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Determination of Enantiomeric Purity of 2-Piperidinemethanamine by HPLC Combined With Pre-Column Derivation

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A new HPLC method has been developed and validated for determination of enantiomeric purity of 2-piperidinemethanamine within a short run time of less than 10 min. The method was based on pre-column derivation of 2-piperidinemethanamine with 3,5-dinitrobenzoic acid and complete separation of enantiomers has been achieved on a CHI-DMB analytical column (250 mm × 4.6 mm) using *n*-hexane: ethanol (85:15 v/v) as mobile phase at a flow rate of 1.0 mL min^{-1} under UV photodiode-array detector detection. Then the effects of mobile phase and temperature on enantioselectivity 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 102.3 % with percentage relative standard deviation less than 1.17 %. The LOD and LOQ for first enantiomer were 13.7 and 46.3 μg mL^{-1} and for second enantiomer were 15.2 and 51.4 μg mL^{-1}, respectively. This method is expected to be accurate, stable, rapid and sensitive for determination of the enantiomeric purity of 2-piperidinemethanamine in bulk samples.

Key Words: Liquid chromatography, Enantiomeric purity, 2-Piperidinemethanamine, Pre-column derivation, Method validation.

INTRODUCTION

2-Piperidinemethanamine (Fig. 1) has one pair of enantiomers with an asymmetric center on the ring. It was found to be a versatile synthon for the preparation of drugs and bioactive compounds. For example, it was used for synthesis of diazaphospholo-iminophosphorane derivatives of zidovudine (AZT)[1], which displayed antioxidant activity. Hayati *et al.*[2] used it for synthesis of annulated N-heterocyclic carbene ligands. Moreover, 2-piperidinemethanamine can be used to synthesize 7-(2-aminomethyl-1-azetidinyl)-4-oxoquinoline-3-carboxylic acids[3], antiarrhythmic flecainide and its intermediates[4] and antibredt molecules[5].

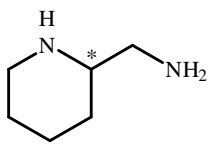

Fig. 1. Chemical structure of 2-piperidinemethanamine

The increasing demand for enantiopure drugs has led to the need for the synthesis of new enantiomerically pure compounds. The optically 2-piperidinemethanamine has been used for synthesis of platinum complexes of unsymmetrical alicyclic diamines[6], which displayed antitumor activities. So it is important to determine the enantiomeric purity of 2-piperidinemethanamine in pharmaceutical preparations. However, there was no resolution report for the enantiomers of 2-piperidinemethanamine. Thus an efficient and economic method is necessary to develop for precisely determination of enantiomeric excess (e.e. %) of the 2-piperidinemethanamine enantiomers. Among the available methods for the separation of enantiomers, the HPLC method with chiral stationary phases (CSPs) is more rapid and efficient in terms of resolution[7-9]. Experimental results indicated that 2-piperidinemethanamine can not be separated directly in the popular chiral stationary phases satisfactorily. So in this paper, it was pretreated with derivatization reagents and then the obtained derivatives were separated with chiral LC column.

EXPERIMENTAL

(*Rac*)-2-piperidinemethanamine (98 % of chemical purity) were purchased from Alfa Aesar (Tianjin, China). *n*-Hexane, ethanol and isopropanol of HPLC grade were supplied by Hangjia Chemical Co. Ltd. (Chengdu, China). Benzoic acid, 4-methoxybenzoic acid, 4-nitrobenzoic acid, 3,5-dinitrobenzoic acid and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Chengdu, China). Other reagents

supplied by Hangjia Chemical Co. Ltd. (Chengdu, China) were all in analytical level.

Analysis was carried out on a Shimadzu series liquid chromatography system, equipped with LC-20AT pump and SPD-M20A photodiode array detector (both from Shimadzu, Kyoto, Japan) and an Automatic Science (Tianjin, China) HCT-360 LC column cooler/heater. Chromatographic parameters such as peak areas, retention times, theoretical plates, *etc*., were calculated using the Class-VP workstation (Shimadzu, Kyoto, Japan).

Chromatographic conditions: Chiralcel OD-H (250 mm × 4.6 mm; particle size 5 μm) (Daicel, Japan), DNB-PG (250 mm × 4.6 mm; particle size 5 μm), Whelk-O1 (250 mm × 4.6 mm; particle size 5 μm) (Regis Technologies, USA) and Kromasil CHI-DMB (250 mm × 4.6 mm; particle size 5 μm) (Akzo Nobel, Sweden) were used for the separation. Mobile phase consisted of *n*-hexane: isopropanol or ethanol (85:15 or other v/v) and the column temperature was at 25 ºC. 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 *ca.* 5 μL. The sample solution was prepared by dissolving the sample in methanol at 200 μg mL^{-1}.

Derivatization procedure: Briefly, 1 mmol of acid (benzoic acid, 4-methoxybenzoic acid, 4-nitrobenzoic acid and 3,5-dinitrobenzoic acid) and 0.494 g of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) were added to 0.12 mL of (±)-2-piperidinemethanamine in 30 mL of dichlormethane. The derivation reaction route was shown in Fig. 2. The reaction mixture was stirred at 30 ºC for 4 h. After that the solvent was washed with 30 mL of 1 M HCl, 30 mL of 1 M NaOH and 20 mL of water, dried with anhydrous sodium sulphate. The mixture was filtered and the filtrate was evaporated to give white powders and used for LC investigation.

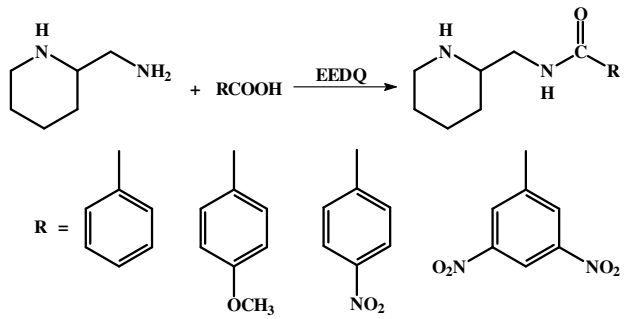

Fig. 2. Derivation reaction route of 2-piperidinemethanamine

Stability studies: Stabilities of the derivatives of 2-piperidinemethanamine were investigated under various conditions as follows. For the reaction under room temperature, the sample solution in a tightly capped volumetric flask was put on a laboratory bench at room temperature for two days. The derivative of 2-piperidinemethanamine content was checked at 6 h intervals during this period. For the thermolysis, the solution was heated at 65 ºC for 1 h. For the acidic and basic hydrolysis, individually 10 mg the derivative of 2-piperidinemethanamine were dissolved in 5 mL methanol in a 10 mL distillation flask and heated at 65 ºC for 1 h after adding: (a) 5 mL 0.1 M HCl for acidic hydrolysis, (b) 5 mL 0.1 M NaOH for basic hydrolysis. After that, the solvent was removed under reduced pressure and the residue was dissolved in methanol, washed with water and dried with anhydrous sodium sulphate.

RESULTS AND DISCUSSION

Derivatization conditions: The type of chiral stationary phases in the experiment is amide, so the amide derivatives were synthesized for investigation. Considering there is no strong ultraviolet absorption and π-π interaction group in the structure, aromatic acids were used as derivatizing agents in order to detect with UV detector conveniently. Moreover, larger molecule could be obtained after derivatization, the steric effect could enhance adsorption with CSP to improve chiral recognition. The derivatization reaction time for the derivative of 2-piperidinemethanamine was investigated as a key factor by integrating peak areas from reaction products taken during a time course of 0-240 min at 30 ºC. The results suggested that the amount of derivatives of 2-piperidinemethanamine enantiomers gradually increased as reaction proceeded towards 2 h. Reaction equilibrium nearly reached after *ca*. 2 h. So, 2 h for the derivatization was proposed.

Choice of the chiral stationary phases: The four samples of derivative of 2-piperidinemethanamine were used in the method development and four different chiral stationary phases were employed as follows: Chiralcel OD-H, CHI-DMB, Whelk-O1 and DNB-PG. The results indicated that only the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine could be separated on CHI-DMB. So, the separation, retention of the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine on CHI-DMB should be further investigated.

Effect of organic modifier: The type and concentration of organic modifier were found to influence the retention and resolution of the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine enantiomers dramatically. The selectivity and resolution of the enantiomers on CHI-DMB column were investigated when isopropanol and ethanol were used as modifiers, respectively (Table-1). Both organic modifiers have shown good selectivity for the enantiomers. However, ethanol has shown better selectivity than ethanol. On decreasing the concentration of organic modifier, the capacity factors as well as resolutions were increased. In ethanol case, sharp peaks with higher resolution and higher sensitivity (higher detections limits) were obtained. Thus, ethanol was chosen as an organic modifier at last. As a compromise between resolution and retention time, 15 % ethanol in *n*-hexane was found to be an optimum mobile phase for analysis purpose. The chromatograms of the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine using ethanol as organic modifier were shown in Figs. 3 and 4.

Effect of temperature: Temperature is an important factor to affect enantiomeric recognition processes[10,11]. The effects of column temperature on selectivity and resolution of the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine enantiomers were studied in the range 288-318 K (15-45 ºC). With the increasing of temperature, the retention decreased. These results could be attributed to the fact that the analytes on molecular level have lower adsorption as temperature increased and therefore migrates rapidly through the column[12]. According to the van't Hoff equation[12-15]:

TABLE-1
EFFECT OF ISOPROPANOL AND ETHANOL ON SELECTIVITY AND RESOLUTION OF THE 3,5-DINITROBENZOIC ACID DERIVATIVES OF 2-PIPERIDINEMETHANAMINE ENANTIOMERS

Mobile phase	k_1	k_2	t_1 (min)	t_2 (min)	α	R_s
n-Hexane: isopropanol (98:2)	9.636	17.089	33.013	56.149	1.773	6.400
n-Hexane: isopropanol (95:5)	5.756	9.606	20.971	32.920	1.677	5.098
n-Hexane: isopropanol (90:10)	2.010	3.293	9.344	13.324	1.647	2.338
n-Hexane: isopropanol (85:15)	1.610	2.611	8.102	11.207	1.627	2.181
n-hexane: isopropanol (80:20)	1.072	1.736	6.432	8.491	1.610	1.910
n-Hexane: isopropanol (75:25)	0.769	1.234	5.491	6.935	1.609	1.740
n-Hexane: ethanol (98:2)	7.577	13.052	26.624	43.616	1.720	6.425
n-Hexane: ethanol (95:5)	3.402	5.478	13.663	20.107	1.613	5.110
n-Hexane: ethanol (90:10)	1.625	2.598	8.149	11.168	1.609	3.494
n-Hexane: ethanol (85:15)	0.899	1.409	5.894	7.476	1.576	2.369
n-Hexane: ethanol (80:20)	0.668	1.045	5.179	6.347	1.565	1.945
n-Hexane: ethanol (75:25)	0.485	0.743	4.608	5.411	1.530	1.625

k_1: Capacity factor of the first enantiomer; k_2: capacity factor of the second enantiomer; t_1: retention time of the first enantiomer; t_2: retention time of the second enantiomer; α: separation factor; R_s: resolution; stationary phase: CHI-DMB; flow rate: 1.0 mL min^{-1}; column temperature: 25 ºC; UV detection wavelength: 254 nm.

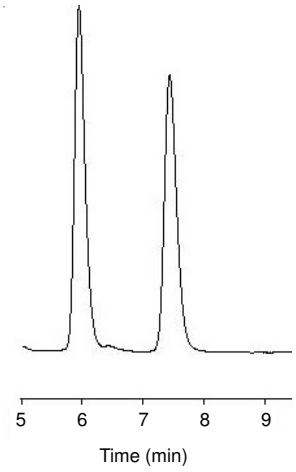

Fig. 3. Chromatograms obtained from the derivatives of 2-piperidinemethanamine enantiomers on CHI-DMB. Conditions: mobile phase, *n*-hexane:ethanol (85:15, v/v); flow rate, 1.0 mL min^{-1}; column temperature, 25 ºC; detection wavelength, 254 nm

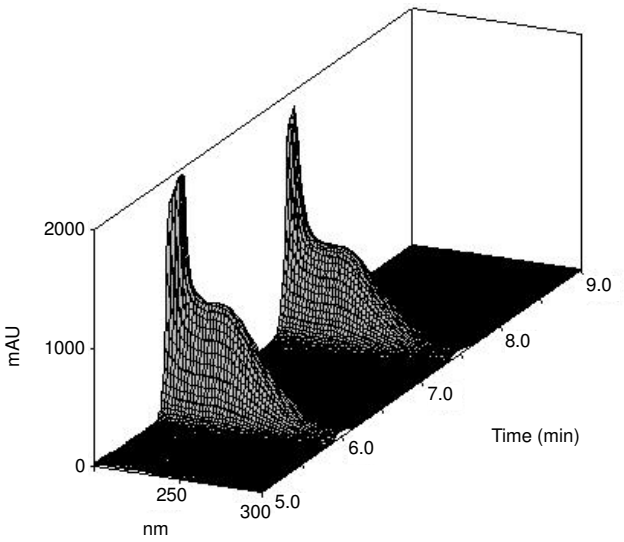

Fig. 4. Chromatogram of the derivatives of 2-piperidinemethanamine enantiomers detected by photodiode-array detector. Conditions: stationary phase: CHI-DMB; mobile phase: *n*-hexane:ethanol (85:15, v/v); flow rate: 1.0 mL min^{-1}; column temperature: 25 ºC; UV detection wavelength: 254 nm

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

where k is the retention factor, R is the gas constant and T is the absolute temperature in Kelvin, ΔH^o and ΔS^o are the molar enthalpy and molar entropy of absorption. 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 (Fig. 5). ΔH^θ and ΔS^θ for the two enantiomers were obtained 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

$$\Delta\Delta G^o = \Delta\Delta H^o - T\Delta\Delta S^o \quad (2)$$

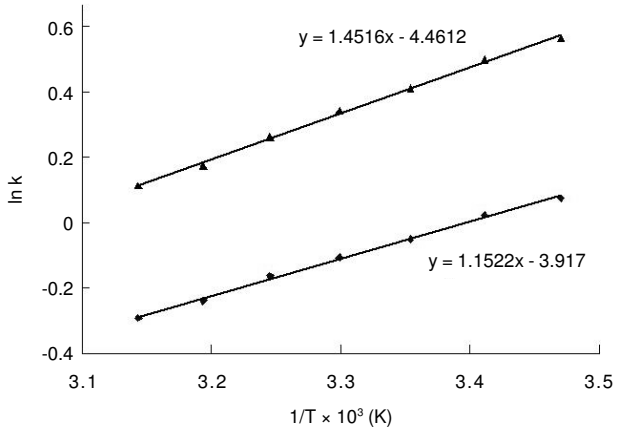

Fig. 5. Plot of ln k *versus* 1/T. Conditions: Mobile phase, *n*-hexane:ethanol (85:15, v/v); flow rate, 1.0 mL min^{-1}; UV detection wavelength, 254 nm

The apparent thermodynamic parameters for the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine enantioseparations were obtained with *n*-hexane:ethanol (85:15 v/v) as the mobile phase. The corresponding data were listed in Table-2, which proved that the processes of enantiomeric recognition were enthalpy-controlled in the study.

Effect of flow rate: The effect of flow rate on resolution of the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine enantiomers was investigated in the range of 0.5-1.5 mL min^{-1} (mobile phase was *n*-hexane:ethanol (85:15

TABLE-2
THERMODYNAMIC DATA CALCULATED FROM THE VAN'T HOFF PLOTS OF THE 3,5-DINITROBENZOIC ACID DERIVATIVES OF 2-PIPERIDINEMETHANAMINE ENANTIOMERS IN TEMPERATURE RANG 15-45 ºC

Enantioner	ΔH^o (kJ mol^{-1})	$\Delta\Delta H^o$ (kJ mol^{-1})	ΔS^o (J K^{-1} mol^{-1})	$\Delta\Delta S^o$ (J K^{-1} mol^{-1})	$\Delta\Delta G^o$ (kJ mol^{-1})
First enantiomer	-9.579	-2.489	-32.566	-4.524	-1.141 (298 K)
Second enantiomer	-12.068		-37.090		

v/v) and column temperature was 25 ºC). The results were shown in Table-3. On increasing the flow rate, the separation factor increased but the resolution decreased. On the comprehensive consideration of separation factor and resolution, 1.0 mL min^{-1} was chosen as the optimal flow rate.

TABLE-3
EFFECT OF FLOW RATES ON ENANTIOSELECTIVITY

Flow rate (mL min^{-1})	k_1	k_2	α	R_s
0.5	2.773	3.701	1.335	2.526
0.8	1.361	1.938	1.424	2.389
1.0	0.899	1.409	1.567	2.361
1.2	0.574	0.956	1.666	2.120
1.5	0.261	0.567	2.170	2.041

k_1: Capacity factor of the first enantiomer; k_2: capacity factor of the second enantiomer; stationary phase: CHI-DMB; column temperature: 25 ºC; mobile phase: *n*-hexane: ethanol (85:15, v/v); UV detection wavelength: 254 nm.

Validation of HPLC method

Precision: Precision of the method was tested with preparing five individual solutions of the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine and making triplicate injections for each solution under the working conditions. The RSD % of the assay was less than 1.13 %. Inter and intra-day assay precisions were performed with analyzing the solutions for five times in a day for 3 days. The RSD % of the assay was less than 1.28 % for both the isomers.

Accuracy: Accuracy studies were performed with spiking the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine solution at six levels with respect to specified level and analyzing each solution in triplicate (n = 3) for 3 days. The recoveries were between 99.1 and 102.3 % with percentage relative standard deviation less than 1.17 %.

Linearity: Good linearity was observed for the first enantiomer and the second enantiomer in the concentration range of 30-2000 µg mL^{-1}. The curves were linear with $r_1^2 = 0.9997$ and $r_2^2 = 0.9996$ and the regression equations for the first enantiomer and the second enantiomer were $y_1 = 1661.3x_1 + 2417.1$ and $y_2 = 1643.7x_2 + 3756.7$, respectively. Linearity was checked over the same concentration ranges for three consecutive days.

Limit of detection (LOD) and limit of quantitation (LOQ): LOD and 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 13.7 and 15.2 µg mL^{-1} for the first enantiomer and the second enantiomer, respectively. LOQ was found to be 46.3 and 51.4 µg mL^{-1} for the the first enantiomer and the second enantiomer, respectively.

Robustness: Robustness of the method was studied with making small deliberate changes in the method parameters. A variation of 2 % of ethanol in the composition of the mobile phase hardly affected the resolution except that retentions were changed. The effect of temperature was studied with analyzing sample at 25 ± 2 ºC. Again retention times varied in the range of 1 min but the resolution remained above 2.0. The effect of flow rate was studied with analyzing the samples in 0.8 and 1.2 mL min^{-1} flow rates. In the both cases the resolution was found to be above 2.0.

Stability studies: The results of room temperature study showed that the RSD of the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine content during experiment was 1.34 %. Hence the solution was stable for at least 48 h. When the sample solution was heated at the boiling point of methanol, no degradants were observed in the chromatogram. However, no peak corresponding to the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine was detected which suggested the the 3,5-dinitrobenzoic acid derivative of 2-piperidinemethanamine was hydrolyzed when treated with acid and base.

Conclusion

A validated pre-column derivatisation HPLC method was developed for the enantiomeric separation and purity determination of 2-piperidinemethanamine. Under optimum derivatization reaction and chromatographic conditions, the enantiomers were well separated on CHI-DMB column. The effects of organic modifiers and temperature on resolution and retention of enantiomers have been investigated to optimize the HPLC conditions. The method was completely validated and shown satisfactory results for all the method validation parameters (precision, accuracy, linearity, LOD, LOQ, robustness and stability studies) tested. The method was proved to be useful for quantitative analysis of enantiomeric purity of the 2-piperidinemethanamine in bulk samples.

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