (kJ/mol)	$\Delta\Delta H^{\theta}$ (kJ/mol)	ΔS^{θ} (J/K/mol)	$\Delta\Delta S^{\theta}$ (J/K/mol)	$\Delta\Delta G^{\theta}$ (kJ/mol)
(+)-γ-lactam	–8.026	–1.237	–27.811	–0.241	–1.165
(–)-γ-lactam	–9.263		–28.052		(298 K)

$$\Delta\Delta G^{\theta} = \Delta\Delta H^{\theta} - T\Delta\Delta S^{\theta} \qquad \text{Eq. 2}$$

The apparent thermodynamic parameters for the γ-lactam enantioseparations were obtained with *n*-hexane–isopropanol (80:20, v/v) as the mobile phase. The corresponding data are listed in Table III, which suggests that the processes of enantiomeric recognition was enthalpy-controlled in the study.

Effect of flow rate

The effect of flow rate on resolution of the γ-lactam enantiomers was investigated in the range of 0.8-1.5 mL/min [mobile phase was *n*-hexane–isopropanol (80:20 v/v) and the column temperature was 30°C]. The results are shown in Table IV. When the flow rate was increased, the separation factor was increased but the resolution decreased. After considering the separation factor and resolution, 1.0 mL/min was chosen as the optimal flow rate.

Validation of HPLC method

Precision

Precision of the method was tested by preparing six individual solutions of the γ-lactam and making triplicate injections for each solution under the working conditions. The RSD% of the assay was less than 1.31%. Inter and intra-day assay precisions were performed with analyzing the solutions five times in a day for three days. The RSD% of the assay was less than 1.46% for both the isomers.

Table IV. Effect of Flow Rates on Enantioselectivity*

Flow rate (mL/min)	k_1	k_2	α	R_s
0.5	2.753	3.873	1.407	2.197
0.8	1.354	2.038	1.505	2.043
1.0	0.879	1.425	1.621	2.013
1.2	0.575	1.017	1.769	1.951
1.5	0.277	0.632	2.280	1.552

* k_1: capacity factor of the first enantiomer peak; k_2: capacity factor of the second enantiomer peak; stationary phase: Chiralcel OD-H; column temperature: 30°C; mobile phase: n-hexane–isopropanol (80:20, v/v); UV detection wavelength: 254 nm.

Table V. Recovery Data (n = 3)

Actual conc. (μg/mL)	Enantiomer	Experimental (μg/mL)	Recovery (%)	RSD (%)
40	(+)	20.1	100.5	1.12
(–)	19.9	99.5	1.08	
60	(+)	30.2	100.7	1.14
(–)	30.1	100.3	1.09	
80	(+)	40.8	102.0	1.05
(–)	39.7	99.2	1.13	
90	(+)	45.1	100.2	1.12
(–)	44.6	99.1	1.10	
100	(+)	50.4	100.8	1.06
(–)	3.26	99.1	1.07	
120	(+)	61.3	102.2	1.13
(–)	59.6	99.3	1.07	

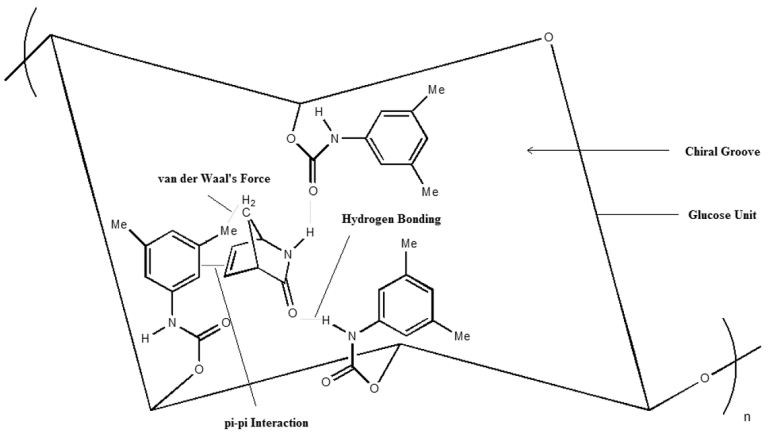

Figure 4. The representation of supra-molecular bindings of the γ-lactam in chiral groove of the cellulose tris-(3,5-dimethylphenylcarbamate) CSP.

Accuracy

Accuracy studies were performed with spiking the (rac)-γ-lactam solution at six levels with respect to specified level and analyzing each solution in triplicate (n = 3) for 3 days. The results were shown in Table V. The recoveries were between 99.1% and 102.2%, with percentage relative standard deviation less than 1.14%.

Linearity

Good linearity was observed for (+)-γ-lactam and (–)-γ-lactam in the concentration range of 5 ~ 320 µg/mL. The curves were linear with r_1^2 = 0.9993 and r_2^2 =0.9998, and the regression equations for the (+)-γ-lactam and (–)-γ-lactam were $y_1 = 8401.5x_1 - 2842.7$ and $y_2 = 8374.6x_2 - 2783.1$, respectively. Linearity was checked over the same concentration ranges for three consecutive days.

Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were estimated at a signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of dilute solutions. LOD was found to be 1.2 and 1.3 µg/mL for the (+)-γ-lactam and (–)-γ-lactam, respectively. LOQ was found to be 4.3 and 4.4 µg/mL for the (+)-γ-lactam and (–)-γ-lactam, respectively.

Robustness

Robustness of the method was studied with making small deliberate changes in the method parameters. A variation of 2% of isopropanol in the composition of the mobile phase hardly affected the resolution except that retentions were changed. The effect of temperature was studied by analyzing sample at 30 ± 2°C. Again retention times varied in the range of 2 min, but the resolution remained above 1.9. The effect of the flow rate was studied with analyzing the samples in 0.8 and 1.2 mL/min flow rates. In the both cases the resolution was found to be above 1.9.

Chiral recognition

Figure 4 represents the possible existing interactions between the γ-lactam and the cellulose tris-(3,5-dimethylphenylcarbamate) CSP, which indicated inner retention mechanism between the CSP and the analyte. Different possible bondings and interactions were hydrogen bondings (between –C = O and amine groups), π-π interactions (between phenyl groups of CSP and unsaturated double bond of γ-lactam) and van der Waal's forces (among alkyl groups of CSP and methylene of γ-lactam). However, steric effects in the chiral groove played significant roles in chiral recognition mechanism. This investigation is awaiting further study and represents future work not included in this article.

Conclusions

A validated and simple HPLC method was developed for the enantiomeric separation of the γ-lactam on Chiralcel OD-H. The elution sequence and absolute configurations were determined. The effects of organic modifiers and temperature on resolution and retention of enantiomers have been evaluated to optimize the HPLC conditions. The method was completely validated and showed satisfactory data for all the method validation parameters (precision, accuracy, linearity, LOD, LOQ, and robustness) tested. The improved method was of use for quantitative analysis of enantiomeric purity of the γ-lactam in bulk samples.

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