

HPLC separation of enantiomeric cathine hydrochloride using a chiral stationary phase

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ABSTRACT

A new chiral liquid chromatographic method was developed for the enantiomeric separation of Cathine Hydrochloride with UV detector. The enantiomers of cathine were baseline resolved on Chiral pack AD-H (250mm X4.6mm, 5 μ m) column using a mobile phase system containing Heptane:Ethanol: Diethylamine (920:80:1 v/v). The presence of Diethylamine in the mobile phase has played an important role in enhancing the chromatographic efficiency and resolution between enantiomers.

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KEYWORDS

Enantiomeric separation;
Chiral;
Cathine hydrochloride;
Resolution.

INTRODUCTION

Cathine, also referred to as norpseudoephedrine, is a sympathomimetic drug. It, as well as its related compounds Phenylephrine, ephedrine, norephedrine and pseudoephedrine, are adrenergic agents widely used in the treatment of asthma, ophthalmia, colds and allergies.

The structure of Cathine contains two asymmetric centers. The compounds of interest are a pair of enantiomers: 1*R*,2*S*-(-)-Cathine and 1*S*,2*R*-(+)-Cathine. Besides hydrogen, a hydroxyl group and a benzene ring are bound onto C₁ of Cathine, while C₂ has a methyl and an amino group.

Resolution of the enantiomers is carried out by chromatography of the racemic mixture on a chiral stationary phase (CSP), which commonly includes crown ether and several types of derivatives of cellulose and amylose. Stationary phases containing crown ether are particularly useful for the chiral resolution of amino

containing compounds and their derivatives^[1-3], and carbamates and benzoates of polysaccharides such as cellulose and amylose are effective and very popular for the optical resolution of racemic drugs^[4-7]. Also frequently used as CSPs are cyclodextrin^[8] and immobilized proteins such as bovine serum albumin^[9] and α -acid glycoprotein^[10], ion-pair chromatographic method for the separation of a racemic mixture of various drugs including β -blockers^[11].

Few reports have been published on the chromatographic resolution of the enantiomers of these adrenergic agents. Norpseudoephedrine (Cathine) has recently been imprinted and the resultant MIP was used for enantiomer separation in chromatography (TLC) mode^[12]. Phenylephrine, phenylpropanolamine, Ephedrine and pseudoephedrine have also been imprinted and the resultant MIP was used for liquid chromatography of enantiomer separation^[13]

In this article, we report on the development of an HPLC method to detect cathine enantiomers using a

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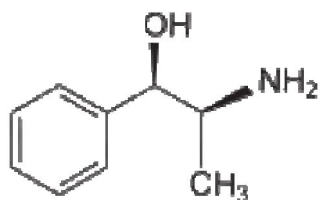


Figure 1 : (1S,2S)-2-amino-1-phenylpropan-1-ol, CAS number 492-39-7, Chemical formula : $C_9H_{13}NO$, Mol. mass: 151.206 g/mol.

Chiral AD-H column that has a cellulose carbamate derivative as CSP. The effect of organic modifiers and temperature on the resolution and retention of cathine enantiomers has been studied and the mobile-phase composition has been optimized.

EXPERIMENTAL

Materials and methods

D-Cathine hydrochloride and L-Cathine hydrochloride were synthesized by Medicinal Chemistry group. Both the compounds were characterized for their identity and purity, their enantiomeric purity was monitored by specific rotation using Polarimeter and were purified till equal opposite values for specific rotation were obtained. HPLC grade n-Heptane and Analytical reagent grade Absolute Ethanol and Diethylamine were obtained from Merck, India.

HPLC system used was Agilent 1200 series system comprised of degasser, quaternary pump, auto injector, and column compartment and variable wavelength detector. The system was controlled through EZ Chrome Elite version 3.2.1.

Preparation of solutions and chromatographic conditions

(A) Standard solution

(a) Impurity stock solution (ISS-1)

Transfer about 10mg of D-cathine hydrochloride Standard, accurately weighed to a 100 ml volumetric flask and add 5ml of ethyl alcohol to dissolve and sonicate for 2 to 5 minutes. Dilute to volume with ethyl alcohol and mix well.

(b) Impurity stock solution (ISS-2)

Transfer about 100 mg of L-Cathine hydrochloride

Standard, to a 10 ml volumetric flask and add 5ml of ethyl alcohol to dissolve (sonicate if required). Dilute to volume with ethyl alcohol and mix well.

(B) System suitability solution

Transfer 10mL of ISS-2 and 1 ml of ISS-1 into a 100mL volumetric flask, dilute to volume with n-Heptane and mix well.

(C) Test solution

Weigh accurately about 100mg of the test substance to be examined to a 10ml volumetric flask, add 5ml of ethyl alcohol to dissolve and dilute to volume with ethyl alcohol and mix. Transfer 1ml of this solution into a 10ml volumetric flask, dilute to volume with n-Heptane and mix.

Liquid chromatography

The chromatographic column used was a Chiralpak AD-H 4.6 mm X 250 mm with 5 μ particle size. Mobile phase consist of a mixture 920ml of n-Heptane, 80 ml of Ethyl alcohol and 1.0 ml of Diethylamine and degas by sonication for 5 minutes. Flow rate of mobile phase was 1.0 ml/min. Column was maintained at 25°C and column eluent was monitored at 220nm. Injection volume was 50 μ l.

Method performance criteria were resolution (R) between two enantiomer peaks should be not less than 2.0

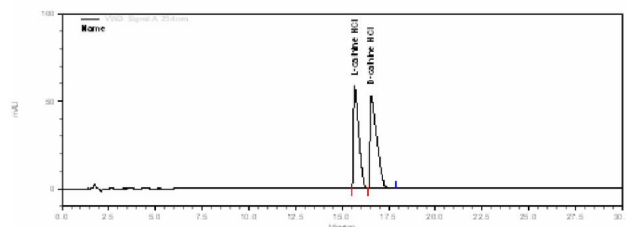
RESULT AND DISCUSSION

Mobile phase optimization

It was well known that the resolution of enantiomers based on a CSPs column were closely associated with the column, mobile phase additives. First, two commonly used CSPs columns, such as chiral pack AD-H and chiral CD-Ph, were applied to separate the cathine. Typical mobile phases for CD-Ph β -cyclodextrin, such as acetonitrile-water, methanol-water, hexane-ethanol and heptanes-2-propanol, were used for tests. No indication of separation was found, even upon addition of some acid or base additives. The commonly used mobile-phase composite for the chiral pack AD-H column, such as heptane-ethanol and heptane-2-propanol, was applied to the enantiomeric separation of cathine. There was no indication that the separation

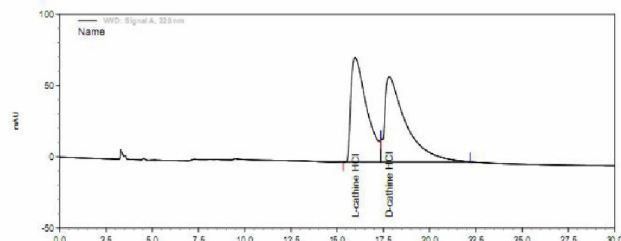
was achieved using different ratios of heptane-ethanol or heptane-2-propanol. However, after the addition of a small quantity of diethylamine as a modifier, limited separation was observed using different ratios of heptane-ethanol or heptane-2-propanol. The Resolution (RS) were increased for the amphoteric character of Diethylamine (DEA), a common additive, was often

n-Heptane 920:Ethanol 80:Diethylamine 0.1mL (a)



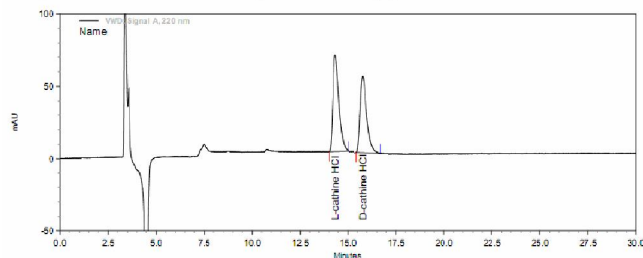
The resolution between D and L Cathine HCl is 0.9

n-Heptane 920: Ethanol 80: Diethylamine 0.2mL (b)



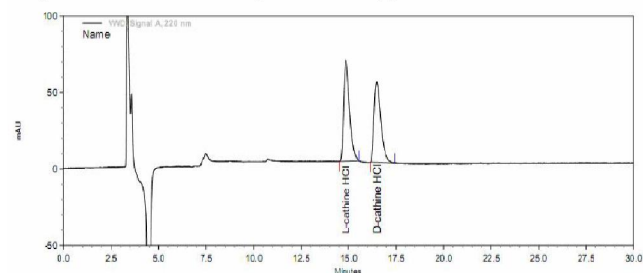
The resolution between D and L Cathine HCl is 1.2

n-Heptane 920: Ethanol 80: Diethylamine 0.5mL (c)



The resolution between D and L Cathine HCl is 2.06

n-Heptane 920: Ethanol 80: Diethylamine 1.0mL (d)



The resolution between D and L Cathine HCl is 2.60

Figure 2 : Effect of Diethylamine on chromatographic separation of cathine Hydrochloride enantiomers using different mobile-phase system, (a) Heptane-ethanol-diethylamine, 92/8/0.1 v/v (b) Heptane-ethanol-diethylamine, 92/8/0.2 v/v (c) Heptane-ethanol-diethylamine, 92/8/0.5 v/v (d) Heptane-ethanol-diethylamine, 92/8/1 v/v

added to the mobile phase in which the analytes contained an amino basic function, but the total content of the additives remained below 2.0% as the instruction manual recommended. When Diethylamine was added, the peak tailing was reduced and RS was enhanced. With reducing the analytical time (Figure 2). Based on the above considerations, mobile phase system containing different ratios of heptane-ethanol-diethylamine and heptane-2-propanol- Diethylamine were investigated. The values of RS indicated that baseline separation ($RS > 2.0$) was obtained using a mobile phase system of heptane-ethanol-diethylamine (92/8/1, v/v). The effect of the column temperature on the resolution and the retention of cathine enantiomers were also studied in the range 25-40°C on chiral pack AD-H column. The selectivity (α) and RS were increased with decreasing of the column temperature at 25°C an acceptable RS value (2.6) with complete separation was achieved with the mobile phase consisting of heptane-ethanol-diethylamine (92/8/1, v/v) (Figure 2 (d)).

Precision, linearity, LOQ and LOD

The precision of the method was studied for instrumental repeatability ($n=6$) and intermediate precision ($n=18$). The results were expressed in terms of the relative standard deviation (RSD) values for the peak area. For instrumental repeatability, the RSD values for the (S)- and (R)-enantiomers were found to be below 4.0 and 5.0%, respectively intermediate precisions for two enantiomers were found to be below 3.5 and 8.0%, respectively. There results indicate a good precision.

The detector response linearity was assessed by preparing six calibration sample solution of (S)-enantiomer and (R)-enantiomer covering from 20 μ g/mL to 1.0 μ g/mL (Figure 3- 10% to 200% with respect to working concentration 10 μ g/mL of (S)-enantiomer and (R)-enantiomer), prepared in the mobile phase from the stock solution. The determination coefficients (r^2) of the plots obtained for both cathine enantiomers (>0.99) demonstrated a good linearity. The RSD value for the slop of the standard calibration curves of (R)-Cathine and (S)-cathine ($n=5$) were 2.9 and 5.7% respectively.

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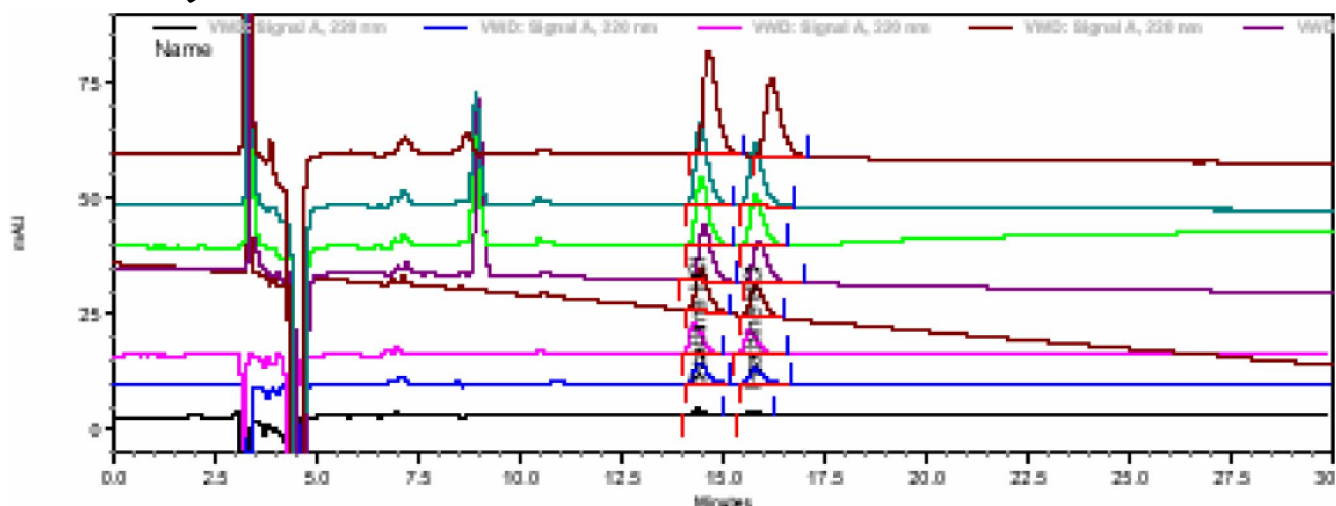


Figure 3 : Linearity chromatograms (10% to 200% with respect to working concentration 10µg/mL of (S)-enantiomer and (R)-enantiomer)

The limit of detection (LOD) and limit of quantification (LOQ) were both considered to be the concentrations that produced signal-to-noise ratios of 3 and 10, respectively. They were determined

they were 1.0µg/mL for two enantiomers (Figure 4).

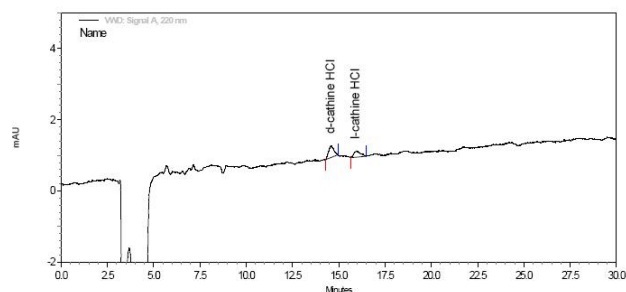
CONCLUSIONS

In the present work, a chiral HPLC separation was developed for the determination of cathine enantiomers, without derivatization, using a cellulose-based stationary phase (Chiral pack AD-H column) in the normal-phase mode. The obtained separation offered good resolution for both enantiomers ($RS=2.60$) with an analytical time of 30 min, using a mobile phase composition, heptane-ethanol-diethylamine (92/8/1.0, v/v) at a flow rate of 1.0 ml/min with a temperature column of 25°C. The method showed good performance regarding the linearity and instrument repeatability

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LOD - 0.3µg/mL for (R)-cathine and (S)-cathine



LOQ were 1.0µg/mL for (R)-cathine and (S)-cathine

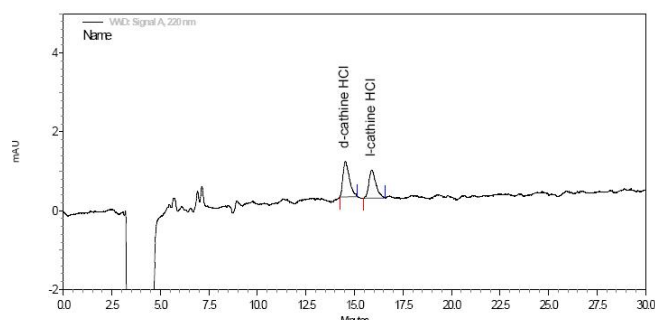


Figure 4 : LOD and LOQ chromatograms (0.3µg/mL and 1.0 µg/mL for (R)-cathine and (S)-cathine)

from linear regression by extrapolation of the signal-to-noise ratio as a function of the concentration. The values obtained for the LOD were 0.3µg/mL for (R)-cathine and (S)-cathine, respectively, and for LOQ

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