INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DEVELOPMENT AND VALIDATION OF LC METHOD FOR THE ESTIMATION OF IRINOTECAN IN PHARMACEUTICAL DOSAGE FORM

Gogineni Ratna Prasad[1*], P. Srinivas Babu[2], Suman Avula[3] and M.V. Ramana [4]

[1]Aurobindo, Unit XII, Quthubullapur, R.R Dist, Andhra Pradesh, India.
[2]Vignan Pharmacy College, Vadlamudi, Guntur, Andhra Pradesh, India.
[3]Cato Research Neuland India, Hydrabad, Andhra Pradesh, India.
[4]Vagdevi College of Pharmacy, Gurazala, Guntur, Andhra Pradesh, India.

*Corresponding Author: rpgogineni@yahoo.co.in

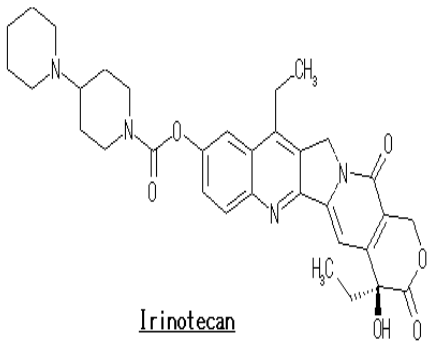
ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Irinotecan in tablet dosage form. An Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing Methanol: Triethylamine: 1% Orthophosphoric acid in the ratio of 60:5:35,(v/v/v) was used. The flow rate was 1.0ml/min and effluents were monitored at 254nm. The retention time for Irinotecan was 5.109min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 0.09ppm and 0.297ppm respectively and recovery of Irinotecan from tablet formulation was found to be 98.304%. The proposed method was successfully applied for the quantitative determination of Irinotecan in tablet formulation.

Keywords: Irinotecan, HPLC, Linearity, Validation.

INTRODUCTION

Irinotecan (Camptosar, Pfizer; Campto, Yakult Honsha) is a drug used for the treatment of cancer. Irinotecan is a topoisomerase 1 inhibitor, which prevents DNA from unwinding. Chemically, it is a semisynthetic analogue of the natural alkaloid camptothecin. Its main use is in colon cancer, particularly in combination with other chemotherapy agents. This includes the regimen FOLFIRI, which consists of infusional 5-fluorouracil, leucovorin, and irinotecan. Irinotecan was approved by the U.S. Food and Drug Administration (FDA) in 1994. During development, it was known as CPT-11.(*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1*H*-pyrano[3',4':6,7]-indolizino[1,2b] quinolin -9-yl-[1,4'bipiperidine]-1'-carboxylate
Molecular Formula $C_{33}H_{38}N_4O_6$
Molecular Mass 586.678g/mol



Irinotecan

Fig. 1: Molecular Structure of Irinotecan

Literature survey revealed that numerous methods have been reported for estimation of Irinotecan in pharmaceutical formulations has been reported.

Present study involves development of LC method using simple mobile phase which is

sensitive and rapid for quantification of Irinotecan in tablet dosage forms as well as subsequent validation of developed method according to ICH guide lines.

EXPERIMENTAL

Instrument

The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Intersil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

Reagents and materials

Methanol of HPLC grade was purchased from E.Merck, Mumbai, India. LC grader water was obtained by double distillation and purification through milli – Q water purification system. Ortho phosphoric acid of analytical grade was procured from qualigens, Mumbai,India.

Preparation of Standard Stock Solution

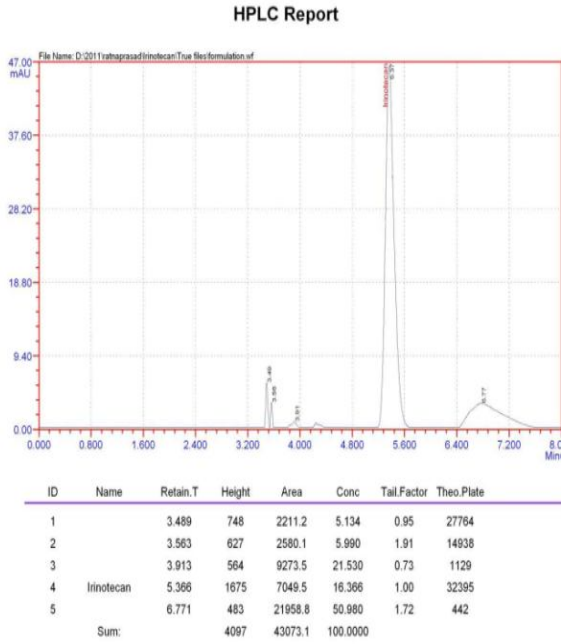
A stock solution of Irinotecan was prepared by accurately weighing 10mg of drug, transferring to 100ml of volumetric flask, dissolving in 25ml of solvent and diluting up to mark with solvent. Appropriate aliquot of this solution was further diluted with solvent to obtain final standard solution of 25ppm of Irinotecan. Resultant solution was filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper.

Preparation of sample Solution

The formulation tablets of Irinotecan were crushed to give finely powdered material. Powder equivalent to 10mg of Irinotecan was taken in 10 ml of volumetric flask containing 5ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 20ppm.

Chromatographic conditions

The mobile phase consisting of Methanol:Triethylamine:1%Orthophosphoric acid were filtered through 0.45µm Ultipor N_{66} Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 60 : 5 : 35,(v/v/v),and was pumped into the column. The flow rate of mobile phase was maintained at 1.0ml/min and detection wavelength was set at 254nm with a run time of 8min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		3.489	748	2211.2	5.134	0.95	27764
2		3.563	627	2580.1	5.990	1.91	14938
3		3.913	564	9273.5	21.530	0.73	1129
4	Irinotecan	5.366	1675	7049.5	16.366	1.00	32395
5		6.771	483	21958.8	50.980	1.72	442
	Sum:		4097	43073.1	100.0000		

Fig. 2: HPLC chromatogram of Irinotecan formulation

Calibration curve

Appropriate aliquots of standard Irinotecan stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 5, 10, 15, 20 and 25ppm of Irinotecan. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Irinotecan was constructed by plotting peak area ratio versus applied concentration of Irinotecan and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Irinotecan in tablet

sample was found out using regression equation.

Method validation
The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness by following procedures.

Accuracy
The accuracy of the method was determined by calculating recovery of Irinotecan by the method of standard addition. Known amount of Irinotecan (10ppm, 5ppm and15ppm.) was added to a pre quantified sample solution and the amount of Irinotecan was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Irinotecan was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Precision
The intra-day precision study of Irinotecan was carried out by estimating the correspondence responses six times on the same day with 25ppm concentration and inter-day precision study of Irinotecan was carried out by estimating the correspondence responses six times next day with 25ppm concentration.

Linearity and range
The linearity of the method was determined at six concentration levels ranging from 5-25ppm for Irinotecan.

Specificity
Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

Limit of detection and limit of quantification
Limit of detection = 0.09ppm
Limit of quantification = 0.297ppm

Stability
In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature.

Robustness
Robustness of the method was studied by changing the composition of organic phase by ±5% and the P^H by ± 0.2, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

RESULTS AND DISCUSSION

The UV spectra of Irinotecan showed that the drug absorbs appreciably at 254nm was selected as the detection wave length in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol: Triethylamine: 1% Orthophosphoric acid in the ratio of 60:5:35,(v / v / v) was used The retention time of Irinotecan was found to be 5.109, which indicates a good base line .

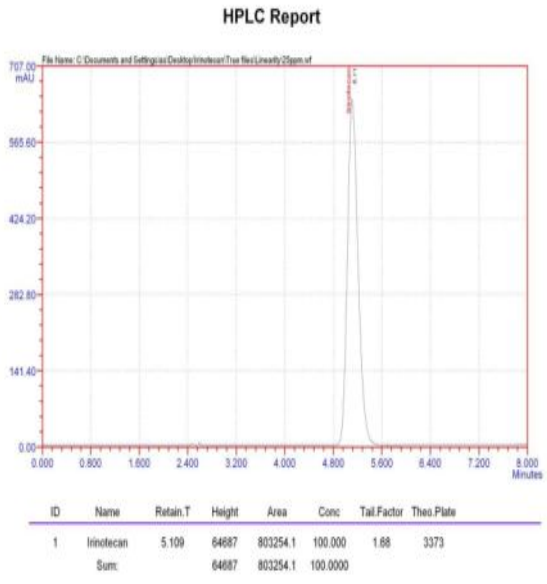


Fig. 3: HPLC chromatogram of Irinotecan

The number of theoretical plates was found to be 3373.31, which indicates efficient performance of the column. The asymmetric factor was found to be 1.68, which indicates asymmetric nature of the peak. The calibration curve for Irinotecan was obtained by plotting the peak area ratio versus the concentration of Irinotecan over the range of 5-25ppm, and it was found to be linear with r^2=0.999. The regression equation of Irinotecan concentration over its peak area ratio was found to be y = 8055.99+ 31572.99 x, where x is the concentration of Irinotecan (ppm) and Y is the respective peak area. The data of regression analysis of the calibration curve was shown in table 1. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantitation for Irinotecan was found to be 0.09ppm and 0.297ppm, indicates the sensitivity of the method. The system suitability and validation parameters were given in table 3. The high percentage of recovery of Irinotecan was found to be 98.304% indicates that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Irinotecan in tablet formulation. The result for Irinotecan was comparable with a corresponding labeled amount (Table 2). The absence of additional peaks indicates no interference of the excipients used in the tablets.

Table 1: Regression analysis of the calibration curve

Parameters	Values
Calibration range (ppm)	5-25ppm
Slope	31572.99
Intercept	8055.99
Correlation coefficient (r^2)	0.999

Table 2: Assay results of tablet formulation

Formulation	Labelled claim (mg)	% of Irinotecan in Tablet
Irnocam	40	9.962

Table 3: System suitability and validation parameters

Parameters	Results
Theoretical plates (N)	3373.31
Retention time (min)	5.109
Asymmetric factor	1.68
LOD (ppm)	0.09
LOQ (ppm)	0.297
Accuracy (%)	99.93
R.S.D. (%)	0.281

CONCLUSION

Proposed study describes new LC method for the estimation of Irinotecan in tablet formulation and serum. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis of estimation of Irinotecan in its tablet formulation and serum.

REFERENCES

1. Innocenti F, Undevia SD, Iyer L. et al. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol. 2004;22(8):1382–8.
2. O'Dwyer PJ and Catalano RB. Uridinediphosphateglucuronosyltransferase (UGT) 1A1 and irinotecan: practical pharmacogenomics arrives in cancer therapy. J Clin Oncol. 2006;24(28):4534–8.
3. Sweetman et al. Martindale: The Complete Drug Reference. 36th edition. 2009. Pharmaceutical Press.
4. British National Formulary. 58th edition. 2009. British Medical Association and Royal Pharmaceutical Society of Great Britain.
5. Electronic Medicines Compendium. (Accessed October 2009).
6. Perry MC. The Chemotherapy Source Book. 4th edition. 2007. Lippincott, Williams and Wilkins.