DEVELOPMENT OF AN EFFICIENT HPLC METHOD WITH PRE-COLUMN DERIVATIZATION FOR DETERMINATION OF ENANTIOMERIC PURITY OF 2-(AMINOMETHYL)-1-ETHYLPYRROLIDINE

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Short Running head: Determination of Enantiomeric Purity of 2-(Aminomethyl)-1-ethylpyrrolidine
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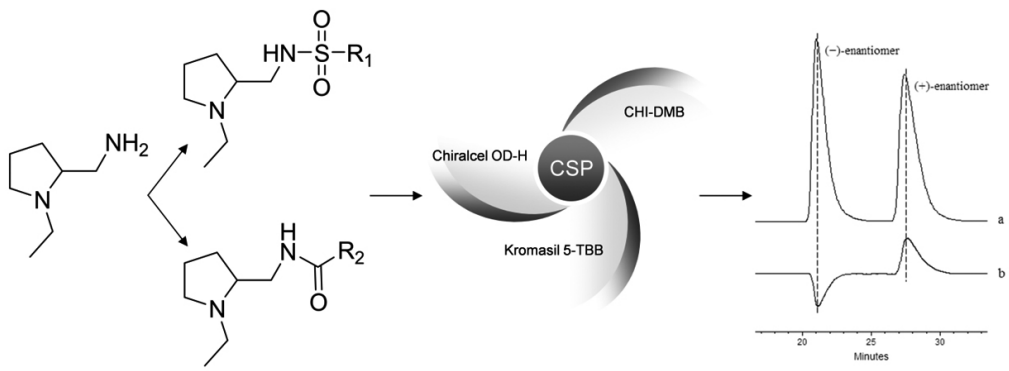
ABSTRACT

The development of a simple and efficient analytical method for determination of enantiomeric purity of 2-(aminomethyl)-1-ethylpyrrolidine was presented. The method was based on pre-column derivatization of 2-(aminomethyl)-1-ethylpyrrolidine with 4-nitrobenzoic acid completing separation of the enantiomers on high performance liquid chromatographic (HPLC) chiral stationary phase (CSP). After optimizing all the effective parameters, the best performances were achieved on a Chiralcel OD-H analytical column (250 × 4.6 mm) using *n*-hexane: ethanol (98:2, *v/v*) containing 0.2% triethylamine as mobile phase with a flow rate of 1.0 mL min^{-1} at 25°C. Online UV and optical rotation (OR) detection was performed at 254 nm. The parameters which have an effect on chiral separation were investigated, including mobile phase additive, organic modifier type and concentration, column temperature and flow rate. The method was tested for precision, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ) and robustness. The present method is expected to be accurate, stable, rapid and sensitive and can be used for the determination of the enantiomeric purity of 2-(aminomethyl)-1-ethylpyrrolidine in bulk samples.

Keywords: HPLC, enantiomeric separation, 2-(aminomethyl)-1-ethylpyrrolidine, pre-column derivatization



1. INTRODUCTION

2-(Aminomethyl)-1-ethylpyrrolidine [(1-ethylpyrrolidin-2-yl) methanamine] (shown in Figure 1) has one pair of enantiomers for an asymmetric center on the 2-position of the pyrrolidine ring. As a versatile synthon in the synthesis of bioactive compounds, it plays a very crucial role for them to exhibit activity. As a result, the derivatives of 2-(aminomethyl)-1-ethylpyrrolidine have continuously attracted the attention of many chemists to search for new biologically active compounds. For example, its derivatives have been developed as potential antipsychotics with selectivity for dopamine D_2 receptors[1,2]. In a search for novel antimalarial compounds, a series of compounds containing *N*-ethylpyrrolidine moiety were synthesized and evaluated as antiplasmodial agents[3,4]. Moreover, 2-(aminomethyl)-1-ethylpyrrolidine can be used to synthesize antiviral agents[5,6], antitumor compounds[7,8], melanin-concentrating hormone (MCH) receptor 1 antagonists[9], and Homo sapiens acetylcholinesteraste (hAChE) inhibitor[10].

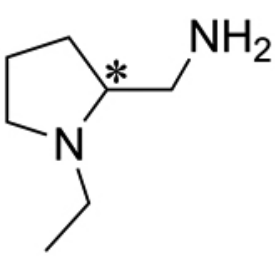


Figure 1. Chemical structure of 2-(aminomethyl)-1-ethylpyrrolidine.

The increasing demand for enantiomerically pure drugs has led to the need for the preparation of new enantiopure compounds. So it is significant to determine the enantiomeric purity of 2-(aminomethyl)-1-ethylpyrrolidine in pharmaceutical preparations. However, there was no resolution report for the 2-(aminomethyl)-1-ethylpyrrolidine enantiomers. Consequently, an efficient and economic method is necessary to be developed for precisely determination of enantiomeric excess (e.e.%) of the 2-(aminomethyl)-1-ethylpyrrolidine enantiomers. Although there are many methods used to isolate enantiomers, liquid chromatography is a simple, sensitive and accurate method. Among the various HPLC methods, the application of chiral stationary phases (CSPs) has been known to be the most accurate, convenient, and economic method in terms of resolution[11]. In method development, the availability of durable, stable and high-performance CSP column is preferable in developing a rugged and reproducible method. Experimental results indicated that 2-(aminomethyl)-1-ethylpyrrolidine can not be separated directly on the popular columns satisfactorily. So in this paper, 2-(aminomethyl)-1-ethylpyrrolidine was pretreated with various derivatization reagents, including aryl sulfonyl chlorides which was a new type of pre-column derivatization reagents for chiral amine, and then the derivatives were separated with commercially available columns. The effects of several parameters, including mobile phase additives, organic modifier type and concentration, column temperature and flow rate, on the retention and enantioseparation were investigated thoroughly.

2. EXPERIMENTAL

2.1 Chemicals and Reagents

(Rac)-2-(aminomethyl)-1-ethylpyrrolidine was purchased from Fluorochem Ltd (Derbyshire, United Kingdom). Ethanol, isopropanol and *n*-hexane of HPLC grade were supplied by Kelong Chemical Reagent Co., Ltd. (Chengdu, China). 3,5-dinitrobenzoic acid was purchased from Kefeng Chemical Reagent Co., Ltd. (Shanghai, China). 2-nitrobenzenesulfonyl chloride, 3-nitrobenzenesulfonyl chloride and 4-nitrobenzenesulfonyl chloride

were purchased from Asta Tech (Chengdu, China). Triethylamine, isobutyl chloroformate, benzoic acid, 4-nitrobenzoic acid, benzenesulfonyl chloride, pyridine and tetrahydrofuran were purchased from Kelong Chemical Reagent Co., Ltd. (Chengdu, China). Other reagents supplied by Kelong Chemical Reagent Co., Ltd. (Chengdu, China) were all in analytical level.

2.2 Instruments and Equipments

Analysis was performed on a Shimadzu series liquid chromatography system, equipped with LC-20AT pump, SPD-M20A photodiode array detector (Kyoto, Japan), JASCO (Japan) model OR-2090 optical rotation detector, and HCT-360 LC column box (Tianjin, China). Chromatographic parameters such as peak areas, retention times, theoretical plates, etc. were calculated by using the Class-VP workstation.

2.3 Chromatographic Conditions

Chiralcel OD-H (250 × 4.6 mm; particle size 5 μm) (Daicel, Japan), Whelk-O1 (250 × 4.6 mm; particle size 5 μm) (Regis Technologies, USA), Kromasil 5-TBB (250 × 4.6 mm; particle size 5 μm) (Akzo Nobel, Sweden), Kromasil CHI-DMB (250 × 4.6 mm; particle size 5 μm) (Akzo Nobel, Sweden) were used for the separation. The composition of mobile phase was *n*-hexane: isopropanol or ethanol with 0.2% triethylamine and the column temperature was set at 25°C. The flow rate was 1.0 mL min^{-1} and the detection wavelength was kept at 254 nm. Void times were determined using ethanol as a marker. The injection volume was about 10 μL. The sample solution was prepared by dissolving the sample in ethanol at 400 μg mL^{-1}.

2.4 Derivatization Procedure

The 7 derivatives of 2-(aminomethyl)-1-ethylpyrrolidine were obtained following the general method outlined in Figure 2, which could be divided into 2 types, i.e., the synthesis of benzenesulfonamides and benzamides. The synthesis of benzenesulfonamides was as follows. A mixture of (rac)-2-(aminomethyl)-1-ethylpyrrolidine (0.70 mL, 5 mmol), aryl sulfonyl chloride (5 mmol) and pyridine (25 mL) was stirred overnight at room temperature. After the completion of the reaction, pyridine was removed under reduced pressure. Further purification of the desired product was accomplished by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether/TEA (1:1:0.04, v/v/v).

The synthesis of benzamides was different from benzenesulfonamides, as shown below. Triethylamine (0.83 mL, 6 mmol) was added to a stirred solution of aromatic acid (5 mmol) in THF (40 mL) at 0°C for 3 min. Then isobutyl chloroformate (0.78 mL, 6 mmol) was added to the solution, and the resulting mixture was stirred for 10 min. Next, (rac)-2-(aminomethyl)-1-ethylpyrrolidine (0.70 mL, 5 mmol) was added to the solution, and the mixture was stirred for another 0.5 h. Subsequently, the mixture was allowed to warm to room temperature overnight. The solvent was evaporated under reduce pressure and the crude product was purified by column chromatography on silica gel using ethyl acetate/petroleum ether/TEA (2:1:0.06, v/v/v) as eluent.

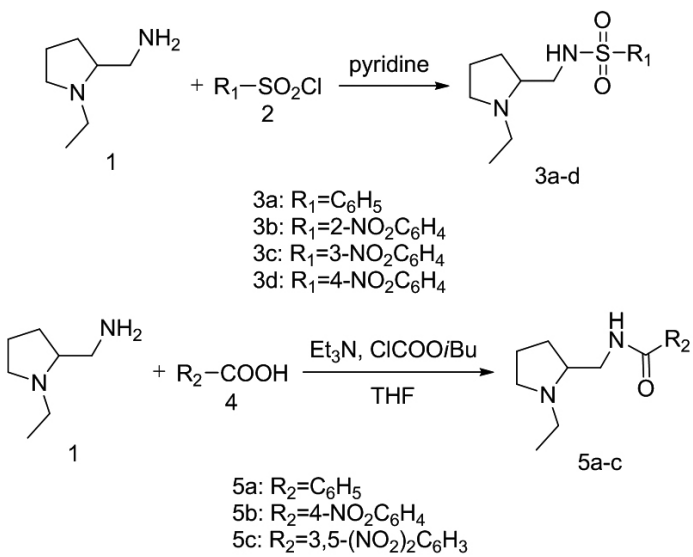


Figure 2. The derivatization reaction routes of 2-(aminomethyl)-1-ethylpyrrolidine.

Table 1. Mass spectra and ^{1}H NMR spectra data of compounds 3a-d and 5a-c

Compd.	ESI-MS *m/z* $[M+H]^+$	^{1}H NMR ($CDCl_3$, 400 MHz)
3a	269.1	7.50-7.88 (5H, m), 2.84-3.10 (3H, m), 2.35-2.51 (2H, m), 2.01-2.14 (2H, m), 1.60-1.83 (4H, m), 0.95 (3H, t, *J*= 7.3 Hz)
3b	314.0	7.72-8.14 (4H, m), 2.98-3.15 (3H, m), 2.49-2.57 (2H, m), 2.08-2.17 (2H, m), 1.63-1.88 (4H, m), 0.99 (3H, t, *J*= 7.2 Hz)
3c	314.1	7.66-8.64 (4H, m), 2.86-3.06 (3H, m), 2.37-2.52 (2H, m), 2.02-2.12 (2H, m), 1.52-1.83 (4H, m), 0.92 (3H, t, *J*= 7.2 Hz)
3d	314.0	8.37 (2H, d, *J*= 8 Hz), 8.05 (2H, d, *J*= 8 Hz), 2.92-3.12 (3H, m), 2.42-2.58 (2H, m), 2.08-2.19 (2H, m), 1.61-1.88 (4H, m), 0.98 (3H, t, *J*= 7.2 Hz)
5a	233.1	7.42-7.80 (5H, m), 6.95 (1H, s), 3.70-3.75 (1H, m), 3.20-3.32 (2H, m), 2.69-2.89 (2H, m), 1.60-2.30 (6H, m), 1.12 (3H, t, *J*= 7.2 Hz)
5b	278.1	7.94-8.31 (4H, m), 7.05 (1H, s), 3.70-3.75 (1H, m), 3.19-3.36 (2H, m), 2.72-2.87 (2H, m), 1.62-2.32 (6H, m), 1.13 (3H, t, *J*= 7.2 Hz)
5c	323.1	9.03-9.16 (3H, m), 7.51 (1H, s), 3.77-3.80 (1H, m), 3.21-3.43 (2H, m), 2.77-2.92 (2H, m), 1.64-2.35 (6H, m), 1.16 (3H, t, *J*= 7.3 Hz)

3. RESULTS AND DISCUSSION

3.1 Derivatization Conditions

In order to achieve enantioseparation, it is necessary to have enantiospecific interactions, i.e., dipole–dipole interactions, H-bonds, van der Waals interactions and π–π interactions, between a chiral selector and the enantiomers of an analyte resulting in transient diastereomeric complexes. Besides, the type of chiral stationary phases in the experiment was amide, it would be better to make 2-(aminomethyl)-1-ethylpyrrolidine into amide derivatives for investigation. Consequently, aromatic acids were used as derivatization reagents, to provide strong ultraviolet absorption and π–π interaction group. Moreover, larger molecule could be gained after derivatization, and the steric effect could enhance adsorption with CSP being useful to chiral recognition. In the experiment, benzenesulfonyl chloride, 2-nitrobenzenesulfonyl chloride, 3-nitrobenzenesulfonyl chloride, 4-nitrobenzenesulfonyl chloride, benzoic acid, 4-nitrobenzoic acid and 3,5-dinitrobenzoic acid were chosen as derivatization reagents, and 2 types of amides were synthesized, i.e., benzenesulfonamides and benzamides.

3.2 Comparison of the Chiral Stationary Phases

The 7 derivatives of 2-(aminomethyl)-1-ethylpyrrolidine were used in the experiment, and 4 different chiral stationary phases were employed as follows: Chiralcel OD-H, Kromasil CHI-DMB, Whelk-O1 and Kromasil 5-TBB. Because of 2-(aminomethyl)-1-ethylpyrrolidine containing an amino basic function group, the peak shape was very poor. So triethylamine was used to reduce peak tailing by masking the residual silanol groups of the CSP as basic mobile phase additive. A great improvement in the peak symmetry and resolution was achieved for the enantiomers when 0.2% triethylamine was added. When the concentration of triethylamine became higher, the peak symmetry and resolution remained almost the same. Hence, triethylamine at a concentration of 0.2% was chosen for the enantioseparation.

After the completion of different attempts, it was found that the 7 derivatives of 2-(aminomethyl)-1-ethylpyrrolidine could be separated on the chiral columns mentioned above except for Whelk-O1 (shown in Table 2). The results indicated that only Chiralcel OD-H column could separate the entire derivatives of 2-(aminomethyl)-1-ethylpyrrolidine to some extent. The 3,5-dinitrobenzoic acid, 4-nitrobenzoic acid and 2-nitrobenzenesulfonyl chloride were more popular derivatization reagents for their versatility on chiral columns. The Kromasil CHI-DMB and Kromasil 5-TBB columns had similar characteristics on the enantioseparation of different analytes. The retention times of the 7 derivatives of 2-(aminomethyl)-1-ethylpyrrolidine on the Kromasil CHI-DMB and Kromasil 5-TBB columns were shorter as compared with that of Chiralcel OD-H column on the same mobile phase. The

4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine **5b** on the Chiralcel OD-H column and 2-nitrobenzenesulfonyl chloride derivative of 2-(aminomethyl)-1-ethylpyrrolidine **3b** on the Kromasil CHI-DMB column were more likely to achieve baseline separation within a shorter period of time, which were selected for further research.

Table 2. Resolution of racemic 2-(aminomethyl)-1-ethylpyrrolidine derivatives using various columns

Chiral column	Compound	t_1(min)	t_2(min)	α	R_s
Chiralcel OD-H	3a	16.299	17.323	1.079	0.436
	3b	27.979	28.907	1.038	0.229
	3c	35.371	40.149	1.149	0.890
	3d	47.157	52.640	1.125	0.900
	5a	18.464	20.053	1.105	0.490
	5b	33.003	41.867	1.298	1.505
	5c*	41.344	44.885	1.093	0.574
Kromasil CHI-DMB	3a	9.824	10.037	1.032	0.184
	3b	14.667	16.085	1.124	0.892
	3c	24.480	24.480	1.000	0.000
	3d	26.603	26.603	1.000	0.000
	5a	8.427	8.427	1.000	0.000
	5b	19.573	20.469	1.055	0.243
	5c	35.883	38.251	1.073	0.462
Kromasil 5-TBB	3a	9.205	9.205	1.000	0.000
	3b	13.536	14.133	1.058	0.441
	3c	22.336	22.336	1.000	0.000
	3d	25.515	25.515	1.000	0.000
	5a	6.944	6.944	1.000	0.000
	5b	15.445	16.267	1.067	0.261
	5c	27.509	30.091	1.106	0.705

t_1: retention time of the first enantiomer; t_2: retention time of the second enantiomer; α: separation factor; R_s: resolution; flow rate: 1.0 mL min^{-1}; column temperature: 25°C; mobile phase: *n*-hexane: isopropanol (98:2, *v/v*) containing 0.2% triethylamine; UV detection wavelength: 254 nm; 5c* mobile phase: *n*-hexane: ethanol (97:3, *v/v*) containing 0.2% triethylamine.

3.3 Effect of Organic Modifier

The type and concentration of organic modifier were found to dramatically influence the retention and resolution on both columns when isopropanol and ethanol were used as modifiers, respectively (shown in Table 3). After the replacement of isopropanol with ethanol, the peak shape became more sharply and the retention times became shorter on both columns, but the trend of selectivity and resolution of the enantiomers on the Chiralcel OD-H and Kromasil CHI-DMB columns were different. The selectivity and resolution of the enantiomers on the Chiralcel OD-H were improved as compared with that of Kromasil CHI-DMB column getting worse. With the decrease of modifier percentages in the mobile phase, the retention factors as well as resolutions were increased. As a compromise between resolution and retention time, *n*-hexane: ethanol (98:2, *v/v*) containing 0.2% triethylamine was found to be an ideal mobile phase for the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine **5b**, and *n*-hexane: isopropanol (99.5:0.5, *v/v*) containing 0.2% triethylamine was found to be an ideal mobile phase for the 2-nitrobenzenesulfonyl chloride derivative of 2-(aminomethyl)-1-ethylpyrrolidine **3b**. Under optimum chromatographic conditions, the performances of the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine **5b** on the Chiralcel OD-H column were better, so it was selected in further experiments. Figure 3 and Figure 4 showed characteristic chromatograms obtained during the method development for the measuring of the enantiomeric purity of the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine enantiomers. It was found that (−)-4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine enantiomer was the first to elute.

Table 3. Effect of mobile phase constitutes on selectivity and resolution of derivative **5b** on the Chiralcel OD-H column and derivative **3b** on the Kromasil CHI-DMB column

Chiral column	Mobile phase	t_1(min)	t_2(min)	α	R_s
Chiralcel OD-H	*n*-hexane: isopropanol 98.5:1.5	42.677	54.304	1.295	1.750
	n-hexane: isopropanol 98:2	33.003	41.867	1.298	1.505
	n-hexane: isopropanol 95:5	16.021	20.288	1.334	0.936
	n-hexane: ethanol 99:1	37.547	49.525	1.349	2.292
	n-hexane: ethanol 98:2	21.056	27.403	1.357	1.571
	n-hexane: ethanol 97:3	15.733	20.203	1.358	1.197
Kromasil CHI-DMB	*n*-hexane: isopropanol 99.5:0.5	25.525	28.885	1.151	1.510
	n-hexane: isopropanol 99:1	18.741	20.789	1.132	1.215
	n-hexane: isopropanol 98.5:1.5	16.107	17.749	1.128	1.004
	n-hexane: isopropanol 98:2	14.667	16.085	1.124	0.945
	n-hexane: ethanol 99.5:0.5	19.541	20.981	1.088	0.821

Mobile phase additive: 0.2% triethylamine; t_1: retention time of the first enantiomer; t_2: retention time of the second enantiomer; α: separation factor; R_s: resolution; flow rate: 1.0 mL min^{-1}; column temperature: 25 °C; UV detection wavelength: 254 nm.

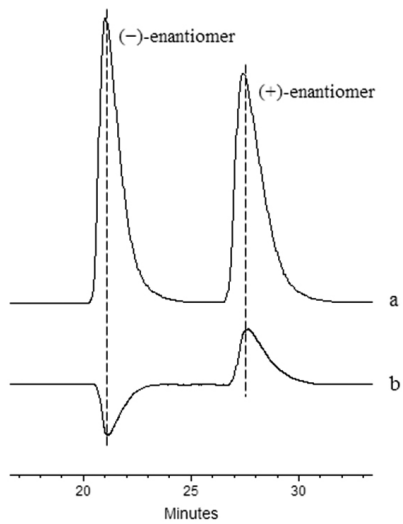


Figure 3. Chromatograms obtained from the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine enantiomers on the Chiralcel OD-H column. Conditions: mobile phase, *n*-hexane: ethanol (98:2, *v/v*) containing 0.2% triethylamine; flow rate, 1.0 mL min^{-1}; column temperature, 25 °C; detection wavelength, 254 nm.

a. UV detection

b. optical rotation detection

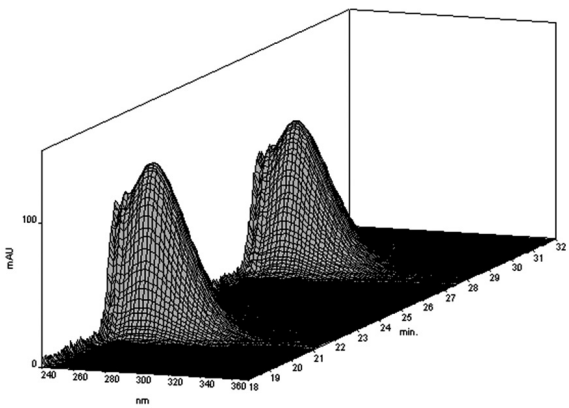


Figure 4. Chromatogram of the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine enantiomers detected by photodiode-array detector. Conditions: stationary phase, Chiralcel OD-H; mobile phase, *n*-hexane: ethanol (98:2, *v/v*) containing 0.2% triethylamine; flow rate, 1.0 mL min^{-1}; column temperature, 25 °C; UV detection wavelength, 254 nm.

3.4 Effect of Temperature

Temperature is an important factor in affecting enantiomeric recognition processes[12]. To study the chiral recognition mechanism further, the effect of varying column temperature on chromatographic parameters of the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine, such as retention factor and selectivity, were investigated. It was found that the retention time decreased with the increasing of temperature. The logical explanation may be that the analytes on molecular level have smaller adsorption as temperature increased and therefore migrate rapidly through the chiral column. In accordance with the Van′t Hoff equation[13-15]:

$$\ln k = -\frac{\Delta H^{\theta}}{RT} + \frac{\Delta S^{\theta}}{R} \qquad (1)$$

where k represents the retention factor, R represents the gas constant and T represents the absolute temperature in Kelvin, ΔH^{θ} and ΔS^{θ} represent the molar enthalpy and molar entropy of adsorption. Van't Hoff plots were drawn for logarithm of retention factor (ln k) versus inverted temperature (1/T) for the two enantiomers, which yielded straight lines in the corresponding temperature range of 288~323K (shown in Figure 5). ΔH^{θ} and ΔS^{θ} for the two enantiomers were calculated from the slope and intercept of the straight lines, respectively. The change in free energy ($\Delta\Delta G^{\theta}$) accompanying the separation of two enantiomers was given by

$$\Delta\Delta G^{\theta} = \Delta\Delta H^{\theta} - T\Delta\Delta S^{\theta} \qquad (2)$$

The apparent thermodynamic parameters for the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine enantioseparation were obtained using *n*-hexane: ethanol (98:2, *v/v*) containing 0.2% triethylamine as the mobile phase. The corresponding data were listed in Table 4, which proved that the enantiomeric recognition processes were enthalpy-controlled in the research.

Table 4. Thermodynamic data calculated from the Van′t Hoff plots of the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine enantiomers in temperature rang 288~323K

Enantioner	ΔH^{θ} (*kJ mol^{-1}*)	$\Delta\Delta H^{\theta}$ (*kJ mol^{-1}*)	ΔS^{θ} (*J K^{-1} mol^{-1}*)	$\Delta\Delta S^{\theta}$ (*J K^{-1} mol^{-1}*)	$\Delta\Delta G^{\theta}$ (*kJ mol^{-1}*)
(−)-enantiomer	-5.289	-3.259	-3.525	-8.349	-0.770 (298K)
(+)-enantiomer	-8.548		-11.875		

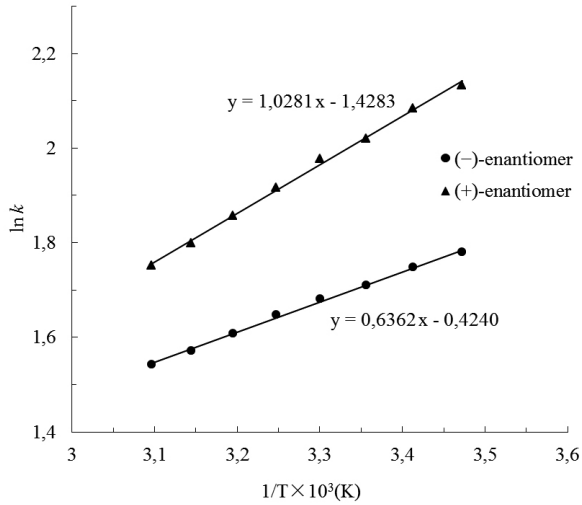


Figure 5. Plot of ln k versus 1/T. Conditions: Mobile phase, *n*-hexane: ethanol (98:2, *v/v*) containing 0.2% triethylamine; flow rate, 1.0 mL min^{-1}; UV detection wavelength, 254 nm.

3.5 Effect of Flow Rate

The effect of flow rate on selectivity and resolution of the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine enantiomers was investigated in the range of 0.5~1.5 mL min^{-1} (shown in Table 5). Experimental results indicated that with the increase of the flow rate, the separation factor increased but the resolution decreased. Based on an overall consideration of separation factor and resolution, 1.0 mL min^{-1} was chosen as the ideal flow rate.

Table 5. Effect of flow rates on enantioselectivity

Flow rate (mL min^{-1})	k_1	k_2	α	R_s
0.5	11.967	15.892	1.328	1.869
0.8	7.069	9.510	1.345	1.651
1.0	5.451	7.396	1.357	1.571
1.2	4.366	5.974	1.368	1.480
1.5	3.271	4.552	1.392	1.216

k_1: capacity factor of the first enantiomer; k_2: capacity factor of the second enantiomer; stationary phase: Chiralcel OD-H; column temperature: 25 °C; mobile phase: *n*-hexane: ethanol (98:2, *v/v*) containing 0.2% triethylamine; UV detection wavelength: 254 nm.

3.6 Validation of HPLC Method

In the validation of HPLC method, the sample solutions from 2-(aminomethyl)-1-ethylpyrrolidine were prepared and analyzed under the optimized derivatization procedure and chromatographic conditions. Following parameters were evaluated one by one to ensure the validity of the HPLC method based on good analytical practice guidelines.

Precision

Precision of the method was determined by preparing five individual solutions of the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine and making triplicate injections for each solution under the optimized chromatographic conditions. The RSD% of the assay was less than 1.28%. Inter and intra-day assay precisions were evaluated by analyzing the solutions for five times in a day for 3 days. The RSD% of the assay was less than 1.41% for both isomers.

Accuracy

Accuracy studies were tested by spiking the 4-nitrobenzoic acid derivative of 2-(aminomethyl)-1-ethylpyrrolidine solution at six levels with respect to specified level and analyzing each solution in triplicate (n = 3) for 3 days. It was found that the recoveries were between 98.9% and 102.2% with percentage relative standard deviation less than 1.32%.

Linearity

Linearity was determined with constructing calibration curve to find out

the relationship between instrumental response and known concentrations of pure sample solution over the concentration range of 50~2000 μg mL^{-1}. The calibration curves shown good linearity with a correlation coefficient of r_1^2 = 0.9998 and r_2^2 = 0.9999 and the regression equations for the first enantiomer and the second enantiomer were y_1 = 10144.9 x_1 – 19089.8 and y_2 = 10097.9 x_2 - 38022.3, respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were estimated using signal/noise (S/N) ratio method by injecting a series of dilute solutions at signal-to-noise ratios of 3:1 and 10:1, respectively. LOD was found to be 10.9 and 14.3 μg mL^{-1} for the first enantiomer and the second enantiomer, separately. LOQ was found to be 36.7 and 47.8 μg mL^{-1} for the first enantiomer and the second enantiomer, separately.

Robustness

The reliability of the analytical procedure with respect to deliberate variations in the method parameters was tested. A variation of 1% of ethanol in the composition of the mobile phase hardly affected the resolution except that retention times were changed. The influence of temperature was investigated with analyzing sample at 25 ± 2°C. Again retention times varied in the range of 1 min but the resolution remained above 1.5. The influence of flow rate was investigated with analyzing the samples in 0.9 and 1.1 mL min^{-1} flow rates. In both cases the resolution was found to be above 1.5.

4. CONCLUSIONS

A simple and efficient HPLC method combined with pre-column derivatization was developed for enantiomeric separation and purity determination of 2-(aminomethyl)-1-ethylpyrrolidine. Considering the overall results of the experiment, the best performances were achieved on the Chiralcel OD-H column. The effects of mobile phase composition, temperature and flow rate on the resolution and retention times of enantiomers were studied to optimize the HPLC conditions. The enantiomeric separation was found to be an enthalpy driven process. The method was tested for precision, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ) and robustness. The method was proved to be useful for quantitative analysis of enantiomeric purity of the 2-(aminomethyl)-1-ethylpyrrolidine in bulk materials in routine quality control laboratories.

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