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Research Article

Synthesis of (1R, 4S)-4-Aminocyclopent-2-en-yl Methanol HCL– An Undesired Isomer of Key Starting Material in the Synthesis of Abacavir Sulphate and Development of Validated Chiral HPLC Method for its Estimation

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ABSTRACT

The Preparative Chiral Separation with Crown ether based Chiral Stationary Phases was utilized for resolving racemic Vince Lactam compound into the desired (-) Lactam and the undesired (+) Lactam. After the resolution of Vince Lactam, (+) Lactam was protected using Di-tert-butyl dicarbonate to form carbonate ester. This carbonate ester then reacts with amines to give tert-butyl (1S, 4R)-3-oxo-2-azabyclo [2.2.1] hept-5-ene-2-carboxylate. Further this protected Lactam is reduced with Sodium Borohydride, to form tert-butyl [(1S, 4R)-4-hydroxymethyl) cyclopent-2-en-1-yl]carbamate. This amide compound is further hydrolyzed to deprotect the (di-tert-butyl dicarbonate) BOC protected amino group using dilute hydrochloric acid to form an undesired isomer of amino alcohol compound - (1R, 4S)-4-Amino cyclopent-2-en-1-yl Methanol HCl. The reverse phase Enantioselective High Performance Liquid Chrsomatography method was developed for the separation of (1R, 4S)-4-Aminocyclopent-2-en-1-yl Methanol HCl and desired isomer (1S,4R) -4-Aminocyclopent-2-en-1-yl Methanol HCl, a key raw material used in the manufacturing processes of Abacavir Sulphate. The enantiomers of 4-Aminocyclopent-2-en-1-yl Methanol were resolved on Daicel Crown pack (+) (15cm x 4.0mm, 5µ) column using a mobile phase 50mM Sodium Perchlorate and pH of the solution was adjusted to 2.0 with perchloric acid. The resolution between the enantiomers was found to be more than 2.0.The method was validated as per International Conference of Harmonization (ICH) guidelines in terms of Limit of detection (LOD), Limit of quantitation (LOQ), linearity, precision, accuracy, specificity, robustness and solution stability. The LOD and LOQ values were found to be 0.6µg/ml and 2.0µg/ml, respectively for 2.5µL injection volume. The sample concentration injected was 5mg/ml. The method is linear within the range of 2.0-7.5µg/ml for (1R, 4S) Isomer.

Keywords: (1R, 4S) -4-Aminocyclopent-2-en-1-yl Methanol HCl; (1S, 4R) -4-Aminocyclopent-2-en-1-yl Methanol HCl; Abacavir sulphate; HPLC ; Chiral Separation.

1. INTRODUCTION

Enantiomers of racemic drugs often show different behavior in pharmacological action and metabolic process. It is not uncommon for one enantiomer to be active while other isomer is toxic in biological system. The pharmaceutical industry has raised its emphasis on the generation of enantiomerically pure compounds before undertaking pharmacokinetic, metabolic, physiological and toxicological evaluation in the search for drugs with greater therapeutic benefits and low toxicity.[1,2] Nowadays, chiral separation plays an important role for the analysis of single Enantiomers in the field of pharmaceutical industry[3]. However, the development of the method for the quantitative analysis of chiral compound and for the assessment of enantiomeric purity is extremely challenging, because the same physical and chemical properties of the two enantiomers makes discrimination and separation very difficult [4].

(1S, 4R)-4-Aminocyclopent-2-en-1-yl Methanol HCl was used in the manufacturing process of Abacavir Sulphate. Abacavir is an Active Pharmaceutical Ingredient. The chemical name of Abacavir is {(1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-

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9-yl] cyclopent-2-en-1-yl} methanol. The Enantiomers of Abacavir Sulphate with the 1S, 4R absolute configuration on the cyclopentene ring are used for the treatment of HIV.

The undesired isomer (1R, 4S) of Abacavir Sulphate is possible if the (1R, 4S)-4-Aminocyclopent-2-en-1-yl Methanol HCL isomer is present as an isomeric impurity in (1S, 4R)-4-Aminocyclopent-2-en-1-yl Methanol HCL.

Hence, in current research work a sensitive, precise, accurate, rugged chiral HPLC method is developed to control the undesired isomer (1R, 4S)-4-Amino-2-cyclopentene-1-methanol HCl in the desired isomer (1S, 4R)-4-Amino-2-cyclopentene-1-methanol HCl[5]. This approach of controlling the undesired isomeric impurity in n-1, n-2 stage is approved and recommended by regulatory bodies like USFDA.

As a result of this, the enantiomer (1R, 4S) isomer of Abacavir will not be observed, since it's origin from (1R, 4S)-4-Amino-2-cyclopentene-1-methanol HCl is controlled in (1S, 4R)-4-Amino-2-cyclopentene-1-methanol HCl. This will also eliminate the purification required in the final stage of (1R, 4S) Abacavir thereby increasing the yield by 5- 10% and eliminating the use of complex chiral reagents required for purification.

Although much more enantiomeric methods have been reported for separation and quantification of Abacavir sulphate using HPLC method, no literature is available for enantiomeric separation of (1S, 4R)-4-Amino-2-cyclopentene-1-methanol.HCl.

2. MATERIALS AND METHODS

2.1 Chemicals

Racemic mixture of Vince Lactam, HPLC grade sodium perchlorate monohydrate and perchloric acid (70%), Di-tert-butyl dicarbonate , Sodium borohydride and Hydrochloric acid was purchased from Sigma Aldrich. Millipore HPLC grade water was used for Mobile phase preparation along with Crown pack ether column and Methanol. Chemical structure of (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol.HCl (Fig.1).

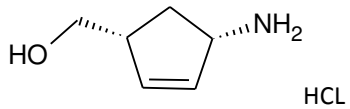


Fig. 1: (1R, 4S)-4-Aminocyclopent-2-en-1yl Methanol. HCL

2.2 Synthesis of (1R, 4S)-4-Aminocyclopent-2-en-1-yl Methanol. HCL

The newly developed column for Preparative Chiral Separation with Crown ether based chiral Stationary Phases, containing a chiral crown ether as a chiral selector coated onto a 5µm silica Support was used for the resolution of 20g of Vince Lactam compound into the desired (-) Lactam (5g) and undesired (+) Lactam (5g).

These columns are the reference columns for achieving amino acid separations, with the advantage that the elution order of the enantiomer can be reversed when necessary (CR(-) column gives the reversed elution order compared to CR(+) column). Acidic mobile phases of Sodium perchlorate and Perchloric acid pH around 3, was used to operate the column under standard conditions and water was used for sample preparation. Methanol was also used to shorten the retention time. After the resolution of Vince Lactam, 5g of (+) Lactam was protected using 12.6g Di-tert-butyl dicarbonate, which is a reagent widely used in organic synthesis.[6,7]

This carbonate ester reacts with amines to give 8g of tert-butyl (1S, 4R)-3-oxo-2-azabyclo[2.2.1]hept-5-ene-2-carboxylate (i.e Protected Lactam) or so-called BOC derivatives. These derivatives do not behave as amines, which allow certain subsequent transformations to occur that would have otherwise affected the amine functional group. The BOC can later be removed from the amine using acids. "Thus, BOC serves as a protective group", for instance in solid phase peptide synthesis. It is unreactive to most bases and nucleophiles. Further this 8gm of protected Lactam is reduced with 1.7g Sodium borohydride, to form 7g of tert-butyl [(1S, 4R)-4-hydroxymethyl)cyclopent-2-en-1-yl]carbamate which is more selective, or a stronger reductant. This compound, obtained by reduction of the amide is further hydrolyzed to deprotect the BOC protected amino group using dilute hydrochloric acid to yield the amino alcohol compound i.e (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol. HCl. (3gm) (Fig.2, 3)[8-11].

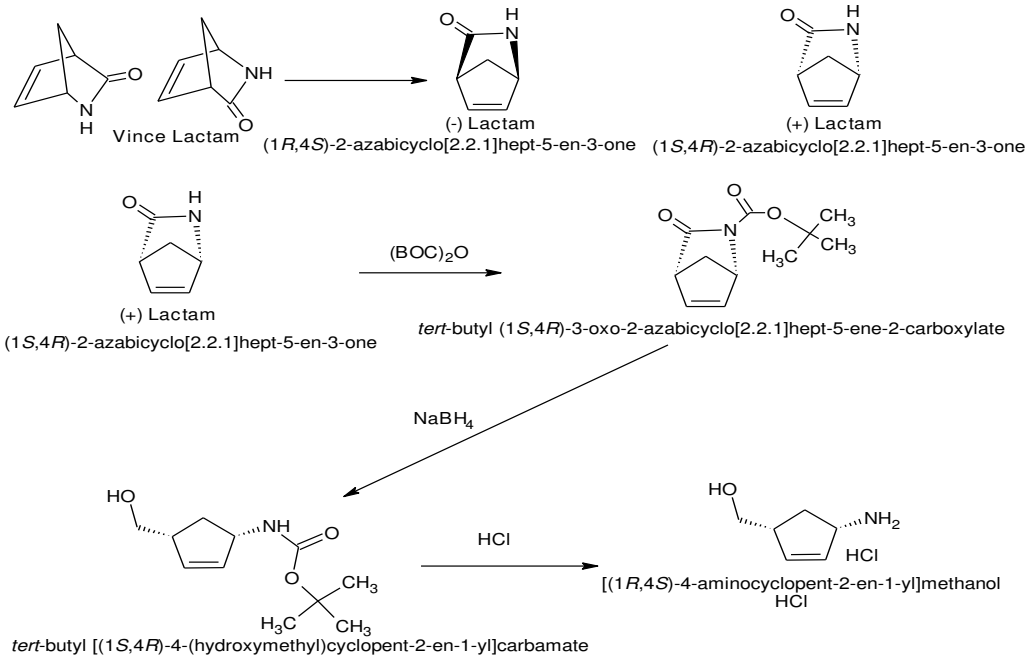
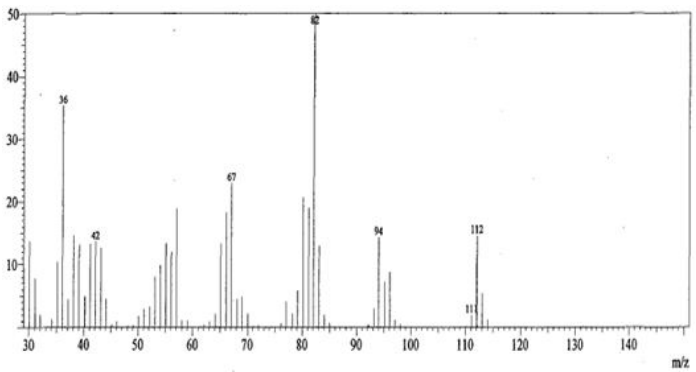
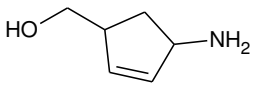


Fig. 2: Reaction scheme for (1R, 4S)-4-Aminocyclopent-2-en-1-yl Methanol. HCL

Hence, $NaBH_4$ was tuned to reduce the lactam function. Some of these reductions occur by virtue of the in-situ generation of boranes from $NaBH_4$. Amides and lactams are not easily reduced to their corresponding amine using chemical hydrides. Moreover, reduction can yield the deoxygenated amine or the corresponding alcohol (+) amine (from amide cleavage).





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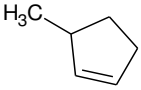

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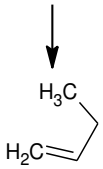

Formula weight 56

Fig.3: GCMS data and fragmentation pattern for (1R, 4S)-4-Aminocyclopent-2-en-1-yl Methanol. HCL

2.3 Instrumentation /Chromatographic conditions

Agilent HPLC 1100 series instrument equipped with column oven, PDA detector was used for analysis and the data was processed using computer program chemstation software. The Chromatographic conditions were optimized using a chiral stationary, Crown Pack CR (+) Column (15cm x 4.0mm, 5µ). The isocratic mobile phase 50mM Sodium Perchlorate (pH adjusted to 2 with perchloric acid), was pumped at a flow rate of 0.25 ml/min. The temperature of the column was maintained at 10°C and the wavelength selected was 200 nm. The injection volume was 2.5 µl.

2.4 Sample Preparation

Stock solution of (1S, 4R)-4-Aminocyclopent-2-en-1-yl methanol.HCl was prepared by dissolving an appropriate amount of the substances in the Mobile phase. The analyte concentration was fixed as 5mg/ml. (1S, 4R)-4- Aminocyclopent-

2-en-1-yl methanol. HCl solution spiked with low levels of the undesired isomer (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol. HCl. Spiked solution was prepared by transferring calculated amount of undesired isomer in (1S, 4R)-4-Aminocyclopent-2-en-1-yl methanol HCl stock solution.

2.5 Calibration standard

Stock racemic mixture was prepared by dissolving an appropriate amount of desired and undesired isomer in the mobile phase to give a final concentration of 2.5mg/ml. Standard racemic solutions were prepared by subsequent dilutions to form 5.0µg/ml.

2.6 Validation of the method

2.6.1. System suitability

A system suitability test should be carried out to determine to see if the operating system is performing properly. System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out. These tests are based on the concept that equipment, electronics, analytical operations and samples to be analyzed constitute an integral system, which can be evaluated as such.

System suitability tests were performed in accordance with ICH guidelines to confirm the reproducibility of the equipment was adequate for the analysis to be performed. System suitability was performed by injecting system suitability solution and determining resolution between undesired isomer (1R, 4S) 4-Amino-2-cyclopentene-1-methanol and desired isomer (1S, 4R) 4-Amino-2-cyclopentene-1-methanol. Also, the % RSD of peak responses of (1R, 4S) 4-Amino-2-cyclopentene-1-methanol and (1S, 4R) 4-Amino-2-cyclopentene-1-methanol in diluted standard solution (less than 5%).

2.6.2. Specificity

The specificity of the method was performed by injecting both the isomers, desired and undesired isomer individually. The system suitability of the method was performed by adding an equal concentration of the undesired and desired isomer (5.0µg/ml). The system suitability was confirmed by using resolution and tailing factor. (Fig.4, 5, 7) Table 1.

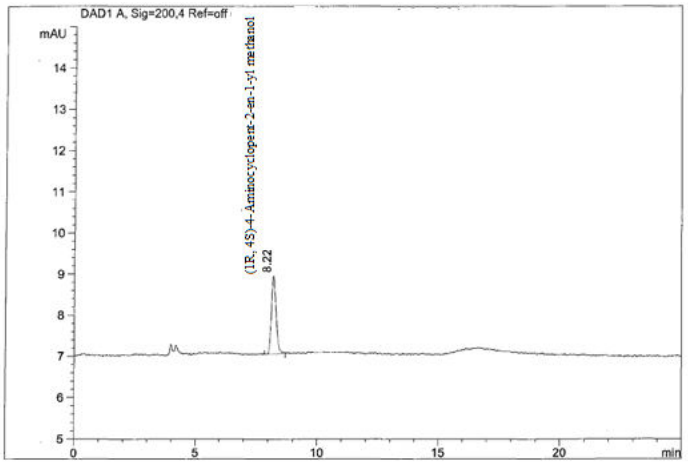

Fig. 4: HPLC Chromatographic of (1R,4S)-4-Aminocyclopent-2-en-1-yl Methanol. HCL

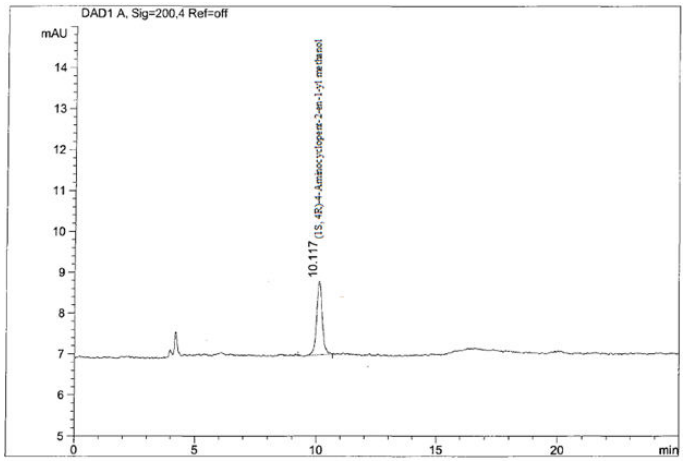

Fig.5: HPLC Chromatographic of (1S,4R)-4-Aminocyclopent-2-en-1-yl Methanol. HCL

Table 1: System suitability

Sr. No	Parameter	Limit	Value
1	Resolution between (1R, 4S)-4-Aminocyclopent-2-en-1-yl Methanol and (1S, 4R)-4-Aminocyclopent-2-en-1-yl Methanol	Not less than 2.0	4.2
2	(1R, 4S)-4-Aminocyclopent-2-en-1-yl Methanol (Tailing factor)	Not more than 2.0	1.06
3	(1S, 4R)-4-Aminocyclopent-2-en-1-yl Methanol (Tailing Factor)	Not more than 2.0	0.96

2.6.3. Precision

Method precision was determined by measuring repeatability and intermediate precision of retention times and peak areas for each isomer. The repeatability of the method was determined by analyzing six replicate injections of the sample (1S, 4R)-4-Aminocyclopent-2-en-1-yl methanol. HCl (5mg/ml) spiked with (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol. HCl (5µg/ml).

2.6.4. Linearity

A linearity calibration curve was constructed using four series of mixture of the desired and undesired isomer concentration level in a range from 2-7.5 µg/ml in diluent. The regression

coefficient was obtained by plotting peak area versus concentration (Fig.6) (Table 2).

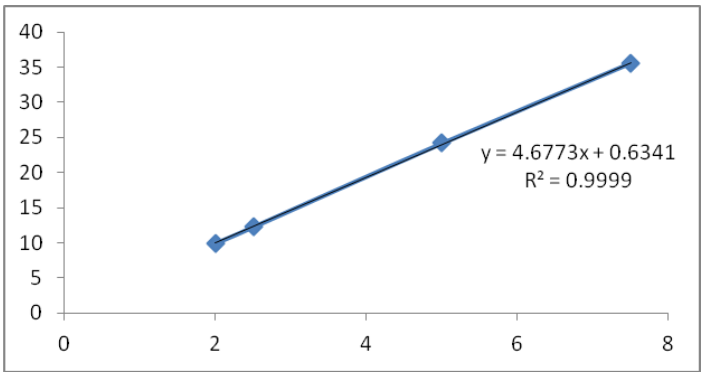

Fig.6: Linearity calibration curve of (1R, 4S)-4-Aminocyclopent-2-en-1-yl Methanol. HCL

Table 2: Linearity data

Parameter	(1R, 4S)-4-Aminocyclopent-2-en-1yl-Methanol
Linearity	2-7.7µg/ml
Regression coefficient	0.999
Y Intercept	2.62

2.6.5. Accuracy

The accuracy of the method was performed by injecting a known concentration of (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol. HCl to the (1S, 4R)-4-Amino-2-cyclopentene-1-methanol HCl. The accuracy was calculated in the range of 2-7.5 µg/ml in diluent (Table 3).

Table 3: Accuracy Table for (1R, 4S)-4-Aminocyclopent-2en-1yl Methanol. HCL

Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)
2.0	1.98	99.00
2.5	2.47	98.80
5.0	4.98	99.60
7.5	7.45	99.33

2.6.6. Solution stability

A solution of CAA HCl containing impurities was prepared and kept at room temperature. This solution was injected at intervals of 0, 4, 8, 12, 16, 20 and 24hrss. Area of (1R, 4S) 4-Amino-2-cyclopentene-1-methanol were nearly identical to that obtained at 0hrs and additional peaks were not observed which indicate solution stability.

2.6.7. Limit of detection

The sensitivity for detection can be demonstrated by determining the limit of detection (LOD). A signal to noise (S/N) ratio between 3 to 10 is generally considered to be acceptable for the estimation of the detection limit. S/N ratios of the individual peaks were determined at different concentrations to estimate LOD and respective %RSD was calculated for replicate injections (n=3). The LOD was found to be 0.012% for (1R, 4S) 4-Amino-2-cyclopentene-1-methanol. The results are shown in the Table 4.

Table 4: LOD and LOQ results of Impurities

Compound Name	LOD (mg/ml)	%RSD	LOQ (mg/ml)	%RSD
(1R, 4S) 4-Amino-2-cyclopentene-1-methanol	0.6µg/ml	NA	2.0µg/ml	3.05

2.6.8 Limit of quantification

The quantification limit is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. A typical S/N ratio of 10-30 is generally considered to be acceptable for estimating the limit of quantification. S/N ratios of the individual peaks were determined at different concentrations to estimate the limit of quantification (LOQ) and the respective %RSD was calculated for the replicate injection (n=6). The LOQ was determined to be 0.045%. The results are shown in Table 4.

3. RESULTS AND DISSCUSSION

3.1 Optimization of Chromatographic Condition

Racemic mixture solutions of (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol HCl and (1S, 4R) 4-Amino-2-cyclopentene-1-methanol (5 mg/ml each) prepared in mobile phase were used for method development. A number of column chemistries supplied by different manufacturers and different mobile phase composition were tried, in order to to get good separation and peak shapes between (1R, 4S) and (1S, 4R) isomer. Poor peak shape and resolution was observed when normal phase with AD-H and OD-H column was used. By using another mixture of Mobile phase (reverse phase) water: Acetonitrile: methanol and column Daicel Chiralpack IA, Daicel Chiralpack IB and Daicel chiralpack IC, (1R, 4S) eluted in close proximity to (1S, 4R). Good peak shape and resolution was achieved using 50mM Sodium perchlorate monohydrate (pH adjusted to 2.0 with perchloric acid) on crown pack CR (+) column (15cm x 4.0mm, 5µ). (Fig. 7, 8)

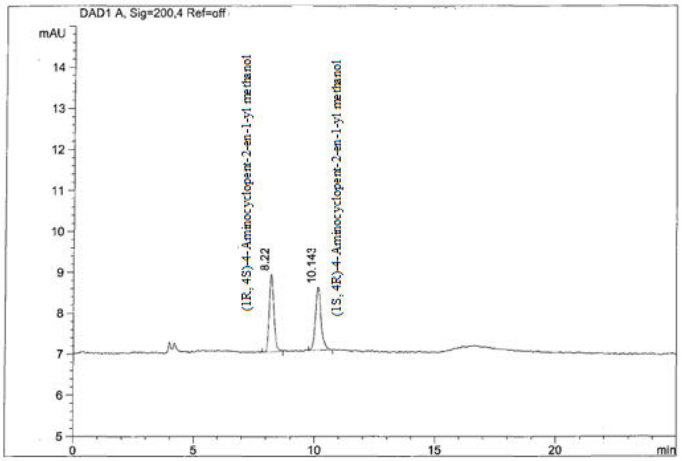

Fig.7: Resolution Chromatographic (1R, 4S))-4-Aminocyclopent-2-en-1-yl methanol and (1S, 4R))-4-Aminocyclopent-2-en-1-yl methanol Isomer of 4-Amino-2-cyclopentene-1-methanol

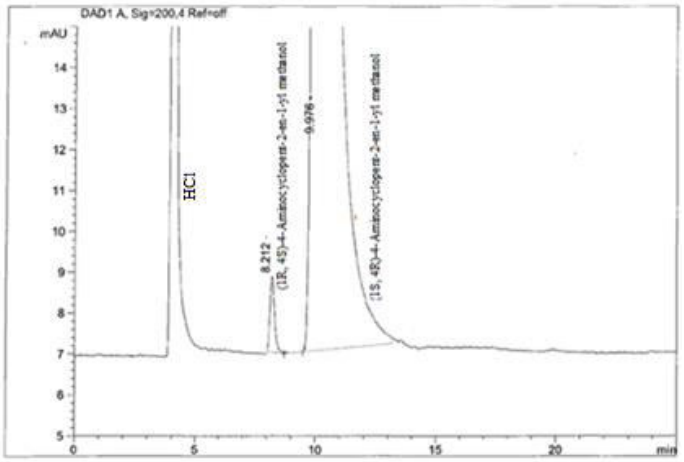

Fig. 8: Sample Chromatographic (1S, 4R)-4-Aminocyclopent-2-en-1-yl methanol Spiked with (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol

3.2 Validation Results of the method

The HPLC condition of the final method was evaluated for its specificity, LOD, LOQ, linearity, accuracy, precision, robustness and stability. The specificity of the method was determined by injecting the undesired isomer and peak purity.

The LOD and LOQ concentrations were estimated as 0.6 µg/ml and 2.0 µg/ml for (1R, 4S) Isomer. Method precision for (1R, 4S) isomer at LOQ was less than 2.0% RSD. Therefore, this method had adequate sensitivity for the detection and estimation of (1R, 4S) isomer in (1S, 4R).

Good linearity of (1R, 4S) isomer was evaluated over four levels of (1R, 4S) isomer solutions from 0.6 µg/ml to 7.5 µg/ml, with the linear regression equation y = mx + c, where x is the concentration in µg/ml, and y is the corresponding peak area of the undesired enantiomer in mV/s. We observed linear results with respect to concentration for (1R, 4S) isomer. The correlation coefficient value is more than 0.999 (Table 1).

The standard addition and recovery experiments were conducted for the (1R, 4S) isomer in samples in duplicate at 2.0 µg/ml to 7.5 µg/ml (2.0, 2.5, 5.0, and 7.5µg/ml). The accuracy was in terms of recovery (%). The recovery was calculated by back calculating concentration at each level in each preparation. The recovery is not less than 90.0% and not more than 110.0%

The repeatability and intermediate precision were expressed as relative standard deviation (RSD). For this study, solution of (1S, 4R)-4-Aminocyclopent-2-en-1-yl methanol. HCL (5000 µg/ml) spiked with (1R, 4S) (0.1%, 5 µg/ml) was analyzed in duplicate injections to establish repeatability. RSD values were better than 1.0% for the retention times of both the Enantiomers. In the intermediate precision study, results show that RSD values were in same order of magnitude than those obtained for repeatability studies. All these values indicated that the method was precise.

The method robustness studies were demonstrated by adjusting flow rate, column temperature and making variations in the mobile phase concentration. The chromatographic resolution of (1S, 4R)-4-Aminocyclopent-2-en-1-yl methanol. HCl and (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol. HCL was more than 2.0 under all separation conditions tested, demonstrated sufficient robustness. The stability of the solution and mobile phase used in this method was tested over a long time. No significant change in (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol. HCL content was observed in (1S, 4R)-4-Amino-2-cyclopent-1-methanol. HCL sample during solution stability and mobile phase stability experiments, and the RSD values were less than 2.0% for (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol HCL peak area. Hence, the (1S, 4R)-4-Aminocyclopent-2-en-1-yl methanol. HCL sample solution and the mobile phase were stable for at least 24hrss. (Fig.9)

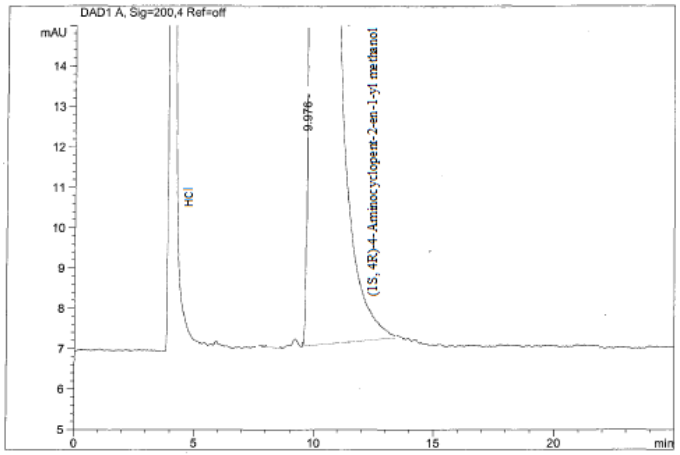

Fig.9: Sample Chromatographic (1S, 4R)-4-Aminocyclopent-2-en-1yl methanol. HCL

4. CONCLUSION

The preparative Chiral column (Crown ether based stationary phase) was used to resolve the raecemic Vince Lactam compound into the desired (-) Lactam and undesired (+) Lactam. After the resolution of Vince Lactam (+) lactam was protected using Di-tert-butyl dicarbonate. Further, this carbonate ester reacts with amines to give tert-butyl (1S, 4R)-3-oxo-2-azabyclo [2.2.1]hept-5-ene-2-carboxylate (BOC Derivative). This protected Lactam is reduced with sodium borohydride to form tert-butyl [(1S, 4R)-4-hydroxymethyl) cyclopent-2-en-1-yl]Carbamate. The compound obtained by reduction of the amide was further hydrolyzed to deprotect the BOC protected amino group using diluted hydrochloric acid to form amino alcohol compound i.e. (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol.HCl.

A simple, specific, linear, accurate and precise HPLC method was developed, which was capable of separating the undesired isomer (1R, 4S)-4-Aminocyclopent-2-en-1-yl methanol. HCl from (1S, 4R)-4-Aminocyclopent-2-en-1-yl methanol.HCl. Crownpack CR (+) was found to be suitable for separation of the enantiomer of (1S, 4R)-4- Aminocyclopent-2-en-1-yl methanol.HCl. The developed and validated method can be used for the chiral purity testing of (1S, 4R)-4-Aminocyclopent-2-en-1-yl methanol. HCl.

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