Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (4): 1698-1704

(International Peer Reviewed Journal)

New RP HPLC Method For The Estimation of Topotecan In Pharmaceutical Dosage Form

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Accepted on 17th July 2014

ABSTRACT

The present paper describes a simple isocratic RP-HPLC method for the estimation of Topotecan in tablet dosage form. Best resolution was obtained with column Zodiac C18 column (250 mm x 4.6 mm, 5µ) at 263nm with retention time of 4.49min. The mobile phase used was Methanol: Acetonitrile: Water 78:17:5 (v/v/v) with flow rate of 1.0 ml min^{-1}. The method for estimation of Topotecan in tablet dosage form was found to be linear, accurate, precise, sensitive and selective. The linearity range was from 10µg mL^{-1} to 40µg mL^{-1}. Method was found to be highly sensitive as LOD and LOQ were found to 0.5µg mL^{-1} and 0.15µg mL^{-1}. The repeatability and reproducibility were within the range i.e. less than 2%.The accuracy of the method between 98.48 to 101.32%. The percentage of assay was calculated for market formulation was 98.93%.

Keywords: Topotecan, HPLC, Linearity, validation.

INTRODUCTION

Topotecan is chemically known as (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano [3',4';6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)dione[1].Topotecan, a water soluble analogue campto thecin, is used to treat ovarian cancer [1], Cervical cancer [2, 3] and lung cancer [4, 5], as well as other cancer types [6-9].

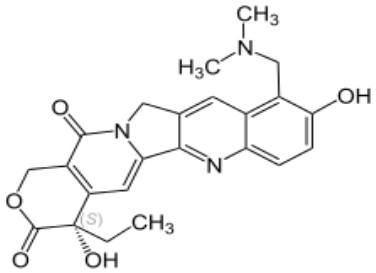

Figure 1. Structure of Topotecan

Topotecan is a natural product extracted from the bark of the tree Camptotheca acuminata. Topoisomerase-I is a nuclear enzyme that relieves torsional strain in DNA by opening single strand breaks. Once topoisomerase-I creates a single strand break, the DNA can rotate in front of the advancing replication fork. Topotecan intercalates between DNA bases. This intercalation disrupts the DNA duplication machinery when it reaches a site where topotecan is intercalated. This disruption prevents DNA replication, and ultimately leads to cell death. Mammalian cells cannot efficiently repair these double strand breaks [10]. This process leads to breaks in the DNA strand resulting in apoptosis. Common side effects for topotecan includes Myelo suppression, Diarrhea, Low blood counts, Susceptibility to infection. Literature survey revealed that a few analytical methods were reported for the determination of topotecan using HPLC[11,12], HPLC with fluorescence detector[13]. In the present investigation attempts have been made to develop a very fast, accurate and precise method for the analysis of topotecan in tablet dosage form.

MATERIALS AND METHODS

Instrumentation: Analysis was carried out by using an isocratic peak HPLC instrument on a Zodiac C18 column (250 mm x 4.6 mm, 5µ). The instrument is equipped with a LC 20AT pump and variable wavelength programmable UV-Visible detector, SPD-10AVP. A 20µL Hamilton syringe was used for injecting the samples. Data was analyzed by using peak software. 2301-UV/Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using a Supertech ultrasonic bath sonicator. A Denver balance was used for weighing the materials.

Chemicals: The standard drug sample of Topotecan was supplied by Cipla, Hyderabad. The tablets were purchased from local market. All the chemicals and solvents used are of HPLC grade and purchased from Merck specialties private ltd, Mumbai.

Chromatographic conditions: A mixture of Methanol: Acetonitrile: Water in the ratio of 78:17:5(v/v/v) was prepared and used as mobile phase. The spectrum of diluted solutions of the Topotecan in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectrum of the Topotecan depicted that at wavelength 263nm drug shows maximum absorbance.

Preparation of stock and standard solution: Accurately weighed 10mg of Topotecan was dissolved in 10ml of methanol in a volumetric flask. Thus the stock solution is obtained in the concentration of 1000µg/ml. From the stock solution, standard solution is prepared by transferring 1ml of 1000µg mL^{-1} sample and diluting it to obtain the concentration of 100µg mL^{-1}. From the standard solution different dilutions were prepared by carrying out the proper dilutions.

Preparation of tablet assay solution: 20 tablets of HYCAMTIN were accurately weighed and average weight was noted. In order to prepare tablet assay solution, tablets were crushed and grinded into fine powder using mortar and pestle. From the powdered tablet form, 10mg was weighed accurately and sample solution was prepared. Different dilutions were made from the above solution. The assay of drug in the tablet dosage forms was effectively estimated by evaluation of data of standard drug and pharmaceutical formulations.

Development of Method: In order to develop the method the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The use of hydrophobic stationary phases usually provides adequate retention of organic non polar molecules. The chromatographic separation was achieved using an RP C18 column for Topotecan with symmetrical peak shape. As the peak shape was obtained satisfactory in Zodiac C18 column, it was used for the analysis. Previous experiments and data reported in the literature showed that both the methanol and acetonitrile could be

used an organic modifier in the mobile phase. The use of acetonitrile as a mobile phase organic modifier resulted in better sensitivity compared to methanol. Tests involving the use of mixtures solvents were used as mobile phase; acetonitrile in addition to other solvents such as Methanol, water were carried out to develop suitable mobile phase for estimation. After many trails it has been found that methanol, Acetonitrile and water in the ratio of 75:17:5v/v was best for the analysis with the flow rate of 1.0mL min^{-1}. The pH of the system was varied in the range of 4.5 to 5.5, it has recorded that the pH of 4.3 was well suited for the estimation of the drug. Our experiments revealed that isocratic elution with simple mobile phase were given good results than gradient with complicated mobile phases. The method has many advantages like simplicity, isocratic conditions, shorter run time, low injection volume, smaller particle size, and less flow rate, inexpensive mobile phases. Under these conditions, the retention time of Topotecan was about 4.49min, with a good peak shape (peak tailing factor < 2), and the run time was 8min. Typical chromatogram of Topotecan standard is shown in figure 2. The results are furnished in table 1.

Table 1. Table showing optimized chromatographic conditions

Mobile phase	Methanol: Acetinitrile: Water 78:17:5 (v/v/v)
Pump mode	Isocratic
Mobile phase pH	5.3
Diluents	Mobile phase
Column	Zodiac C18 column (250 mm x 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	263nm
Injection Volume	20 µl
Flow rate	1.0 mL/min
Run time	8 min
Retention Time	4.49 min
Pump pressure	9.5±5 MPa

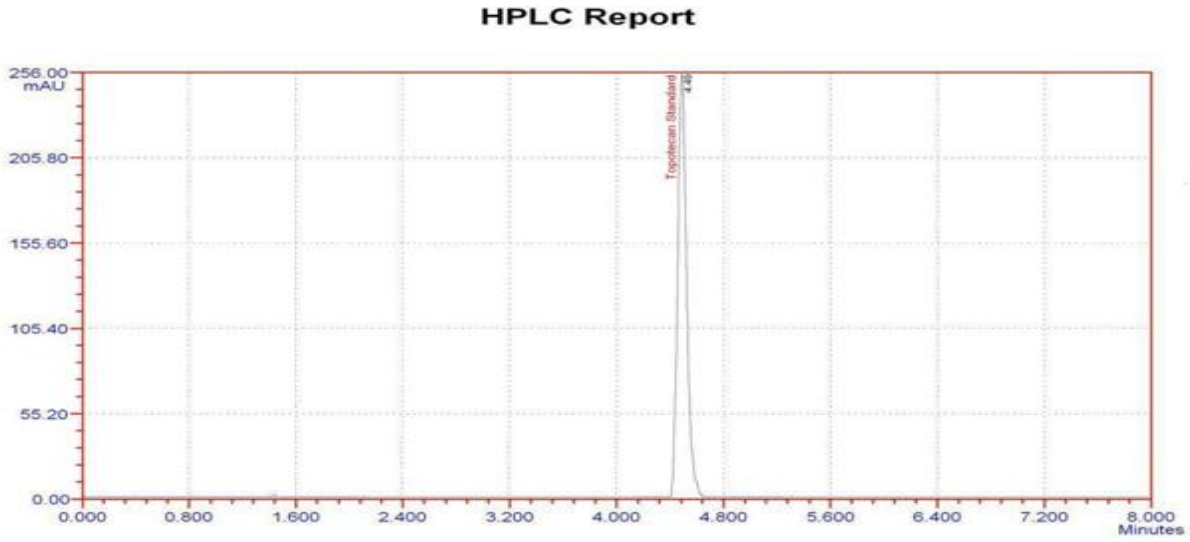

Figure 2: Standard chromatogram of Topotecan

Validation Method: The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method as per the ICH guidelines for the estimation of Topotecan [14]. The system suitability parameters like Theoretical plates (N), Resolution (R), Tailing factor (T), LOD (µg/ml) and LOQ (µg mL^{-1}) were calculated and compared with the standard values to ascertain whether the proposed

RP-HPLC method for the estimation of Topotecan in pharmaceutical formulations was validated or not. Linearity is determined by replicate injections of seven concentrations level within the range of 10–40µg mL^{-1}. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation. Linearity is evaluated graphically by plotting a graph of the relative responses on the y-axis and the corresponding concentrations on the x-axis. A linear regression equation is applied to the results to evaluate correlation coefficient. In addition, y-intercept, slope of the regression line and residual sum of squares should also calculate. The range of an analytical method is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range is normally expressed in the same units as the test results (e.g., percentage, parts per million) obtained by the analytical method. In the present study we calculated intraday and inter day precision by analyzing the samples on same day of preparation and consecutive days. The accuracy of the proposed method was also further assessed by performing recovery experiments using the standard additional method known amount of the pure Topotecan was added to pre-analyzed formulation and the total concentration was once again determined by the proposed method. If the obtained mean recoveries and relative standard deviations were in the range 90-110%and 0.001–1.99%, respectively, the method is said to be accurate. In the present analysis, it was found that %recoveries lie in the range of 98.48-101.32%.

For the determination of a method's robustness, method parameters like pH, flow rate, column temperature, column lot or mobile phase composition are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 30µg mL^{-1} of Topotecan using the same instrument by two different analysts under the same optimized conditions at different days. In order estimate LOD values, drug solution was diluted and injected until the chromatogram, failed its system suitable conditions. And LOQ was measured by calculating the 3.3 × LOD and tested for precise result. The results are furnished in table 3.

Formulation assay: The commercial brand tablets (Hycamtin-1.0 mg) were chosen for testing suitability of proposed method to estimate Topotecan in pharmaceutical dosage forms. Solution obtained from tablet powder was diluted with diluents to obtain in the range of linearity previously for the pure drug determined. Sample solution was injected under the chromatographic conditions and chromatogram was recorded. The amount of Topotecan present in the tablet formulation was determined by comparing the peak area from the standard. In both cases assay obtained is more than 98% and no interference of impurity peak observed in Topotecan peak.

RESULTS AND DISCUSSION

Method development: A reverse phase HPLC method has been developed in order to estimate Topotecan in pharmaceutical dosage forms. Several trails have been done in order to find the suitable chromatographic conditions. Most essential chromatographic parameters like column, mobile phase composition, flow rate, pH of the system, UV detection wavelength etc, were judged in this phase of analysis. It has been analyzed that the method developed is very simple and rapid because in the ease of mobile phase composition. The column was selected based upon the polarity of interacting molecules. After analysis it has been found that Zodiac C-18 column was most suitable one. Isocratic mode of elution was used for the analysis. The mobile was selected as Methanol: Acetonitrile: Water 78:17:5 (v/v/v). The flow rate of the mobile phase was maintained as 1.0mL min^{-1}. The pH of the medium was maintained as 5.3. The ambient temperature was most suitable for analysis. The drug was eluted at 4.49min which is most suitable for the routine analysis of the drug in pharmaceutical dosage forms.

Method validation: The proposed method was validated according to ICH Guidelines. All the crucial validation parameters like linearity, precision, accuracy, ruggedness, robustness and sensitivity were verified and their values were obtained within the acceptable range. The linearity range was found to be 10-40µg mL^{-1}. The calibration curve showed good linearity and regression equation was obtained as y=5551.117x-140.316. The points in the calibration plot good co-relation among them, so the co-relation coefficient was obtained as 0.999.It was concluded that the method was linear. Results are given in table2

Table 2: Table showing linearity results

Level	Concentration of Topotecan (in µg/ml)	Mean peak area
Level -1	10	57636
Level -2	15	84163
Level -3	20	109638
Level -4	25	135263
Level -5	30	163252
Level -6	35	195635
Level -7	40	224736
Linearity Range: 10 to 40µg/ml	**Slope** **Intercept** **Correlation coefficient**	**5556** **285.6** **0.998**

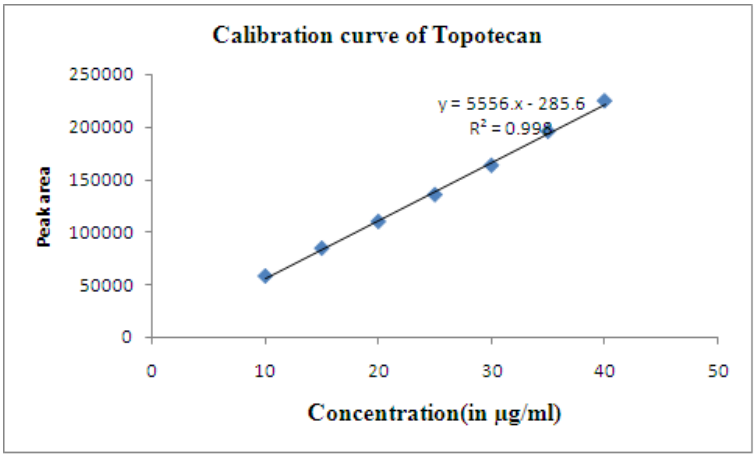

Figure 3: Calibration curve of Topotecan

The precision depicts the repeatability of the method. Precision was carried out on the same day which was referred to as intraday precision and inter day precision was carried on next consecutive days. %RSD value depicts that the method was precise as %RSD were found to be 0.20 and 0.40 for intraday and inter day precision respectively. The extents of losses during the analysis were estimated in the recovery studies. It was reported that %recoveries lie in the range of 98.48%-101.32%. It depicts that the method was accurate and can be effectively used for the routine analysis of drug in the pharmaceutical dosage forms. The ruggedness of the method was proved by %RSD values of six replicate injections, it was found to be 0.55. The chromatographic conditions like wavelength of the detector, mobile phase composition and flow rate of the mobile phase were varied slightly, but there were no considerable changes in the elution time and shape of the peak. It shows that in spite of deliberate changes in the chromatographic conditions results were unaffected. Hence, it was proved that the method was robust and rugged. The developed method can be efficiently used for analyzing the samples containing very low amount of drug content in it, as the limit of detection of the method was found to be 0.5µg mL^{-1} and limit of quantitation was 0.15µg mL^{-1}. It shows that method was sensitive and can be used to detect and quantify low concentrated drug samples. Finally,

we can conclude that the proposed and developed method can be routinely used for the determination of Topotecan in pharmaceutical formulations. The results of validation parameters were illustrated in table 3.

Table 3. Summary of validation results

Validation parameter		**Result**
Precision (RSD)	Intraday precision	0.20
	Interday precision	0.405
Recovery range (%)		98.48-101.32
Robustness (% of change)		0.55-1.67
Ruggedness (RSD)		0.55
Limit of Quantification		0.5µg/ml
Limit of Detection		0.15µg/ml
Formulation assay (%)		98.933

APPLICATIONS

The developed HPLC method can be used for the routine analysis of Topotecan in pharmaceutical formulations. This method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

CONCLUSIONS

In the proposed study, HPLC method was developed for the estimation of Topotecan and it was validated as per ICH guidelines. Statistical analysis proved that method was accurate, precise, and repeatable. The proposed method for the assay of Topotecan in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer illustrating simplicity of the method. The method was validated for specificity, linearity, precision, accuracy and robustness.

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