International Journal of Multidisciplinary Research and Growth Evaluation.

RP-HPLC method development and validation for the estimation of irinotecan in bulk form and marketed pharmaceutical dosage form

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Article Info

ISSN (online): 2582-7138
Volume: 04
Issue: 03
May-June 2023
Received: 16-04-2023;
Accepted: 02-05-2023
Page No: 419-425

Abstract

A simple, Accurate, precise method was developed for the estimation of the Irinotecan in API and Marketed Pharmaceutical Dosage forms. Chromatogram was run through Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm. Mobile phase containing Acetonitrile, Methanol and 0.1% Ortho Phosphoric Acid taken in the ratio 60:30:10% v/v/v was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.1% OPA. Temperature was maintained at Ambient. Optimized wavelength selected was 235 nm. Retention time of Irinotecan was found to be 2.570min. %RSD of the Method Precision for Irinotecan were and found to be 0.768. %Recovery was obtained as 100.59% for Irinotecan. LOD, LOQ values obtained from regression equations of Irinotecan were 0.08 and 0.24 respectively. Regression equation of Irinotecan is y = 19423x + 5444.4 of Irinotecan. Retention time was decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

DOI: https://doi.org/10.54660/.IJMRGE.2023.4.3.419-425

Keywords: Irinotecan, RP-HPLC, Method Development, Validation, Accuracy, Precision

Introduction

Irinotecan is an antineoplastic enzyme inhibitor primarily used in the treatment of colorectal cancer. It is a derivative of camptothecin that inhibits the action of topoisomerase I. Irinotecan [1] prevents religation of the DNA strand by binding to topoisomerase I-DNA complex, and causes double-strand DNA breakage and cell death. It is a derivative of camptothecin. Irinotecan was approved for the treatment of advanced pancreatic cancer in October, 2015 (irinotecan liposome injection, trade name Onivyde). For the treatment of metastatic colorectal cancer (first-line therapy when administered with 5-fluorouracil and leucovorin). Also used in combination with Cisplatin for the treatment of extensive small cell lung cancer. Irinotecan [2] is currently under investigation for the treatment of metastatic or recurrent cervical cancer. Also used in combination with fluorouracil and leucovorin for the treatment of patients with metastatic adenocarcinoma of the pancreas after disease progression following gemcitabine-based therapy. Irinotecan inhibits the action of topoisomerase I. Irinotecan [3] prevents religation of the DNA strand by binding to topoisomerase I-DNA complex. The formation of this ternary complex interferes with the moving replication fork, which induces replication arrest and lethal double-stranded breaks in DNA. Irinotecan inhibits the action of topoisomerase I. Irinotecan prevents religation of the DNA strand by binding to topoisomerase I-DNA complex. The formation of this ternary complex interferes with the moving replication fork, which induces replication arrest and lethal double-stranded breaks in DNA. As a result, DNA damage is not efficiently repaired and apoptosis (programmed cell death) occurs. The IUPAC Name of Irinotecan is [(19S)-10, 19-diethyl-19-hydroxy-14, 18-dioxo-17-oxa-3, 13-diaza penta cyclo [11.8.0.02, 11.04, 9.015, 20] henicosa-1(21), 2, 4(9), 5, 7, 10, 15(20)-heptaen-7-yl] 4-piperidin-1-yl piperidine-1-carboxylate. The Chemical Structure of Irinotecan is as follows.

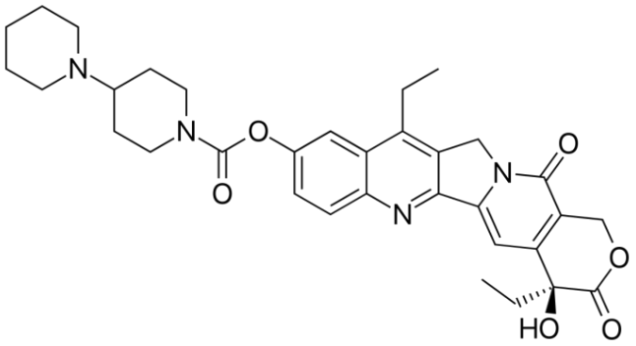

Fig 1: Chemical Structure of Irinotecan

Materials and Methods

Instruments Used

Table 1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**T60-LAB INDIA** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

Chemicals / Reagents Used

Table 2: List of Chemicals used

S. No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric acid	99.9%	A.R.	Loba Chem; Mumbai.
8.	Hydrogen Peroxide	99.9%	A.R.	Loba Chem; Mumbai.

Solubility Study

Table 3: Lists of solvents

Solvents	Solubility
DMSO	Soluble
Ethanol	Soluble
DMF	Soluble
Water	Soluble
Methanol	Freely Soluble
Dichloro Methane	Slightly Soluble
Acetonitrile	Soluble

Method Development and its Validation for Irinotecan by RP-HPLC

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase [4] diluting with the same solvent. (After optimization [5] of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Irinotecan, so that the same wave number can be utilized in HPLC UV detector for estimating the Irinotecan. The scanned UV spectrum is attached in the following page.

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Irinotecan standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Optimization of Chromatographic Conditions

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength [6] & different diluents for sample preparation [7] etc.

Table 4: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol : Acetonitrile = 40 : 60	1.0ml/min	235nm	Very Low response	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol : Acetonitrile = 55 : 45	1.0ml/min	235nm	Low response	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Acetonitrile : Water = 50:50	1.0ml/min	235nm	Tailing peaks	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol : Water = 70:30	1.0ml/min	235nm	Resolution was not good	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN : Methanol: 0.1% OPA = 70:25:5	1.0ml/min	235nm	Tailing peak	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN : Methanol: 0.1% OPA = 60:30:10	1.0ml/min	235nm	Nice peak	Method accepted

Preparation of Mobile Phase

600ml of HPLC Grade Acetonitrile, 300ml of HPLC Grade Methanol and 100ml 0.1% OPA were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration [8].

Method Validation

System Suitability Parameters: The theory of chromatography [9] has been used as the basis for system-suitability tests [10-12], which are set of quantitative criteria that test the suitability of the chromatographic system to identify and quantify drug related samples by HPLC at any step of the pharmaceutical analysis.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity [13] of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

Accuracy: The accuracy [14] of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision [15] may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

a) Repeatability: Repeatability [16] expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

b) Intermediate precision: Intermediate precision[17] expresses within-laboratories variations: different days, different analysts, different equipment, etc.

c) Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Detection Limit: The detection limit [18] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation Limit: The quantitation limit [19] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Linearity: The linearity [20] of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Range: The range [21] of an analytical procedure is the interval between the upper and lower concentration (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.

Robustness: The robustness [22] of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Results and Discussion

Method Development

Selection of Wavelength

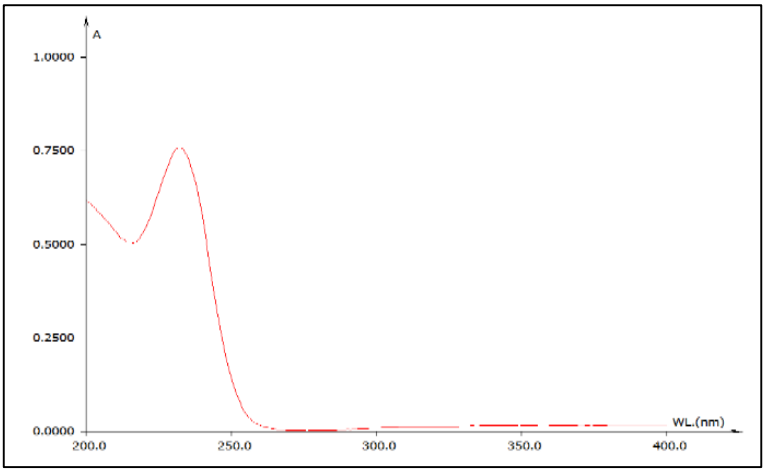

Fig 2: UV Spectrum for Irinotecan

Observation: While scanning the Irinotecan solution we observed the maxima at 235nm. The UV spectrum [23] has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions [24] obtained from experiments can be summarized as below:

Table 5: Summary of optimised Chromatographic conditions

Mobile phase	ACN : Methanol: 0.1% OPA = 60:30:10
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	235 nm
Flow rate	1.0 ml/ min.
Run time	06 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic
Retention time	2.570 minutes

Final Result & Discussion: The selected and optimized mobile phase was ACN: Methanol: 0.1% OPA = 60:30:10 and conditions optimized were flow rate (1.0 ml/minute), wavelength (235nm), Run time was 06 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry [25]. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

Validation of Method

The proposed method was validated according to the ICH guidelines [31] for system suitability, specificity, recovery, precision, linearity, and robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

1. Accuracy

Recovery study

To decide the exactness of the proposed strategy, recuperation thinks about were done by including diverse sums (80%, 100%, and 120%) of unadulterated medication of IRINOTECAN were taken and added to the pre-broke down plan of fixation 10µg/ml. From that rate recuperation esteems were computed. The outcomes were appeared in table-6.

Table 6: Readings of Accuracy

Conc. In ppm	Conc. Found	Peak Area		% Recovery
8	8.035	161523		100.437
8	8.153	163815		101.912
8	8.061	162023		100.762
			Avg.	101.037
			S.D	0.775
			%RSD	0.767046
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
10	9.930	198315		99.30
10	10.033	200320		100.33
10	10.044	200540		100.44
			Avg.	100.0233
			S.D	0.628835
			%RSD	0.628688
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
12	11.981	238151		99.841
12	12.066	239819		100.55
12	12.215	242712		101.791
			Avg.	100.7273
			S.D	0.987021
			%RSD	0.979894

2. Precision

2.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Irinotecan (API). The percent relative standard deviation [26] was calculated for Irinotecan are presented in the table-7.

Table 7: Readings of Repeatability

HPLC Injection Replicates of Irinotecan	Retention Time (Minutes)	Peak Area (AUC)
Replicate – 1	2.572	197236
Replicate – 2	2.570	197762
Replicate – 3	2.573	195969
Replicate – 4	2.570	194724
Replicate – 5	2.574	198327
Replicate – 6	2.573	198711
Average		**197121.5**
Standard Deviation		**1515.213**
% RSD		**0.768667**

Intermediate Precision

Intra-assay & inter-assay

The intra & inter day variation [27] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Irinotecan revealed that the proposed method is precise.

Table 8: Results of Intra-Assay & Inter-Assay

Conc. of Irinotecan (API) (µg/ml)	Observed Conc. of Irinotecan (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.46	0.62	8.05	0.96
10	10.87	0.85	9.43	0.71
12	11.81	0.92	12.04	0.65

3. Linearity & Range

The calibration curve showed good linearity in the range of 6 – 14 µg/ml, for Irinotecan (API) with correlation coefficient [28] (r^2) of 0.999 (Fig-3). A typical calibration curve [29] has the regression equation of y = 19423x + 5444 for Irinotecan.

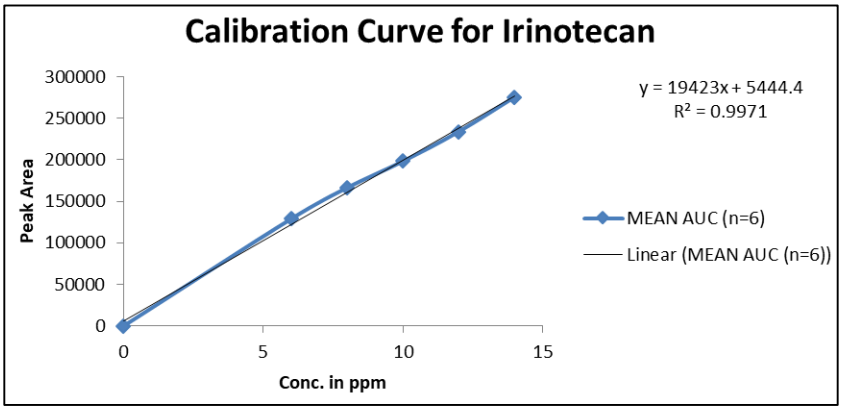

Fig 3: Calibration Curve of Irinotecan (API)

Table 9: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0ppm	0
6ppm	129013
8ppm	166523
10ppm	198315
12ppm	234151
14ppm	275819

Table 10: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.68
Flow (0.9 ml/min)	0.39
More Organic	0.54
Less Organic	0.63
Wavelength of Detection (237 nm)	0.91
Wavelength of detection (233 nm)	0.93

4. Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & organic phase in mobile phase (±5%) studied to determine the robustness of the method are also in favour of (Table-10, % RSD < 2%) the developed RP-HPLC method[30] for the analysis of Irinotecan (API).

5. LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.08 & 0.24µg/ml respectively.

6. System Suitability Parameter

Framework appropriateness testing is an essential piece of numerous scientific techniques.

The tests depend on the idea that the gear, hardware, explanatory activities and tests to be broke down establish a vital framework that can be assessed all things considered. Following framework appropriateness test parameters were built up. The information is appeared in Table-11.

Table-11: Data of System Suitability Parameter

S. No.	Parameter	Limit	Result
1	Resolution	Rs > 2	8.47
2	Asymmetry	T ≤ 2	Irinotecan=0.23
3	Theoretical plate	N > 2000	Irinotecan=2987
4	Tailing Factor	T<2	Irinotecan=1.17

7. Estimation of Irinotecan in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. strategy was taken after to decide the normal weight. Above measured tablets were at last powdered and triturated well. An amount of powder proportionate to 25 mg of medications were exchanged to 25 ml volumetric flagon, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with versatile stage. The arrangement was separated through a layer channel (0.45 µm) and sonicated to degas. The arrangement arranged was infused in five reproduces into the HPLC framework and the perceptions were recorded.

A copy infusion of the standard arrangement was additionally infused into the HPLC framework and the peak regions were recorded. The information is appeared in Table-12.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of medication acquired with test arrangement

AS = Peak Area of medication acquired with standard arrangement

WS = Weight of working standard taken in mg

WT = Weight of test taken in mg

DS = Dilution of Standard arrangement

DT = Dilution of test arrangement

P = Percentage virtue of working standard

Table 12: Recovery Data for estimation Irinotecan in Campto

Brand Name of Irinotecan	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Campto Tablets (Pfizer)	100mg	99.789 (±0.586)	99.89 (±0.578)

Result & Discussion: The amount of drug in Campto Tablets was found to be 99.789 (±0.586) mg/tab for Irinotecan & % assay was 99.89 %.

Summary and Conclusion

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Irinotecan, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Symmetry ODS RP C_{18}, 5µm, 15mmx4.6mm i.d. Column was preferred because using this column peak shape, resolution and absorbance were good.

Discovery wavelength was chosen in the wake of examining the standard arrangement of medication more than 200 to 400nm. From the U.V range of Irinotecan it is apparent that a large portion of the HPLC works can be proficient in the wavelength scope of 210-300 nm helpfully. Further, a stream rate of 1 ml/min and an infusion volume of 10µl were observed to be the best investigation. The outcome demonstrates the created technique is amazingly, one more reasonable strategy for measure and dependability related debasement examines which can help in the investigation of Irinotecan in various details.

A sensitive& selective RP-HPLC method has been developed & validated for the analysis of Irinotecan. Encourage the proposed RP-HPLC technique has astounding affectability, accuracy and reproducibility. The outcome demonstrates the created technique is amazingly, one more appropriate strategy for test, immaculateness and solidness which can help in the examination of Irinotecan in various definitions.

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