Research Article

VOL 1, ISSUE (2)

INTERNATIONAL JOURNAL OF RESEARCH AND REVIEWS IN PHARMACY AND APPLIED SCIENCES

A RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF IRINOTECAN IN PHARMACEUTICAL DOSAGE FORMS

Bhadri.Sivaram, Chenna.Swetha , Kollipara.Siva Anusha, Mallidi.Ammi Reddy, E.V.N Raju*,M.I.T.S COLLEGE OF PHARMACY,MADHIRANAGAR,KODAD,NALGONDA(DT),A.P

Article Received on: 18.05.2011 *Article Accepted on: 05-07-2011*

Name: E.V.N Raju

Address: KODAD

Emai: evnrazbiotech@gmail.com

Phone: +919014137291

Corresponding Author

ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Irinotecan in tablet dosage form. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of acetonitrile: methanol 80:20(V/V). The UV detection wavelength was at 210nm. Linearity was observed in concentration range of 10-100ppm. The retention time for Irinotecan was 3.38 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Irinotecan in pharmaceutical dosage forms.

Keywords: Irinotecan, UV detection 210 nm, recovery; precise; C18 Column.

INTRODUCTION

Irinotecan is a drug used for the treatment of cancer. Irinotecan is a topoisomerase 1 inhibitor, which prevents DNA from unwinding. In chemical terms, it is a semisynthetic analogue of the natural alkaloid camptothecin.Its main use is in colon cancer, in particular, 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.

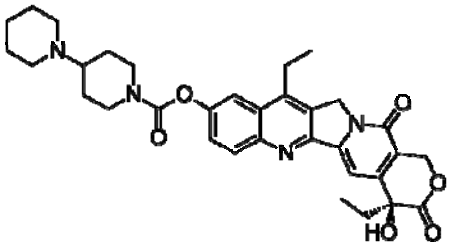


Figure 1: Structure of Irinotecan

EXPERIMENTAL

Chemicals and reagents

Standard drug sample of Irinotecan was provided by V.V.Med Labs. Tablets of combined dosage form were procured from the local market. Other reagents used like Acetonitrile, Methanol which are of HPLC grade were purchased from E.Merck, Mumbai, India

Instrumentation and analytical conditions

The analysis of the drug was carried out on 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 Inertsil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with acetonitrile: methanol 80:20 (V/V) was selected with a flow rate of 1.0 ml min^{-1}.The detection wavelength was set at 210 nm with a runtime of 6 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions

An accurately weighed sample of 100mg of Irinotecan (working standard) was transferred into a 100ml volumetric flask. The solvent methanol was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with methanol. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 100-10 ppm working standard solutions. Calibration solutions were prepared and analyzed immediately after preparation.

The formulation tablets of Irinotecan were crushed to give finely powdered material. Powder equivalent to 10 mg of drug was taken in 10 ml of volumetric flask containing 5 ml 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 60 ppm.

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

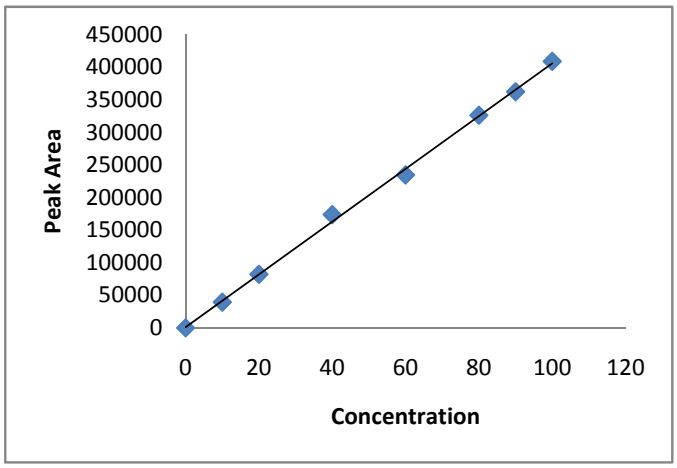
Linearity

The developed method has been validated as per ICH guidelines (Zucman D, 2007). Working standard solutions of Irinotecan in the mass concentration range of 10 ppm to 100 ppm was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Irinotecan was obtained by plotting the peak area ratio versus the applied concentrations of Irinotecan. The linear correlation coefficient was found to be 0.998.

Table 1: Linearity of Irinotecan

S.NO	CONC (ppm)	AREA
1	10	39254
2	20	822001
3	40	173798
4	60	234693
5	80	326356
6	90	36241
7	100	409105

Figure 2: Calibration curve of Irinotecan



Precision

Repeatability of the method was checked by injecting replicate injections of 60 ppm of the solution for six times on the same day as intra day precision study of Irinotecan and the RSD was found to be 1.43.

Table 2: Precision parameters of Irinotecan

CONC 60ppm		
INJECTION	AREA	T.P
1	298196	3713
2	298205	3731
3	296232	3685
4	290149	3406
5	295250	3375
6	304467	3502

Table 3: Linear Regression Data for Calibration curve

Drug	**Irinotecan**
Concentration range	10-100ppm
Slope (m)	4051.1
Intercept (b)	982.7
Correlation coefficient	0.997
% RSD	1.55

Accuracy

The accuracy of the method was determined by calculating recovery of Irinotecan by the method of standard addition. Known amount of Irinotecan (20ppm, 40ppm and 60ppm) 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.

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 2.5 ppm and 8 ppm respectively as per ICH guide-lines.

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied.

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

System Suitability Parameter:

System suitability tests were carried out on freshly prepared standard stock solutions of Irinotecan and it was calculated by determining the standard deviation of Irinotecan standards by injecting standards in six replicates at 6 minutes interval and the values were recorded.

Table 4: System suitability parameters of Irinotecan

Parameters	Values
λ max (nm)	210
Beer's law limit (µg/ml)	10-100
Correlation coefficient	0.998
Retention time	3.38 min
Theoretical plates	5902
Tailing factor	1.19
Limit of detection	2.5 ppm
Limit of quantification	8 ppm

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Irinotecan being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. The elution of the compound from the column was influenced by polar mobile phase. The concentration of the methanol and acetonitrile were optimized to give symmetric peak with short run time 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 acetonitrile: methanol in the ratio of 80:20 (v/v).The retention time of Irinotecan was found to be 3.38 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability and validation parameters are given in Table 5. The high percentage of recovery of Irinotecan was found to be 96.482 indicating 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 labelled amount (Table 5). The absence of additional peaks indicates no interference of the excipients used in the tablets.

CONCLUSION

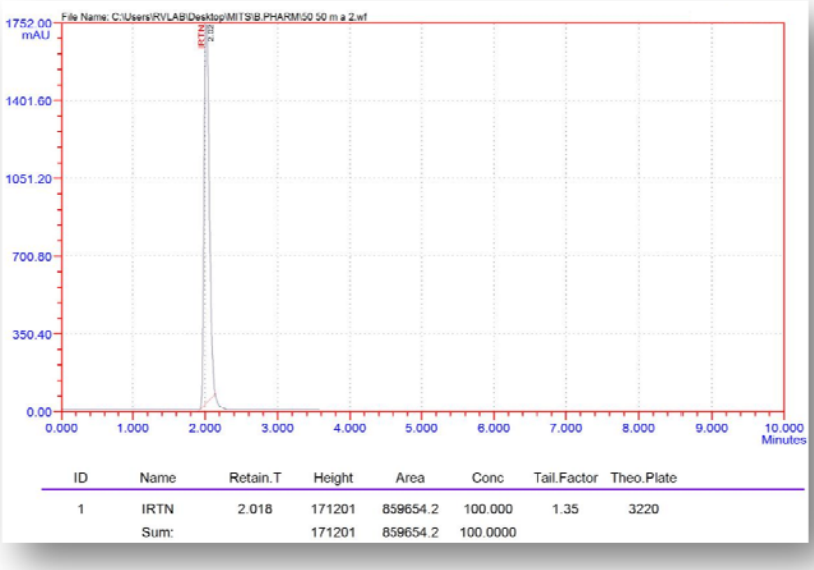
A validated RP-HPLC method has been developed for the determination of Irinotecan in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Irinotecan in pharmaceutical dosage form.

Table 5: Assay results of formulation

Formulation	Labelled claim (mg)	% of Irinotecan in Tablet
Empetus	100	11..224

Table 6: Chromatographic conditions of Irinotecan

S.NO	TEST	RESULT
	CONDITIONS	
1	ELUTION	ISOCRATIC
2	A.P.I CONC	20 ppm
3	MOBILE PHASE	Acetonitrile: Methanol:(50:50)
4	pH	5.4
5	COLUMN	C18
6	WAVE LENGTH	210 nm
7	FLOW	1ml/min
8	RUNTIME	6 minutes
9	RETENTIONTIME	2.018
10	AREA	859654.2
11	TH.PLATES	3220
12	TAILING FACTOR	1.35
13	PUMP PRESSURE	9.5mpa



REFERENCES

1. Kasper, Braun Wald, Fauci, Hauser, Longo,Jameson. Harrison's Principles of Internal Medicine, USA, 16th ed., 2004, 1132.
2. Carolina Lupi Dias, Ana Maria Bergold, Pedro Eduardo Froehlich. UV-Derivative Spectrophotometric determination of Irinotecan Capsules and Comparison with LC Method. Anal Letrs 2009; 42(12):1900-1910.
3. Vaishali P. Nagulwar, Kishore P. Bhusari. Simultaneous Estimation of Irinotecan and Lopinavir by Absorption ratio (Q-analysis) UV Spectrophotometric Method in Combined Tablet Dosage Form. Der Pharmacia Lettre, 2010: 2 (1) 196-200.
4. Dias CL, Rossi RC, Donato EM, Bergold AM and Froehlich PE. LC Determination of Irinotecan, a HIV Protease Inhibitor, in Soft Gelatine Capsules. J chromatographia 2005; 62:589-593.
5. Richard M, Hoetelmans W, Marjolijn Van Essenberg, Monique Profijt, Pieter L. Meenhorst, Jan W. Mulder, *et al*. High-Performance Liquid Chromatographic determination of Irinotecan in human plasma, cerebrospinal fluid and saliva. Journal of Chromatography B: Biomedical Sciences and Applications 1998; 705(1):119-126.
6. Rebiere Herve, Mazel Bernard, Civade Corinne, Bonnet Pierre-Antoine. Determination of 19 antiretroviral agents in pharmaceuticals or suspected products with two methods using High-Performance Liquid Chromatography. Journal of chromatography B 2007; 850:376-383.
7. Yekkala RS, Ashenafi D, Marien I, Xin H, Haghedooren E, Hoogmartens J *et al*. Evaluation of an International Pharmacopoeia method for the analysis of Irinotecan by Liquid Chromatography. Journal of pharmaceutical and biomedical analysis 2008; 48(3):1050-4.
8. Veronica Albert, Pilar Modamio, Cecilia FL and Eduardo LM. Determination of Saquinavir and Irinotecan in human plasma by RP-HPLC and the analytical error function. Journal of Pharmaceutical and Biomedical Analysis 2004; 36(4):835-840.
9. Usami Yoshiko, Tsuyoshi OK, Naka Masahiko, Sagisaka Masafumi, Kaneda Tsuguhiro. A simple HPLC method for simultaneous determination of Lopinavir, Irinotecan and Efavirenz. Journal-Chemical and pharmaceutical bulletin 2003; 51:715-718.
10. Proust V, Toth K, Hulin A, Taburet AM, Gimenez F, Singlas E. Simultaneous High-Performance Liquid Chromatographic determination of the antiretroviral agents' Amprenavir, Nelfinavir, Irinotecan Saquinavir, Delavirdine and Efavirenz in human plasma. Journal of chromatography B 2000; 742:453-458.
11. Sulebhavikar AV, Pawar UD, Mangoankar KV, Prabhunavelkar ND. HPTLC Method for Simultaneous Determination of Lopinavir and Irinotecan in Capsule Dosage Form. E J Chem2008; 5(4):706-712.