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Development and Validation of Stability Indicating RP-HPLC Method for the Determination of Irrinotecan in Bulk and It's Pharmaceutical Dosage Form

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ABSTRACT

A simple, sensitive, and specific reverse phase high performance liquid chromatographic method was developed and validated for the determination of Irinotecan in bulk and tablet dosage forms. The HPLC separation was carried out by reverse phase chromatography on Waters (HPLC-2695) model with Inertsil ODS 250 X 4.6 mm and 5µ particle size column .The mobile phase was consist of 0.02 M KH_2PO_4 Buffer (pH was adjusted to 3.2 with Ortho phosphoric acid) and Acetonitrile mixed in the ratio of 40: 60 v/v . The mobile phase flow rate was set as 1 ml/min and total analysis was performed at wave length 222 nm with PDA Detector at ambient temperature. The developed HPLC method was validated and stability studies were conducted under different conditions. Irinotecan compound was eluted at 2.1 min and the calibration curve was linear over the concentration range 40 – 120 µg/ml. Correlation coefficient was found to be 0.9999. % RSD of irinotecan content was found to be < 0.5%. Recovery was found to be 99.1%. The developed HPLC method was Robust and Rugged and successfully followed for the estimation of Irinotecan content present in injection dosage form as well in API.

Keywords: Irinotecan, Mobile phase, HPLC analysis, Method validation.

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1. Introduction

Irinotecan is an antineoplastic agent primarily used in the treatment of Metastatic colorectal cancer. Irinotecan is a semi synthetic derivative of Comptothecin. Camptothecin is an alkaloid extracted from plant such as Camptotheca accuminata belongs to family nyssaceae. Camptothecin is treated with phosgene gives 7-ethyl -10-(chloro carbonyl

oxy) camptothecin which is then condensed with 4-(1-piperidyl) piperidine gives Irrinotecan (T.Myaska). Its main use is in colon cancer, in particular, in combination with other chemotherapy agents.

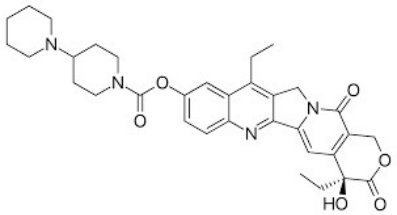

Fig 1: Chemical Structure of Irrinotecan

Literature survey reveals that very few methods have been reported for the determination of Irrinotecan in individually and combination with other drug. There is no reported for the RP-HPLC methods. So, I need to develop simple, precise, accurate and robust RP-HPLC method for the determination of Irrinotecan in bulk and it's Pharmaceutical dosage form according to ICH guidelines.

2. Materials and Methods

Chemicals: The following chemicals and reagents are used for the determination of Irrinotecan by RP-HPLC.

Table 1: List of Chemicals and Reagents

Chemical Name	Company name
Irinotecan reference standard	Dr. Reddy's Laboratories LTD.
Irinotecan injection 5 ml	Purchased commercial sample from the market
Acetronitrile	Spectrochem PVT. LTD, Mumbai
De ionized water	In house LABCONCO water purification system
KH_2PO_4 Buffer	Merck Pvt. Ltd, Mumbai
Ortho phosphoric acid	Merck Pvt. Ltd. Mumbai
H_2O_2 (3 % w/v)	Ranbaxy fine chemicals, Mumbai
NaOH	Loba chemie pvt. Ltd. Mumbai
Zinc	Merck Pvt. Ltd., Mumbai
HCl	Merck pvt. Ltd. Mumbai

Instrument:

Waters HPLC system equipped with quaternary pump, column oven, PDA detection and Empower -2 software for data acquisition. pH instrument for adjustment of buffer pH.

Methods

Preparation of standard stock solution:

Weigh accurately 5 mg of Irinotecan standard and transferred to a 25 ml volumetric flask. Dissolved with about 15ml of mobile phase by sonication and finally filled up to the mark with mobile phase. From the stock solution further dilutions were prepared with mobile phase to the required concentrations.

Preparation of test stock solution:

An accurately measured volume of injection equivalent to 5 mg of Irinotecan i.e., 0.25 ml was transferred into 25ml volumetric flask. Dissolved it with 15ml of Mobile phase by sonication and finally made up the volume up to the mark. From this test stock solution further dilutions were prepared by diluting the appropriate volume of stock solution with mobile phase.

Preparation of KH_2PO_4 buffer solution:

Buffer solution with 0.02 M was prepared and the pH of the buffer was adjusted with ortho phosphoric acid to pH 3.2 and was adjusted by using pH meter.

Preparation of Mobile phase:

KH_2PO_4 buffer solution and acetonitrile were mixed in the ratio of 40:60v/v and it was filtered through 0.45µm membrane filter and degassed for 10 min with sonication.

Selection of the column:

Inertsil ODS (250 x 4.6mm; 5µ) column was selected as the column as it is one of the most robust, reproducible and reliable RP-HPLC columns. This column was found to be stable at the desired pH and temperature. It offers good peak symmetry. Columns with 5µm particle size give the best compromise of the efficiency.

Selection of Wavelength:

The wavelength of maximum absorption (λ_{max}) of the drug 10µg/ml solution of the drugs in methanol were scanned using UV-visible spectrophotometer within the wavelength region of 200-400nm against methanol as blank. The resulting spectra are shows characteristic absorption maxima at 222nm for the combination.

Optimized Chromatographic Conditions:

Column : Inertsil ODS (250 x 4.6mm; 5µ)
Mobile phase : Buffer: Acetonitrile 40: 60
Mode of separation : Isocratic
Buffer and strength : KH_2PO_4 buffer with 0.02M
PH of buffer : pH 3.2 with ortho phosphoric acid
Flow rate : 1 ml/ min
Column temperature : Ambient
Run time : 10 min
Injection Load : 20 µl
Wave length : 222 nm with UV Detection

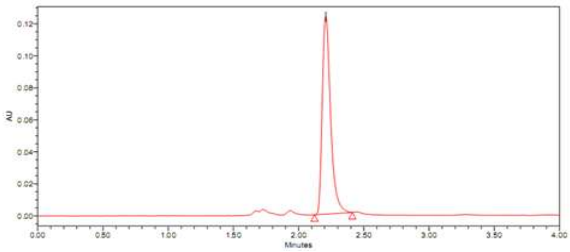

Fig 2: Optimized chromatogram of Irrinotecan

Method Validation:

Validation of an analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the intended analytical application. Typical analytical parameters used in assay validation are system suitability, specificity, linearity, accuracy, precision, LOD & LOQ and Robustness. Validation was carried on according to ICH guidelines Q2(R1).

3. Results and Discussion

System suitability:

It is essential for the assurance of the quality performance of chromatographic system. System suitability tests were carried out on freshly prepared standards stock solution of Irinotecan. Equal volume of standard concentration was mixed well. From the prepared solution 20µl of the sample was injected into HPLC system and results obtained were used to express the system suitability of the developed method. The ascertain the performance of the method a number of parameters such as retention time , resolution, peak asymmetry, theoretical plate have been calculated with the observed reading and the results are recorded in the table 2.

Specificity:

The method specificity was assessed by comparing the chromatograms obtained from a placebo solution containing a mixture of most commonly used excipients without the drug and another solution containing the excpeints with the drug. These solutions were prepared in the diluent. The drug to excipient ratio used was similar to that in the commercial formulation. The mixtures were filtered through a 0.45 µ membrane filter before injection. The placebo solution and the sample solution (placebo and the drug) were injected into HPLC system separately in triplicate and the relevant chromatograms observed. There was no interference from blank and placebo at the retention time of analyte peak. The absence of additional peaks in the chromatogram indicates noninterference of the commonly used excipients in the tablets and hence the method is specific.

Interference from degradation products:

Accelerated degradation studies like heat, acid, base, photolytic, oxidation, water and reduction degradation studies were conducted to demonstrate the specificity. The degradation products were well resolved from main peak and degradation peaks were eluted approximately 4.1, 1.1 and 6.2 min and 5.2. In case of oxidative and heat degradation do not form any interference. Results were given in table 3 & 4.

Linearity:

To demonstrate linearity 5 standard solution were prepared ranging from 50 -150 % to the target test assay concentration (80µg/ml). The calibration curve was constructed by taking the concentration on X axis and response on Y axis. And it was evaluated by its correlation coefficient. The analyte peak area was linear within the range of 40 to 120µg/ml. And correlation coefficient was found to be 0.9999. So it has strong correlation between the area and the concentration under the curve. The results were given in table 5. Calibration curve was obtained by plotting peak area verses concentration Fig. 5.

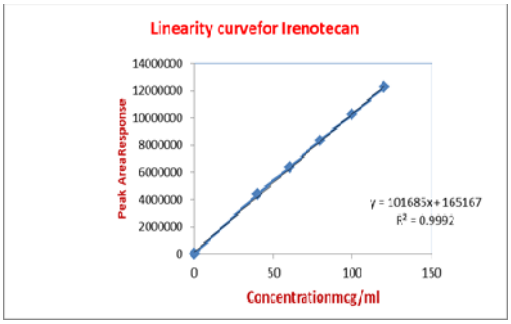

Fig 5: Linearity curve of Irinotecan

Precision:

The method precision was estimated by injecting six times of sample of a single batch having same concentration of standard (80µg/mL of Irinotecan) and recorded the response of the peak areas. The results are reported in Table No.6.

Accuracy

The accuracy of method was expressed in terms of recovery of added compound and % recovery was calculated by multiplying the ratio of measured concentration with 100 so as to give the %recovery. Calculated mean % recovery and % RSD. These were found to be 99.1 and 0.46. Based on obtained results the method represents HPLC method was accurate. The results were given below in table form 7.

LOD & LOQ:

The parameters such as LOD and LOQ were determined on the basis of signal to noise ratio. LOD and LOQ were calculated approximately LOD and LOQ was found to be 0.8ppm and 2ppm respectively.

Robustness:

System suitability tests were performed after making the deliberate changes in flow rate; buffer PH, mobile phase composition. The results were given below and observed as per accepted level. So developed HPLC method was robust %). Results were represented in below given table.8.

Ruggedness:

Rugged ness of assay method experiments were conducted on analyst to analyst, column to column, system to system and day to day variability as per test method. Samples were analysed by replicate analysis of 6 times and calculated the %assay %RSD. Results were given below and obtained results were with in accepted level. Average assay and %RSD of 6 replicate analyses from both variations were found to be 99.25, 99.3 and 1.01, 0.79.The overall assay of %RSD was found to be 0.9%.The results were should not vary more than 2% from one variation to another variations. These results indicate that the developed HPLC analytical method was rugged and provide consistent and reliable results. %). Results were represented in below given table 9.

Table 2: System Suitability Results

Injection	Retention time (min)	Area of analyte	Plate count	Tailing factor
1	2.158	8370847	2295	1.22
2	2.142	8372813	2289	1.21
3	2.167	8376715	2320	1.22

4	2.156	8371638	2290	1.23
5	2.154	8371824	2323	1.21
6	2.159	8372438	2315	1.24
Mean	2.156	8372713	2305	1.22
S D	0.081	2075.08	15.68	0.01169
% RSD	0.375	0.024	0.680	0.958

Table 3: Physical degradation results

Condition	**Heat stress**	**UV light stress**
Temp/intensity	105º C	UV light
Time	6 hrs	7 days
Analyte Rt (min)	2.15	2.15
Impurities Rt (min)	4.5	5.0

Table 4: Chemical degradation Results

	Acid degradation	**Base degradation**	**Oxidation degradation**	**Reduction degradation**	**Water stress**
Concentration	1 N HCl	1 N NaOH	30% w/v H2O2	1 N HCL in zinc	Water
Reflection time (min)	60	60	60	60	60
Reflux temp (ºC)	60	60	60	60	60
Analyte Rt	2.15	2.15	2.15	2.15	2.15
Impurity Rt	4.1	1.9, 2.7	4.7	5.1	1.1

Table 5: Linearity Results

Injection	**% conc.**	**Conc. of analyte (µg/ml)**	**Response**	**% purity**
1	50	40	4393513	98.8
2	75	60	6359898	98.89
3	100	80	8370847	99.6
4	125	100	10258390	99.8
5	150	120	12282459	99.8
Correlation coefficient 0.9999, Slope: 1.01636E-05.				

Table 6: Precision Results

	Irinotecan	
S.No.	Retention time	Peak Area
1	2.158	8370847
2	2.142	8372813
3	2.167	8376715
4	2.156	8371638
5	2.154	8371824
6	2.159	8372438
AVG	2.154	8374152
SD	23.02	532.0
%RSD	0.023	0.035

Table 7: Accuracy Results

Injection	**% assay**	**mg added**	**mg recovered**	**% recovered**	**Mean% recovered**
50 %prep.1	99.6	0.064	0.06374	99.5	99.2
50% prep.2	98.5	0.064	0.06304	98.5	
50% prep.3	99.8	0.064	0.06387	99.7	
100%prep.1	98.3	0.080	0.07864	98.3	98.6
100%prep.2	98.6	0.080	0.07888	98.6	
100%prep.3	99.1	0.080	0.07928	99.1	
150%prep.1	99.4	0.096	0.09542	99.3	99.5
150%prep.2	99.1	0.096	0.09513	99.5	
150%prep.3	98.2	0.096	0.09427	99.7	
Mean					99.1

SD	0.458
% RSD	0.462

Table 8: Robustness Results

Parameter		% RSD of % recovery	Tailing factor	Plate count	% Recovery
Flow rate ± 0.2	1.2 ml	0.92	1.3	2345	99.2
	0.8 ml	1.1	1.4	2549	99.5
Temperature± 5 ºC	20	0.64	1.2	2285	99.1
	30	0.70	1.3	2299	99.3
Mobile phase composition ± 5 ml	45:55	1.1	1.3	2472	98.6
	35:65	1.3	1.2	2283	98.7
pH of buffer ± 0.2	3.4	0.83	1.4	2457	99.5
	3.0	1.01	1.2	2269	99.8

Table 9: Ruggedness Results

Parameter	Variation	
Analyst	Analyst – 1	Analyst – 2
HPLC	System 1	System 2
Column ID	Inertsil ODS	Chromosil ODS
Day	27-04-09	04-05-09
Injections	% Assay	% Assay
1	99.2	98.2
2	98.1	100.3
3	98.5	98.6
4	100.7	99.8
5	100.2	99.4
6	98.8	99.7

4. Conclusion

A novel simple chromatographic method developed according to ICH principle recommended conditions. The total analysis time completed within 4 min respectively and results exhibited that an good correlation exists between peak area and concentration of Irinotecan and calibration curves were linear showing R^2between 0.9999-1.0 over a concentration range of 40-120 µg/ml for Irinotecan respectively. Sample recovery studies shows in all the formulations were in good agreement with their respective label claim and their suggestive no interference of formulation excipients in the estimation. Low values of standard deviation that indicates that high precision of the method. The robustness test results confirms that the test preparation solution was not affected and it was in accordance with that of actual. The proposed method is sensitive enough for the quantitative detection of analytes in the pharmaceutical preparation.

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