Oriental Journal of Chemistry Vol. **25**(4), 913-922 (2009)

Validated reverse phase HPLC method for the determination of topotecan in pharmaceutical dosage forms

MURALI BALARAM VARANASI[1], MUSHRAFF ALI KHAN, VENKATESWARA RAO JANGALA* and BULUSU BHANU TEJA[2]

[1]Department Of Pharmaceutical Analysis, Sultan-Ul-Uloom College Of Pharmacy, Banjara Hills,Hyderabad - 34 (India).
[2]TherDose Pharma Pvt. Ltd, IE, Kukatpally, Hyderabad - 500 072 (India).

(Received: August 20, 2009; Accepted: October 03, 2009)

ABSTRACT

A simple and gradient reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for quantitative determination of topotecan in bulk drug samples and formulations. The method was validated for accuracy, precision, linearity, specificity, limit of detection and limit of quantitation. Topotecan was analyzed by using Zorbax SB – C_{18} (250 mm x 4.6 mm, 5 ìm) at ambient temperature, with gradient elution of Water,Acetonitrile and TFA as a mobile phase (85:15:0.1). The flow rate was set 1.0 ml/min and the analysis was performed at a wavelength of 267 nm using Photo Diode Array (PDA) detector. Efficient UV detection at 267 nm enabled determination of topotecan without any interference from injectable solution excipients or solvents. The retention time (RT) for Topotecan was around 21.50 min. The calibration curves were linear over a concentration range from 0.05 mg to 0.15 mg/ml. Limit of detection (LOD) for Topotecan was 0.000023 mg/ml and Limit of quantitation (LOQ) Topotecan was 0.000070 mg/ml. The developed method was successfully applied to estimate the amount of topotecan in formulations.

Key words: Topotecan, high performance liquid chromatography, reverse phase liquid chromatography, validation.

INTRODUCTION

Topotecan is an antineoplastic agent used to treat ovarian cancer. It works by inhibiting DNA topoisomerases, type I. It is chemically (S)-10-[(dimethylamino) methyl]-4-ethyl-4, 9-dihydroxy-1H-pyrano [3', 4']. The drug is official in Martindale, the Extra Pharmacopoeia [1]. It is available as capsules and intravenous infusions. Its chemical structure is shown in Fig. 1.

Topotecan, a semi-synthetic derivative of camptothecin (a plant alkaloid obtained from the *Camptotheca acuminata* tree), is an anti-tumor drug with topoisomerase I-inhibitory activity similar to irinotecan. Topotecan interferes with the growth of cancer cells, which are eventually destroyed. Since the growth of normal cells may also be affected by the medicine, other effects may also occur. Unlike irinotecan, topotecan is found predominantly in the inactive carboxylate form at neutral pH and it is not a prodrug. Topoisomerase I relieves torsional strain in DNA by inducing reversible single strand breaks. Topotecan binds to the topoisomerase I-DNA complex and prevents religation of these single strand breaks. The cytotoxicity of topotecan is thought to be due to double strand DNA damage produced during DNA synthesis when replication

enzymes interact with the ternary complex formed by topotecan, topoisomerase I and DNA. Mammalian cells cannot efficiently repair these double strand breaks [1].

Aim of the work

Few HPLC methods for quantitative determination of topotecan in formulations were reported in the literature and these reports mainly included the determination of topotecan and its metabolites in biological fluids[2-7]. The aim of the work is to develop and validate a rapid, economical and sensitive HPLC method for quantitative determination of topotecan in bulk drug samples and injectable preparations. In order to minimize batch-to-batch variation there is an immense need for developing a rapid, sensitive and validated analytical method for day-to-day analysis of the drug in pharmaceutical dosage forms.

MATERIAL AND METHODS

Chemicals and reagents

Topotecan bulk drug (99.80 % purity) and formulations were kind gifts from TherDose Pharma Pvt. Ltd.,Hyderabd, India. Acetonitrile (HPLC grade) was obtained from Rankem, India,TFA,pure grade was obtained from Sisco.

Instrumentation

The HPLC system consisted of a Shimadzu LC–2010 A_{HT} module with PDA Detector. Data acquisition was performed by LC solutions software operated on a Pentium® IV microprocessor. Zorbax SB – C_{18} (250 mm × 4.6 mm 5 ìm) at ambient temperature, with gradient elution of Water, Acetonitrile and TFA as a mobile phase (85:15:0.1). The flow rate was set 1.0 ml/min and the analysis was performed at a wavelength of 267 nm using Photo Diode Array (PDA) detector. The mobile phase was degassed and filtered through 0.2 µm membrane filter before pumping into HPLC system. Preparation of solutions:

Preparation of drug stock solution

The stock solution of topotecan was prepared by dissolving accurately weighed quantity of 10.10 mg of the drug in 100 ml of Mobile Phase Water : Acetonitrile: TFA (85:15:0.1) (concentration, 0.1010 mg/ml).

Method validation

System suitability

The system suitability was assessed by replicate analysis of six injections of the drug at a concentration of 0.1010 mg/ml. The acceptance criteria were not more than 2% for the percentage relative standard deviation (% RSD) for the peak area and 1.5% for retention time of topotecan peaks. The number of theoretical plates should not be less than 2500.

Determination of Limit of Detection and Limit of Quantitation (Sensitivity)

The limit of detection is determined by calculating the signal to noise ratio and by comparing test results from samples with known concentrations of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. The LOD and LOQ of the method were demonstrated by preparing 3 level concentrations of 0.05%, 0.10% and 0.15% of the target concentration of 0.1 mg/ml.

About 50 ml of Topotecan level solutions was injected onto the chromatographic system for 6 times and the mean and standard deviation were calculated. Two different graphs were plotted. (1) Between concentration and average area. (2) Between concentration and the standard deviation for the above level concentrations and calculated the Correlation Coefficient, Slope and Y-intercept.

Calculation

Limit of Detection	=	$3.3\ \sigma/S$
Limit of Quantification	=	$10\ \sigma/S$

Where

σ is the standard deviation at concentration 0

S is the slope of the regression line.

Linearity (Calibration curve)

Standard Stock solution

Accurately weighed 50.82 mg of Topotecan drug substance and transferred to a 50 ml volumetric flask. Dissolved and brought to volume with the mobile phase and mixed. (Concentration 1.0164 mg/ml)

Level solutions

Prepared a series of solutions in 5 concentrations of 50%, 75%, 100%, 125%, and 150% using the standard stock solution. Transferred accurately the volume of stock solution mentioned below into the volumetric flask of specified capacity and brought to volume with the mobile phase as diluent.

Level 51%

Transferred 0.50 ml of standard stock solution in to the 10ml volumetric flask and brought to volume with the diluent (Theoretical Concentration 0.050820 mg/ml). This was prepared in duplicate preparations.

Level 76%

Transferred 0.75ml of standard stock solution in to the 10ml volumetric flask and brought to volume with the diluent (Theoretical Concentration 0.076230 mg/ml). This was prepared in triplicate preparations.

Level 102%

Transferred 1.00mL of standard stock solution in to the 10 ml volumetric flask and brought to volume with the diluent (Theoretical Concentration 0.101640 mg/ml). This was prepared in triplicate preparations.

Level 127%

Transferred 1.25 ml of standard stock solution in to the 10ml volumetric flask and brought to volume with the diluent (Theoretical Concentration 0.127050 mg/ml). This was prepared in triplicate preparations.

Level 152%

Transferred 1.50 ml of standard stock solution in to the 10ml volumetric flask and brought to volume with the diluent (Theoretical Concentration0.152460 mg/ml). This was prepared in duplicate preparations.

The linearity of the method was demonstrated by above prepared level concentration of 51, 76, 102, 127 and 152% of the target concentration of 0.1 mg/ml.

About 50 ml of each of the above prepared solutions was injected onto the chromatographic system connected to ZORBAX SB-C18 column (in duplicate) and calculated the average area in each case.

Accuracy and precision

Accuracy was calculated with respect to above prepared solution at the levels of 75%, 100% and 125% of the normal or target concentration. The accuracy of the method was demonstrated through recovery experiment on 3 samples at concentration 76%, 102% and 127% of the actual concentration employed in the usual procedure. The actual concentration employed in the determination was 0.1 mg/ml of topotecan containing the excipients used in the inventor formulation and the recovery was calculated in each of the case using the regression line equation.

Demonstration of precision was done under two categories. The injection reproducibility was assessed by injecting six replicate injections of the standard solution of topotecan and the relative standard deviation of the replicate injections was calculated.

Injection Reproducibility

Relative standard deviation of RT and Area of the above system suitability solution were calculated for injection reproducibility.

Method Precision

Six individual preparations of Topotecan drug substance were prepared with target concentration of about 0.1 mg/ml for Method Precision.

Preparation 1

Weighed accurately 9.98mg of Topotecan and transferred in to 100ml volumetric flask. Dissolved and brought to volume with the diluent. Labelled as Preparation – 1. (Conc. – 0.0998mg/ml).

Preparation 2

Weighed accurately 10.16mg of Topotecan and transferred in to 100ml volumetric flask. Dissolved and brought to volume with the diluent. Labelled as Preparation – 2. (Conc. – 0.1016mg/ml).

Preparation 3

Weighed accurately 10.20mg of Topotecan and transferred in to 100ml volumetric flask. Dissolved and brought to volume with the diluent. Labelled as Preparation – 3. (Conc. – 0.1020mg/ml).

Preparation 4

Weighed accurately 10.00mg of Topotecan and transferred in to 100ml volumetric flask. Dissolved and brought to volume with the diluent. Labelled as Preparation – 4. (Conc. – 0.1000mg/ml).

Preparation 5

Weighed accurately 10.16mg of Topotecan and transferred in to 100ml volumetric flask. Dissolved and brought to volume with the diluent. Labelled as Preparation – 5. (Conc. – 0.1016mg/ml).

Preparation 6

Weighed accurately 10.18mg of Topotecan and transferred in to 100ml volumetric flask. Dissolved and brought to volume with the diluent. Labelled as Preparation – 6. (Conc. – 0.1018mg/ml).

Procedure

About 50 ml of each of above preparations was injected onto the chromatographic system connected to a Zorbax SB-C18 column in duplicate and recorded the chromatograms. Peak areas were calculated due to Topotecan peak.

Application of the Method to Dosage Forms
Statistical Analysis

Calculated the Mean, Standard Deviation and the Relative Standard Deviation for the assay of 6 preparations.

$$\frac{A_T}{A_s} \times \frac{W_s}{W_T} XP$$

Calculations

Calculated the content of the Topotecan in the sample solution using the following formula:

Where,

A_T= Average area of the sample preparation

A_S = Average area of the standard preparation

P = Potency of the standard

W_T= Concentration (mg/ml) of the sample solution

W_S = Concentration (mg/ml) of the standard solution

Stability

The validity of the assay solutions was demonstrated for a period of 72 hours by chromatographing the same solution at periodic intervals.

Procedure

About 50 ml of the standard solution was injected at 0, and 72 hrs onto the column of the chromatographic system and recorded the peak areas in the chromatograms.

Calculations

Observed the areas of the peaks of Topotecan injected at the mentioned time intervals and calculated their corresponding concentrations.

Specificity

The specificity of the method was demonstrated by interference check by injecting the diluent blank and placebo solution to determine whether any peaks in the diluent and placebo solution are co-eluting with topotecan peaks.

RESULTS AND DISCUSSION

Method development and optimization

Topotecan is freely soluble in isopropyl alcohol. The drug can be separated on a Zorbax SB – C_{18} column in reverse phase mode. The optimization of the method development was done by changing mobile composition by gradient elution. The following gradient program was used for method development

Mobile PhaseA Water

Acetonitrile: TFA (85:15:0.1)

Mobile Phase–B Water

Acetonitrile: TFA (60:40:0.1)

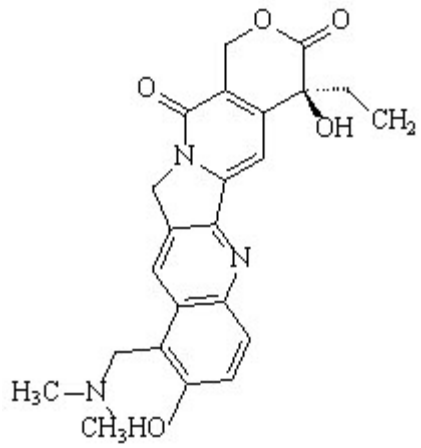

Fig. 1: Chemical structure of topotecan

Gradient programme

Time	Pump-A	Pump-B
0.01	BCNC	0
16.00	BCNC	0
41.00	BCNC	100
60.00	BCNC	0
60.01	STOP	

The peak shape and symmetry were good with above gradient elution and topotecan peaks were resolved with greater than 1.0 resolution at a flow rate of 1.5 ml/min.

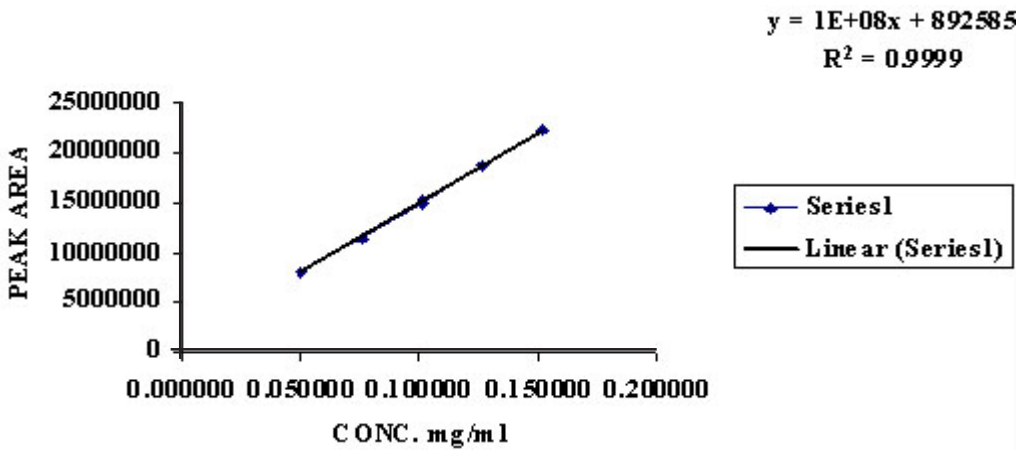

Fig. 2: Linearity range for topotecan

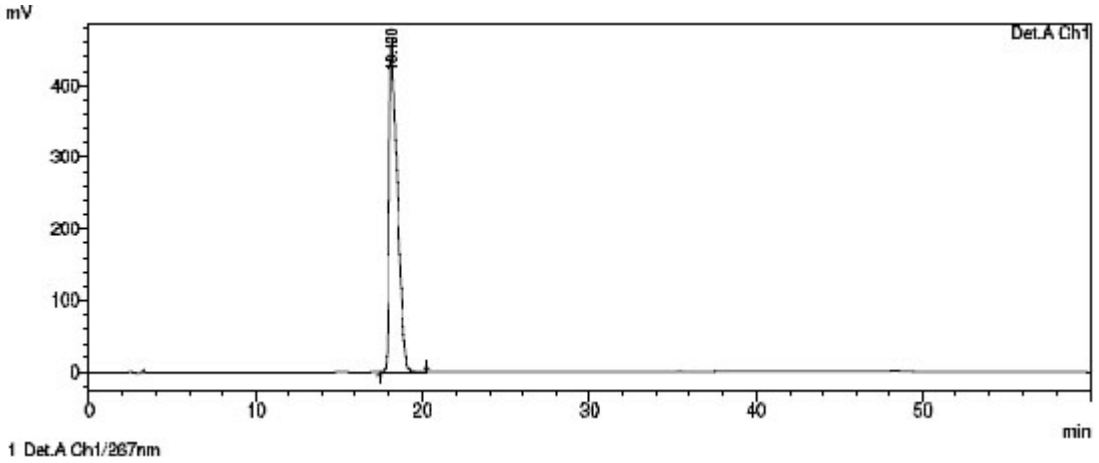

Fig. 3: QC sample chromatogram of Topotecan extracted from formulation

Table 1: System suitability study of topotecan

Injection Number	Retention Time	Peak Area	Theoretical plates	Tailing factor
Injection – 1	21.18	14862098	8933	1.61
Injection – 2	21.41	14771370	11331	1.68
Injection – 3	21.50	14743289	12364	1.72
Injection – 4	21.59	14715498	13216	1.73
Injection – 5	21.64	14704506	13923	1.74
Injection – 6	21.67	14692190	14157	1.75
Mean	21.50	14748159	12321	1.71
RSD	0.84	0.43	-	-

Table 2: Linearity of Topotecan

S. No	Linear Solution Range	Actual Range	Concentration (mg/ml)	Peak Area	Mean Area
1	50.0%	51.0%	0.050820	8046471 8073384	8059928
2	75.0%	76.0%	0.076230	11523419 11551337	11537378
3	100.0%	102.0%	0.101640	15333620 15187776	15260698
4	125.0%	127.0%	0.127050	18837709 18747442	18792576
5	150.0%	152.0%	0.152460	22263663 22282430	22273047

Table 3: Recovery Data of Topotecan

S. No.	Amount Added	Peak Area -1	Peak Area -2	Mean Area	Amount Found	Recovery
1	0.076230	11515972	11530865	11523419	0.07622	100
	0.076230	11548522	11554152	11551337	0.07642	100.2
	0.076230	11428866	11490351	11459608	0.07577	99.4
2	0.101640	15337279	15329961	15333620	0.10317	101.5
	0.101640	15206543	15169009	15187776	0.10214	100.5
	0.101640	14923829	14899836	14911833	0.10019	98.6
3	0.127050	18849268	18826149	18837709	0.12795	100.7
	0.127050	18767311	18727573	18747442	0.12732	100.2
	0.127050	18515749	18492040	18503895	0.12559	98.9
					Mean	100
					SD	0.92
					% of RSD	0.92

Calibration curve equation values:

Slope : 141379260

Intercept : 747616

R^2 Value : 0.9979

Method Validation

System Suitability

Resolution was not less than 1.0, number of theoretical plates was not less than 2500, and percentage relative standard deviation (%RSD) for RT was not more than 1.5% and Peak Area was not more than 2.0 % for topotecan peaks.

The %RSD of peak area and RT for the drug are within 2% indicating the suitability of the system (Table 1). The efficiency of the column as expressed by number of theoretical plates for the 6 replicate injections was 12321 and the tailing factor was 1.71.

Table 4: Method Precision for Topotecan

S.No	Solution ID	Conc. (mg/m/L)	Peak Area	Mean Area	Assay
1	Standard solution	0.0997	14606252 14599794 14576183 14551987 14539849 14516824	14565148	100.0
2	Preparation – 1	0.0998	14419224 14427966	14423595	98.9
3	Preparation – 2	0.1016	14892319 14915644	14903982	100.4
4	Preparation – 3	0.1020	14954356 14979308	14966832	100.4
5	Preparation – 4	0.1000	14477400 14530959	14504180	99.3
6	Preparation – 5	0.1016	14907983 14901921	14904952	100.4
7	Preparation – 6	0.1018	14930970 14919689	14925330	100.4
				Mean	100.0
				SD	0.68
				% of RSD	0.68

Table 5: Assay content of Topotecan

S.No	Solution ID	Conc. (mg/mL)	Mean Area	Assay
01.	Standard solution	0.0997	14565148	100.0%
02.	Sample solution	0.1009	14771478	100.0%

Table 6: Solution stability data of topotecan

Time period	Peak Area	Assay Percent
Initial	14686328	100
34hrs	14785541	100
48hrs	14753607	100
72hrs	14802178	100

Determination of Limit of Detection and Limit of Quantitation (Sensitivity)

Different graphs were plotted. (1) Between concentration and average area. (2) Between

concentration and the standard deviation for the above mentioned level concentrations and Limit of detection (LOD) for Topotecan was found to be 0.000023 mg/ml and Limit of quantitation (LOQ) Topotecan was 0.000070 mg/ml.

Linearity

The calibration curve constructed was evaluated by its correlation coefficient. The peak area of the drug was linear in the range of 0.05 to 0.15 mg/ml.

Statistical Evaluation

A graph between the concentration and the average area was plotted. Points for linearity were observed. Using method of least squares a line of best fit was taken and calculated the Correlation Coefficient, Slope, and Y-intercept. Average areas were shown in table 2 and the plot was shown in figure 2.

These experiments indicated that there was a linear relationship between the amounts of analyte and the areas within the range studied.The chromatograms of topotecan extracted from the formulation and pure topotecan can be observed in Figures 3 and 4.

Accuracy

Accuracy of the method was determined by recovery experiments. Accuracy was calculated with respect to above prepared solution at the levels of 75%, 100% and 125% of the normal or target concentration. The accuracy of the method was demonstrated through recovery experiment on 3 samples at concentration 76%, 102% and 127% of the actual concentration employed in the usual procedure. The actual concentration employed in the determination was 0.1 mg/ml of Topotecan.

The amount of Topotecan found in each of these test solutions was calculated using the calibration curve. The table 3 summarizes the amount added vs. amount found and calculated using the calibration curve and percentage recovery. The results revealed that there was a strong correlation between the amount added and amount found for topotecan.

Precision

The precision of the method was demonstrated through two parameters which are injection reproducibility and the method precision.

For method precision, 6 individual samples were prepared from the same batch of the drug substance and measured the individual peak retention time and peak area. The relative standard deviation was calculated for these six samples. For injection reproducibility, six injections from the same

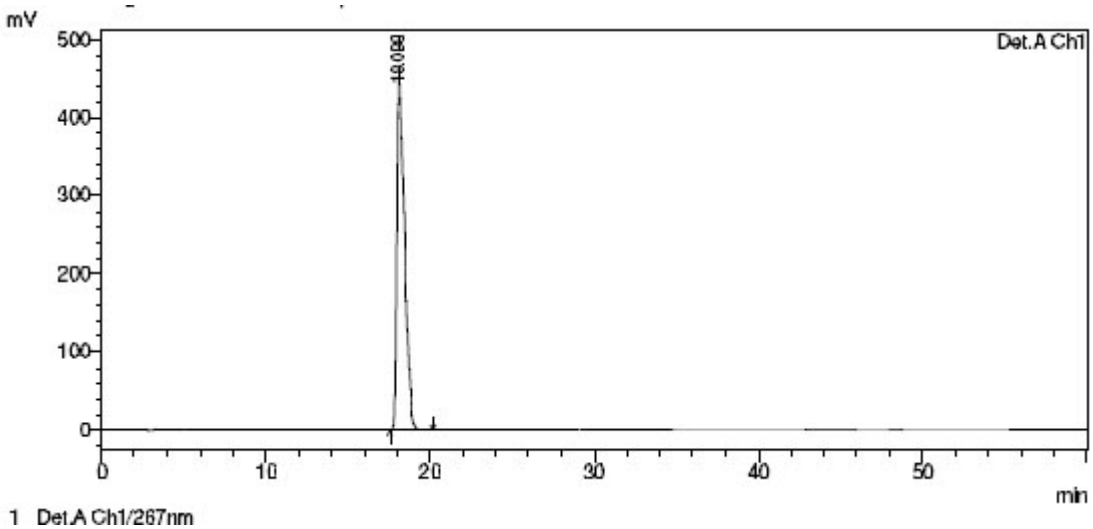

Fig. 4: Chromatogram of topotecan extracted from standard solution

standard preparations were made and the relative standard deviation for the replicate injections was calculated. The results for method precision were given in table 4.

Application of the method to dosage forms

The HPLC method developed is sensitive and specific for the quantitative determination of topotecan. The method is validated for different parameters and hence has been applied for the estimation of drug in pharmaceutical dosage forms. Injections of inventor formulation from TherDose Pharma Pvt. Ltd, India, were evaluated for the amount of topotecan present in the formulation. Each sample was analyzed in triplicate and the amount of topotecan in the formulation was 100 % (Table 5). None of the injection excipients interfered with the analyte peak as seen in the figures 3 and 4.

Stability

The validity of the assay solutions was demonstrated for a period of 72 hours by chromatographing the same solution at periodic intervals.

Procedure

About 50 ml of the standard solution was injected at 0, and 72 hrs onto the column of the chromatographic system and recorded the peak areas in the chromatograms.

Calculations

Observed the areas of the peaks of Topotecan injected at the mentioned time intervals and calculated their corresponding concentrations (Table 6).

Specificity

The specificity of the method was demonstrated by checking the interference of any other peaks with drug peaks. This was performed by injecting the diluent blank and placebo solution to determine whether any impurity peaks in the diluent and placebo solution peaks are co-eluting with topotecan peaks. No interference of peaks eluted in the blank and placebo solution with topotecan peaks was observed.

CONCLUSION

A simple and gradient reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for quantitative determination of topotecan in bulk drug samples and formulations. The method was validated for accuracy, precision, linearity, specificity, limit of detection and limit of quantitation. Topotecan was analyzed by using Zorbax SB – C_{18} (250 mm x 4.6 mm, 5 ìm) at ambient temperature, with gradient elution of Water,Acetonitrile and TFA as a mobile phase (85:15:0.1). The flow rate was set 1.0 ml/min and the analysis was performed at a wavelength of 267 nm using Photo Diode Array (PDA) detector. Efficient UV detection at 267 nm enabled determination of topotecan without any interference from injectable solution excipients or solvents. The retention time (RT) for Topotecan was around 21.50 min. The calibration curves were linear over a concentration range from 0.05 mg to 0.15 mg/ml. Limit of detection (LOD) for Topotecan was 0.000023 mg/ml and Limit of quantitation (LOQ) Topotecan was 0.000070 mg/ml. The developed method was successfully applied to estimate the amount of topotecan in formulations. The proposed HPLC method is precise, sensitive, accurate, specific and efficient and can be used in routine analysis in quality control laboratories.

ACKNOWLEDGEMENTS

Authors are thankful to the management of the college and also to Dr.S.Rama Krishna, IICT, Hyderabad for his support and cooperation in completion of this research work.

REFERENCES

1. Martindale, the Extra Pharmacopoeia, 31st Edition, *Royal Pharmaceutical Society*, 604 (1996).
2. rd KE, Schaiquevich P, Bai F, Fraga CH, Miller L, Panetta JC,Stewart CF, **23**: 707 (2009).
3. de Vries NA, Ouwehand M, Buckle T, Beijnen JH, van Tellingen O, *Biomed Chromatogr*., **21**: 1191 (2007).
4. Gravel E, Bourget P, Mercier L, Paci A, *J Pharm Biomed Anal*., **39**: 581 (2005).
5. Vali AM, Shafaghi B, Dadashzadeh S, *J Chromatogr B Analyt Technol Biomed Life Sci*, **818**: 205 (2005).
6. Chen J, Balthasar JP, *J Chromatogr B Analyt Technol Biomed Life Sci*, **816**: 183 (2005).
7. Bai F, Kirstein MN, Hanna SK, Iacono LC, Johnston B, Stewart CF, *J Chromatogr B Analyt Technol Biomed Life Sci,* **784**: 225 (2003).