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Development and Validation for the Determination of Related Substance in Irinotecan HCl formulation and its Stability Indicating Assay by RP- HPLC method

Rajasekaran Aiyalu[1]*, Rambabu Cherukuri[1], Dharuman Joghee[1].

1.Department of Pharmaceutical Analysis, KMCH College of Pharmacy, India

ABSTRACT

A simple isocratic RP-HPLC stability indicating method has been developed and subsequently validated for the determination of Irinotecan HCl and its related substance (SN-38) in pharmaceutical dosage forms as per ICH guidelines. The separation achieved on a reversed phase Phenomenex Luna C_{18} Column (5µ, 250 × 4.60 mm) as a stationary phase and 0.5% trichloro acetic acid: Acetonitrile: Methanol (60: 20: 20 v/v/v) as mobile phase at a flow rate of 1.0 ml/min. The UV detection was performed at 372 nm. The retention time for Irinotecan HCl and SN-38 was found to be 8.65 and 7.30 min respectively. The detector response was linear in the concentration range of 30-150 µg/ml. The respective linear regression equation being Y= 5233.x + 13299 with R^2 = 0.999. The percentage of Irinotecan HCl in pharmaceutical dosage form was found to be 100.5% and the percentage of related substance (SN-38) in formulation was found to be 0.19%. The limit of detection and the limit of quantification were found to be 0.014 µg/ml and 0.045 µg/ml respectively. The results of the study showed that, the proposed RP-HPLC method was simple, rapid, precise, accurate and stability indicating, which can be used for the routine determination of Irinotecan HCl and its related substance (SN-38) in pharmaceutical dosage form.

Keywords: Irinotecan HCl, Related substance, RP-HPLC, SN-38.

*Corresponding Author Email: rsekaran2001in@yahoo.co.in

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INTRODUCTION

Irinotecan HCl, is a synthetic analogue of the natural alkaloid, camptothecin chemically called as S)-10-[4-(-piperidino) piperidinocarbonyl oxoyl]-4,7-diethyl-4-hydroxy-1H-pyrano[3,4:6,7]indolizino[1,2-b]diethyl-3,14[4H,12H]-dione. It is a chemotherapeutic agent, which has the potency to inhibit the action of topoisomerase I[1]. Irinotecan prevents religation of the DNA strand by binding to topoisomerase I-DNA complex, and mainly used as a drug of choice in colon cancer. The most significant adverse effects of irinotecan are severe diarrhea and extreme suppression of the immune systems. Irinotecan- associated diarrhea is severe and clinically significant, sometimes leading to severe dehydration requiring hospitalization. Also, the immune system is adversely impacted which is reflected in dramatically lowered white blood cell counts in blood. SN-38 (7-ethyl-10-Hydroxycamptothecin) is one of the metabolite/related substance present in irinotecan HCl[2,3]. Hence, it's an indispensable to quantify the related substance in Irinotecan HCl formulation and its stability indicating studies. Chemical structure of Irinotecan HCl and SN-38 are given in Figure.1 and 2. Irinotecan HCl is officially listed in Indian Pharmacopeia (2007), which describes a HPLC method for its estimation in injection formulation. Several HPLC methods[4-15], LS-MS methods[16,17], HPTLC method[18], visible spectrophtometric method[19] and a cyclic voltametric method[20] has been reported for the determination of Irinotecan HCl. No studies had reported on the estimation of related substance and forced degradation studies of Irinotecan HCl by RP-HPLC. Hence the present study was under taken to develop and validate a method for determination of related substance in Irinotecan HCl formulation and its stability indicating studies by RP-HPLC.

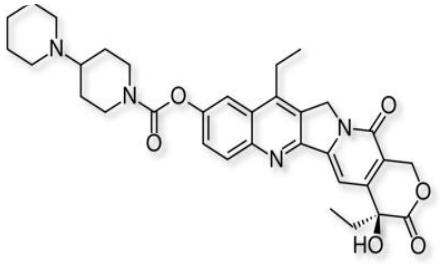

Figure.1 Irinotecan

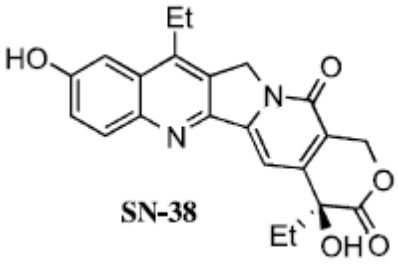

Figure 2 SN-38 (7-ethyl 10-hydroxy camptothecin)

MATERIALS AND METHODS

Materials

Acetonitrile, Water and Methanol were of HPLC grade. Dihydrogen sulphoxide and trichloro acetic were of Analytical grade. Drug samples, Irinotecan HCl (Irinocam) was obtained from Dr.

Reddy's Laboratories and -ethyl 10-hydroxy camptothecin (SN-38) was obtained from Henan Dongtai Pharm Co.Ltd, China.

Instruments used

Elico pH meter LI 127, Shimadzu LC-20 AT HPLC, Shimadzu 1700 UV Spectrophotometer, Sonica Ultrasonic cleaner, solvent filtration unit – Millipore, Shimadzu electronic balance AX 200.

Methods

A literature survey revealed that estimation of Irinotecan HCl was done by various analytical methods such as Visible Spectrophotometric method (Balaram *et al*., 2011), RP-UPLC method (Saini *et al*., 2009) and HPLC method (Shende *et al*., 2009; Satyanarayana et al., 2009; Venkateswara rao *et al*., 2007; B. Mohammed Ishaq *et al*., 2010; Gogineni Ratna Prasad *et al*., 2011). Estimation of related substance in Irinotecan HCl was done mostly in human plasma (Iman Barilero *et al*., 1995; Iman Barilero *et al*., 1992; wei Zhang *et al*., 2009 and Owens *et al*., 2008). Sushama Talegaonkar *et al*., 2011, developed the stability indicating studies of Irinotecan HCl by HPTLC. Ali mohammad *et al*., 2010, developed a simultaneous estimation of Irinotecan HCl and SN-38 by RP-HPLC. No studies had reported on the estimation of related substance and forced degradation studies of Irinotecan HCl by RP-HPLC. Hence the present study was under taken to develop and validate a method for determination of related substance in Irinotecan HCl formulation and its stability indicating studies by RP-HPLC.

Standard solutions and HPLC conditions

The separation achieved on a reversed phase Phenomenex Luna C_{18} Column (5µ, 250 × 4.60 mm) as a stationary phase and filtered and degassed mixture of acetonitrile, methanol and 0.5% trichloro acetic acid (20: 20: 60 v/v/v) was employed as mobile phase at a flow rate of 1.0 ml/min. UV Detection was carried at 372 nm at ambient temperature.

Preparation of standard solution

Standard stock solution a (10 µg/ml) was prepared by dissolving 1 mg of Irinotecan HCl in 2 ml methanol and made up to 100 ml with mobile phase. Stock solution B (10 µg/ml) was prepared by dissolving 1 mg of SN-38 with few 2 ml of dihydrogen sulphoxide until the sample dissolved completely and made up to 10 ml with mobile phase. Standard solutions of Irinotecan HCl were prepared to 30 µg, 60 µg, 90 µg, 120 µg and 150 µg/ml with mobile phase. All the solutions were sonicated for 20 min before injection.

Preparation of sample solution

One ml of Irinotecan HCl injection (20 mg) was diluted to 10 ml of methanol and then serial

dilutions were made with mobile phase to get final concentrations of 60 and 120 µg/ml. The contents were then filtered through 0.45 µm membrane filter and sonicated for 20 minutes. With an optimized chromatographic conditions a steady baseline was recorded after stabilization, the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas were found satisfactory and finally 20 µl of the standard solution of the individual samples of Irinotecan HCl and SN-38 and the chromatograms were recorded (Figure.3 and 4). Successive aliquots of 20 µl of mixed standard solutions were injected and the chromatograms were recorded for linearity, this procedure was repeated using the sample solution and the chromatogram was shown in (Figure. 5). The peak areas were noted for the standard and sample solutions and compared. The elution order of mixture was found to be Irinotecan HCl (retention time 8.6 min) and SN-38 (retention time 7.3 min).

RESULTS AND DISCUSSION

Method development

The drugs selected in the present study are in polar nature thus RP-HPLC method was chosen because of its simplicity and suitability. Preliminary studies with C_{18} and various mobile phases with different ratios were tried for the effective separation of Irinotecan HCl and SN-38. Phenomenex Luna C_{18} column (250 mm x 4.6 mm with 5 µm inner diameter) eluted with a mobile phase of acetonitrile: methanol: 0.5% trichloro acetic acid (20: 20: 60 v/v/v) at a flow rate of 1.0 ml/min and optimum wavelength of analytes was found 372 nm for best separation of Irinotecan HCl and SN-38.

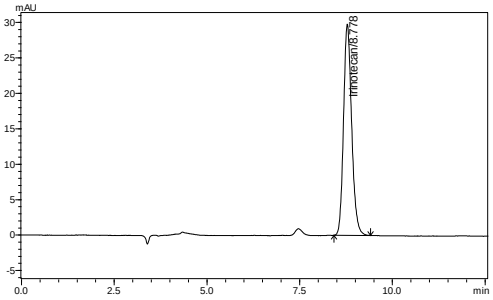

Figure. 3 HPLC chromatogram for standard Irinotecan HCl

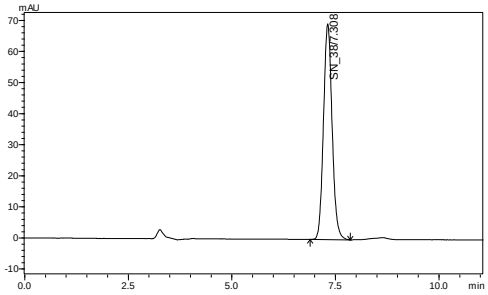

Figure4 HPLC chromatogram for standard SN-38

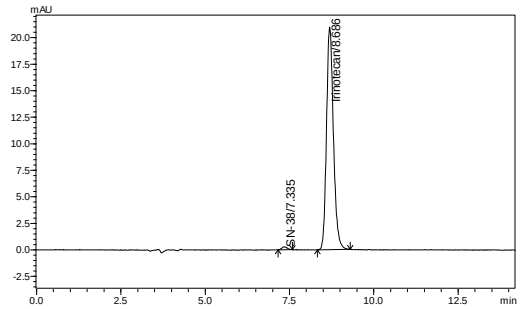

Figure 5 Linearity for Irinotecan HCl

Method validation

After method development of HPLC for the estimation of Irinotecan HCl in a dosage form, the method was validated according to USP guidelines. Analytical method for quantification of finished products includes accuracy, precision, linearity and range, limit of detection (LOD) and limit of quantification (LOQ), robustness and ruggedness and system suitability studies.

Linearity and range

To evaluate linearity of the proposed method with different concentrations of analytes in the range of 30-150 µg/ml for Irinotecan HCl (Table 1) and the linearity between the peak-area and the concentration was examined for each analytes. Correlation coefficient was found to be 0.999 for Irinotecan HCl and 1.000 for SN-38. The linearity study was in the range of 30 to 150 µg/ml with the correlation coefficient of 0.999 for Irinotecan HCl and 1.000 for SN-38.

Table. 1 Linearity studies for irinotecan HCl

S.NO.	Conc. of Irinotecan (µg/ml)	Peak areas of Irinotecan	Peak areas of SN-38
1	30	178350	38796
2	60	335147	77592
3	90	490370	117399
4	120	640000	155184
5	150	791000	193980

Accuracy (recovery)

The accuracy of the method was determined by recovery studies. A known quantity of the pure drug sample was added to the pre-analyzed same formulation at 50%, 100% and 150% levels. The recovery studies were carried out 6 times at each level and the percentage recovery was found to be 98.54%, 99.10% and 99.28%. Percentage relative standard deviation of the percentage recovery was found to be 0.40% (Table 2).

Results of accuracy have shown that the mean recovery of the assay is within the specified limits and the %RSD is lower than 1.0%. The percentage %RSD values for the precision study were 0.45% (system precision) and 0.34% (method precision)

Table. 2 Accuracy studies for Irinotecan HCl

Drug	Label Claim mg/ml	Spike Level (%)	Amount of drug added (mg)	Amount of drug recovered (mg)	Mean Peak area	Percentage Recovery ± SD	%RSD*
Irinotecan	20 mg	50	50	49.27	330386	98.54 ± 0.45	0.45
		100	100	99.10	670722	99.10 ± 0.40	0.40
		150	150	148.93	998654	99.28 ± 0.35	0.36

*-Each value is the mean of six observations.

Table. 3 Intraday stability studies for Irinotecan HCl

No of Injection	Conc. of Irinotecan(µg/ml)	Peak Area	%RSD*
6	60	330193 332491 334384 330569 331726 332549	0.45

*-Each value is the mean of six observations.

Table. 4 Interday stability studies for Irinotecan HCl

Day	Conc. of Irinotecan(µg/ml)	Peak Area	% RSD*
Day 1	120	522253 524775 526365 522786 524543 525465	0.29
Day 2	120	523153 525675 527765 522786 525543 525465	0.35
Day 3	120	522153 525775 527865 522786 525543 525465	0.40

*-Each value is the mean of six observations.

Precision

The precision of this method was determined by studying repeatability and reproducibility. The RSD of the peak areas of six replicates was found 0.45 for Irinotecan HCl in intraday studies

(Table 3). Furthermore inter day studies also performed RSD was found to be 0.34 (Table 4). The %RSD values were found to be less than 1%, confirming good precision of the method.

Selectivity

Selectivity of the proposed method was demonstrated with good separation of the two analytes from each other, the additional peaks were observed in the chromatogram of the formulation which may be due to excipients present in the formulation.

Robustness

Robustness of an analytical method 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. robustness of the current method was investigated by analyzing samples of the drug product using the same chromatographic conditions set forth in method development but with a small change in the following chromatographic parameters: (a) flow rate: 1.2 ml/min instead of 1.0 ml/min, (b) detection wavelength: 375 nm instead of 372 nm, and (c) change of Column: Column C_{18} (Nucleosil) instead of Column C_{18} (Phenomenex), the results are reported in Table 5.

Table. 5 Robustness studies for Irinotecan HCl

Chromatographic Condition	Irinotecan HCl Retention Time(R_t)	% Assay
Wavelength at 372	8.68 ± 0.05	100.5%
Wavelength at 375	8.59 ± 0.05	97.82%
Column C_{18} (Nucleosil)	7.32 ± 0.07	98.27%
Column C_{18} (Phenomenex)	8.68 ± 0.05	99.12%
Flow rate 1.0 ml/min	8.65 ± 0.06	98.89%
Flow rate 1.2 ml/min	6.67 ± 0.06	95.58%

Ruggedness

Ruggedness of the current method was further demonstrated by analyzing six samples (assay) of injection formulation by two analysts in the same laboratory at different days. The RSD for the 12 samples were found to be 0.45% and 0.44% (Table 6). System stability studies were also conducted and the data are reported in Table 7.

Table. 6 Ruggedness studies for Irinotecan HCl

Drug name	Concentration (µg/ml)	Mean peak area	%RSD*
Day-1 analyst-1 Irinotecan HCl	60	332549	0.450
Day-2 analyst-2 Irinotecan HCl	60	332495	0.443

*-Each value is the mean of six observations.

Table.7 System suitability studies for Irinotecan HCl

Validation Parameters	*Irinotecan HCl*
Linearity range (µg/ml)	30-150
Correlation co-efficient (R^2)	0.999
LOD (µg/ml)	0.014
LOQ (µg/ml)	0.045
Intraday (%RSD)*	0.45
Interday (%RSD)*	0.34
Repeatability (%RSD)*	0.40
Accuracy (%)	98.23-100.50
Peak purity index	1.0000
Resolution factor (Rs)	3.2
No. of theoretical plates (N)	8439.4
Capacity factor (K')	2.125
High equivalent to theoretical plates (HETP)	17.60
Tailing factor	0.91

*-Each value is the mean of six observations.

Analysis of formulation

By using the developed and validated method, the percentage of related substance in Irinotecan HCl formulation was found to be 0.19% and the percentage of Irinotecan HCl was found to be 100.5%. (Table 8 and 9) The method was found to be highly specific, since the peaks obtained did not show any interfere with the standard peaks. The method was found to be rugged since the %RSD values calculated were less than 0.55%.

Table. 8 Assay for Irinotecan HCl in formulation

Drug	Label Claim(mg/ml)	Estimated Amount(mg/ml)	% Label Claim	%RSD*
Irinotecan	20 mg	20.10 mg	100.5%	0.45

*-Each value is the mean of six observations.

Table 9. Assay for SN-38 in formulation

Related substance	Estimated amount (mg/ml)	% Amount present
SN-38	0.0389 mg	0.19%

FORCED DEGRADATION OR STABILITY INDICATING STUDIES

The specificity of the method was demonstrated through forced degradation studies conducted on the standard and sample using Acid, alkaline, oxidative, reductive and photolytic degradations. The sample was exposed to these conditions and the percentage peak area was calculated for Irinotecan peak, SN-38 peak and other peaks (Table 10).

Samples preparation for degradation studies

Acid Degradation:

About 10 ml of the 20 µg/ml of Irinotecan HCl solution (pure) and test solution was transferred to a 50 ml volumetric flask individually. About 10 ml of 1N hydrochloric acid was added. The volumetric flask was placed on a water bath maintained at 60°C for 8 h and cooled then this solution was neutralized with 1 ml of sodium hydroxide solution.

Table 10. Forced degradation studies for Irinotecan HCl

Type of degradation	Standard (pure)			Formulation (Test)		
	% drug remained	% degradation to R.S	% degradation Unknown	% drug remained	% degradation to R.S	% degradation Unknown
Acid degradation	41.81%	1.35%	56.83%	45.71%	NIL	54.28%
Alkaline degradation	1.29%	76.98%	21.73%	NIL	77.22%	22.78%
Oxidative degradation	2.63%	3.77%	93.6%	1.51%	NIL	98.49%
Photolytic degradation	NIL	NIL	100%	NIL	NIL	100%
Thermal degradation	NIL	NIL	100%	***	***	***

Alkaline Degradation:

About 10 ml of the 20 µg/ml of Irinotecan HCl solution (pure) and test solution was transferred to a 50 ml volumetric flask individually. Ten milliliter (ml) of 1N sodium hydroxide solution was added. The volumetric flask was then placed on a water bath maintained at 60°C for 8 h. Then it was cooled and neutralized with 1 ml of hydrochloric acid.

Oxidative Degradation:

About 10 ml of the 20 µg/ml of Irinotecan HCl solution (pure) and test solution was transferred to a 50 ml volumetric flask individually. Ten ml of 3% hydrogen peroxide was added. The volumetric flask was placed on a water bath maintained at 60°C for 8 h then cooled to room temperature.

Photolytic Degradation:

About 50 ml of the 20 µg/ml of Irinotecan HCl solution (pure) and test solution was transferred to a 50 ml volumetric flask individually. Then the solutions were exposed to sun light for 24 h.

Thermal (or) dry heat degradation:

About 100 mg of the irinotecan pure drug sample was taken in Petri dish and kept in a hot air oven at 60°C for 8 h to study the heat degradation. A solution of 20 µg/ml of the dry heat degraded sample was prepared.

The percentage of Irinotecan HCl (NLT 98.0% and NMT 102.0%) and related substance (NMT 0.2%) in formulation were found to be within the specified limits. Thus indicating that the method effectively separated the degradation products from the Irinotecan active ingredient and forced degradation studies was found to be satisfactory.

CONCLUSION

The developed RP-HPLC method was found to be very simple, reliable and selective for providing satisfactory accuracy and precision and hence can be used for routine quantitative analysis in pharmaceutical dosage forms. A stability indicating assay has been established by adapting the recommendations of ICH guidelines[21,22].

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