



Topotecan enantio-specific resolution by liquid chromatography

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Abstract — Enantiomer specific method development is essential to ensure the safety and efficacy of the drug. Topotecan is the first topoisomerase inhibitor developed for ovarian cancer and lung cancer. Simple, rapid enantio specific chromatographic method was developed for R (-) enantiomer control in topotecan with immobilized chiral stationary phase (CSP). Chiral pak IA 250 mm long, 4.6 mm inner diameter and 5 µm particle packed with amylose tris (3,5-dimethylphenyl-carbamate) immobilized on silica gel used as a stationary phase. Mobile phase was containing n-hexane: ethanol: diethyl amine: glacial acetic acid in the ratio of 60:40:0.8:0.8 (v/v/v/v) in an isocratic mode of elution. Good resolution is achieved between the enantiomers i.e. > 4. Proposed method was proven for indented purpose. (i.e., Validated in terms of specificity, precision, linearity, limit of detection, limits of quantification and accuracy according to ICH Q2 guideline.) The linearity of a method was studied in the range of 2.5 to 7.5 µg/ml. Regression coefficient of the calibration curve r^2 was >0.999. Recoveries were observed in the range of 90.8-109.6%. The limit of detection (LOD) and quantification (LOQ) was 0.5, 2.5 µg/ml for R-enantiomer. Proposed method can be used for routine analysis.

Keywords: Topotecan, Liquid chromatography Enantiomer, n-hexane, Ethanol

I. INTRODUCTION

Topotecan chemically known as (S)-10-[(dimethyl amino) methyl] -4-ethyl-4,9dihydroxy-1H-Pyran [3,4,6,7] indolizino [1,2-b]quinoline-3,14(4H,12H)dione hydrochloride, (Fig.1) is one of the first topoisomerase inhibitor developed for Ovarian cancer and lung cancer. Topotecan is one the most potent therapeutic agents used for treatment of ovarian cancer, lung cancer and other cancer. Topotecan have one asymmetric carbon function resulting in pair of enantiomers. However, for therapeutic applications it is administered as S-enantiomer [1, 2]. Numerous methods were available to control achiral impurities in bulk drug and injection [3-5, 7-8]. Regulatory authorities are insisting that stereospecific method should be used from the initial development of the drug. Stereo specific method was

proposed by Dhakane et-al [6] for enantiomer estimation in Topotecan with coated “Chiral stationary phase” (CSP) such as Chiralpak AD-H, Coated CSP’s are limitations of solvent usage during the in-process check, even trace level of few solvents will destroy the coated phase column. To overcome this limitations proposed method was developed in “immobilized CSP”, such as chiral pak IA which is robust and no solvent limitation. We can use this method for in-process as well. Method were proved be reproducible, linear, sensitive and accurate, Proposed method can be used to estimate the enantiomer excess in topotecan drug substance and drug product for quality control release and generic development.

II. INSTRUMENT AND REAGENTS

HPLC system was composed of Agilent 1200 series pump, auto-injector, column thermostat and DAD detector (Agilent, Germany) with Chromeleon system controller. Chiral pak IA column (250mm long, 4-6 mm inner diameter, 5µm particle size (Daicel chemical Industries, Tokyo, Japan) were used for separation. HPLC grade n-hexane, glacial acetic acid procured from S.D.fine chem Ltd, T.V. Industrial estate, 248-Worli road, Mumbai-30, India. Ethanol procured from Hayman Ltd, east ways park, Witham, Essex, CM83YE, England. Diethyl amine was purchased from Sigma Aldrich, 3050 Spruce Street, St Louis, M063103 US 314-771-5765). Topotecan were manufactured from M/s Cipla Ltd, Research and development, Virgonagar, Bangalore, India-560049. R-Topotecan was procured from Avra laboratories (P) ltd, Nacharam, Hyderabad, India-501 507.

III. METHOD DEVELOPMENT STRATEGY

Few trails were carried out to select the column and mobile phase.

A. Column & mobile phase selectivity

A preliminary experiment was carried out on Cyclodextrin column using phosphate buffer and

methanol as the solvent. However that experiment not useful to separate the enantiomers. Immobilized Polysaccharide based column more investigated for method development, Because of their broad applicability in resolution in a number of racemic compounds. Amylose, Cellulose derivatives of 3,5-dimethylphenyl carbamate CSP was selected for further studies. n-Hexane and ethanol was used as mobile phase in different concentration with Chiralpak IA stationary phase. In 6:4 ratio of n-Hexane and ethanol combination enantiomers were separated but poor resolution and broad peak observed. Improve the peak shape diethyl amine was added into the mobile phase. Amylose based chiral pak IA column was selected. The enantiomers were separated with sharp peaks and good resolution. Thus it was continued for further optimization.

B. Operating conditions

Chromatographic separation was achieved on Chiralpak IA column 250 mm long, 4.6 mm inner diameter, 5 μ m particle size using a mobile phase consisting of n-hexane: ethanol: Diethyl amine: glacial acetic acid in the ratio of (60:40:0.8:0.8 v/v/v/v) at a flow rate of 1.0 ml/min, column oven temperature was set at 25°C and detection was made at 270 nm.

C. Method validation

Proposed method was validated to prove the intended purpose. Acceptance criteria of validation parameters set as per international conference on harmonization quality guide line Q2A. To prove the repeatability and reproducibility precision study was carried out. Linearity of the method was evaluated from lower to higher concentrations; Sensitivity of the method was arrived from known serial dilutions. Accuracy of the proposed was verified by known addition method.

IV. RESULTS AND DISCUSSION

A. System suitability & Precision

Enantiomers are separated with baseline separation of > 4. System suitability chromatogram presented in Fig.2. Precision of the method was tested by preparing six individual solutions of topotecan. R-isomer content in topotecan was estimated by area normalization method. Percentage relative standard deviation (%RSD) calculated between the replicates of R-isomer. Acceptance criteria set for precision study was not more than 5% RSD. The percentage of R-isomer was calculated from the peak areas of enantiomers and the %RSD found to be less than 1.0 %. Reproducibility study was conducted in different day in different instrument, no variation observed between inter-intraday results, Hence the proposed was reproducible.

B. Sensitivity

Sensitivity of the method was estimated by limit of detection (LOD) and quantification (LOQ). Known serial dilution of the analytes was injected in a chromatographic system. LOD was determined by measuring signal to noise (S/n) ratio of the analyte peak. S/n ratio ≥ 3 was considered as LOD. LOD for R-isomer was 0.5 μ g/ml. LOQ was determined by measuring S/n ratio with precision and accuracy. LOQ was taken as the concentration of the analyte where S/n was more than 10 and it was to be 2.5 μ g/ml for R-isomer with the precision of 1.74% RSD.

C. Linearity

Linearity of the method was proved by injecting five linear concentrations from LOQ to 7.5 μ g/ml. In each level triplicates was injected, average value were used for computing the calibration curve. Calibration curve were drawn in the range of 2.5-7.5 μ g/ml of R-isomer. Solutions are prepared and injected freshly. Curves were linear with $r^2 > 0.999$ and the regression equations for enantiomer and topotecan was $Y = 34.563x + 26990$ and $Y = 38.292x + 36428$ respectively.

D. Accuracy

The accuracy of the method was determined by known addition method. Enantiomer was spiked in topotecan at three different levels in the range of 50-150% with respect to the specified concentration of 500 μ g/ml. Accuracy solutions were injected in triplicate. Percentage recovery calculated as spiked concentration versus observed concentration. Limit set for percentage recovery was between 85,115 with less than 5% RSD of precision. The recoveries were in between 90.82 and 109.59 % with %RSD less than 3.5 %. Hence the method is accurate.

V. CONCLUSION

Topotecan is a relatively new topoisomerase inhibitor for ovarian cancer, lung cancer and other cancer. S-isomer is administrated as a drug, because of its efficacy and safety. So enantio specific method is essential in topotecan purity. Immobilized amylose-based chiral pak IA has shown better resolution and no solvent is reported to damage this CSP. Because of this advantage same method can be used to evaluate the racemization during stress study. Developed method was found to be simple, rapid and sensitive for enantio selective separation and determination of topotecan enantiomers in bulk drugs and pharmaceuticals, method can be used for routine quality control release, Process development and stress studies.

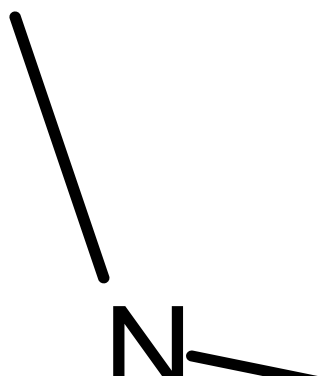


Fig. 1. Structure of Topotecan and its enantiomer

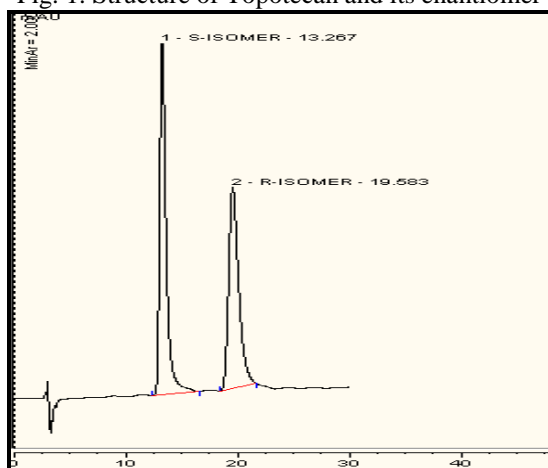


Fig. 2. Typical system suitability chromatogram

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