Available online on 15.05.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Determination of Irinotecan enantiomer impurity in Irinotecan Hydrochloride API by using reverse-phase liquid chromatography

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Article Info:

Article History:

Received 10 March 2023
Reviewed 16 April 2023
Accepted 29 April 2023
Published 15 May 2023

Cite this article as:

Srinivas P V, Manjusha K G, Vinukonda A, Determination of Irinotecan enantiomer impurity in Irinotecan Hydrochloride API by using reverse-phase liquid chromatography, Journal of Drug Delivery and Therapeutics. 2023; 13(5):41-46

DOI: *http://dx.doi.org/10.22270/jddt.v13i5.5815*

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Abstract

To evaluate and quantify Irinotecan (R-enantiomer) / Irinotecan related compound D in Irinotecan hydrochloride trihydrate API, a high stereo-specific liquid chromatography technique was developed and validated. The partition was accomplished on ChiralpakIC-3 (150 x 4.6 mm 3µm) through a mobile fragment comprising 0.1 % v/v Formic acid in water and acetonitrile with 1mL/min, 25^{0}C, 20µL, 5^{0}C and 370 nmas flow rate, column temparture, injection volume, sample cooler temperature and detection wavelength. At 8.903 and 9.75 min, the retention time of Irinotecan (R-enantiomer) and Irinotecan (S-enantiomer) was determined. The resolution between Irinotecan (R-enantiomer) and Irinotecan (S-enantiomer) was found to be 2.4. The impurity acceptance limit is 0.2 %. The established method's precision, accuracy, sensitivity, linearity, specificity, and ruggedness were all verified in accordance with ICH recommendations. The qualifying sample LOQ was found to be 0.4 g/ml, while the minimal amount of sample needed for LOD detection was found to be 0.12 g/ml. The proposed reversed-phase method has been sophisticated and authenticated in accordance with ICH criteria and is capable of quantifying irinotecan enantiomer in irinotecan hydrochloride trihydrate API at trace level concentration. The specificity, linearity, and accuracy of the approach were used to guarantee its efficacy; as a result, it is appropriate for the task at hand, may be used successfully for routine laboratory analysis, and can be utilised for quality control.

Keywords: Irinotecan, liquid chromatography technique, enantiomer impurity

INTRODUCTION

Colorectal cancer is treated with the anti-cancer drug irinotecan (molecular formula C33H38N4O6). It is (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]-dionemono hydrochloride trihydrate chemically (Fig.1). Irinotecan is a semisynthetic camptothecin derivative available under the brand names Camptosar and Onivyde. [1]

Topoisomerase I is prevented from functioning by irinotecan. Irinotecan binds to the topoisomerase I-DNA complex and prevents the DNA strand from religating. When this ternary complex forms, it disrupts the replication fork, causing it to move slowly and leading to deadly double-stranded DNA breaks. Due to ineffective DNA damage repair, apoptosis (programmed cell death) takes place.

For the treatment of metastatic cancer of the colon or rectum, irinotecan is utilised as a first-line therapy along with fluorouracil and leucovorin[2].

The undesired compounds that remain with active pharmaceutical ingredients (APIs), emerge during formulation, or appear as formulations age are known as impurities in pharmaceuticals. The efficacy and safety of pharmaceutical products may be impacted by the presence of these undesirable substances, even in trace concentrations. Impurities found in APIs are of ever-increasing interest. Purity and impurity profiles have recently become crucial due to numerous regulatory requirements[3].

Most of the drug substances single enantiomer is active. In such cases, the inactive enantiomer is considered an impurity.Chiral separation, as well as the determination of the optical purity of chiral pharmaceuticals, has attracted a great deal of attention from the healthcare and pharmaceutical industries[4].

High-performance liquid chromatography (HPLC) on chiral stationary phases (CSPs), which is widely used and one of the most effective, direct, and simple techniques for the determination of the optical purity and analytical separation of several enantiomeric drugs and pharmaceutical preparations[5–12], is one of the chiral analytical techniques currently used to achieve chiral separation of chiral mixtures. Due to the commercial accessibility of a number of CSPs for the direct separation of enantiomers[5,13], it is now standard practise to utilise HPLC to evaluate the chiral purity of medicines, their synthetic intermediates, and raw materials.

Few HPLC methods for the quantitative determination of R-enantiomer of irinotecan were reported in the literature. Hence the study aimed to develop a sensitive and rapid LC method to estimate the R-enantiomer of irinotecan[14].

A simple sensitive, reliable, robust and rapidhigh-performance liquid chromatography method was developed and validated for the estimation of Irinotecan enantiomer in Irinotecan Hydrochloride trihydrate API using reverse phase chromatography. Chromatography was accomplished using thechiralPak IC-3 150 x 4.6 mm 3µ column and ultraviolet (UV) detection and gradient mobile phase consisting of 0.1 %V/V Formic acid and Acetonitrile. The linear range of quantitation for the compound was 0.2 – 8 µg/mL. RP HPLC methods significantly improve the retention behaviour and separation of Irinotecan and irinotecan enantiomer the runtime was no more than 20 minutes. The method has the requisite accuracy, sensitivity and precision in both pharmaceutical dosage forms and bulk API.

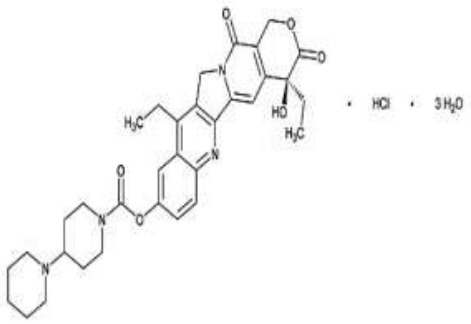
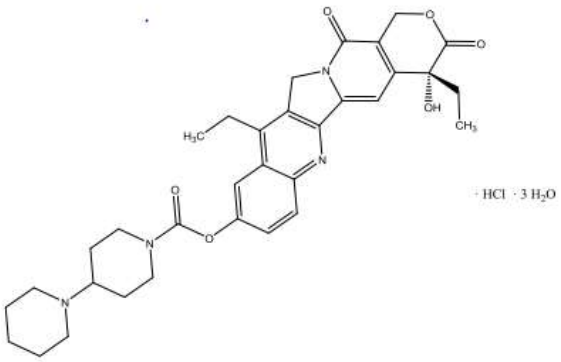


Figure 1 Irinotecan Hydrochloride trihydrate



Irinotecan Hydrochloride enantiomer / Irinotecan Impurity D

METHODS

Chemicals and reagents

Formic acid AR grade and Acetonitrile HPLC grade were procured from India. Irinotecan and Irinotecan enantiomer was procured from TLCchemicals.

Mobile phase A

Transfer 1 mL of formic acid in 100 ml volumetric flask containing 50 ml of water and make up to the volume with 100 ml of water

Mobile phase

Acetonitrile

Preparation of diluent

Prepare a mixture of 0.1 % formic acid in the water, Acetonitrile and Methanol in A ratio of 60:20:20 (v/v/v).

Conditions of Chromatography

Shimadzu CHT2030 chiral Pak IC3 Cellulose tris (3,5-dichlorophenylcarbamate) immobilised on 3m silica-gel (150 x 4.6 mm, 3) column was used as immobile phase, mobile phase consisting of 0.1 percent in water and acetonitrile during reverse-phase HPLC analysis. Gradient was provided by the mobile phase at 1.0 mL/min. The UV detection's wavelength was set to 370 nm. 20 L was chosen as the injection volume, and temperatures of 25 C for the column heater and 5 C for the autosampler were employed

Gradient program: Time / B%: 0/5, 10/ 85, 15/5, 16/5, 20/5.

Preparation of blank

Diluent is used as blank

Preparation of Irinotecan Hydrochloride enantiomer stock solution

Precisely weigh and transfer 3mg of irinotecan Hydrochloride enantiomer standard into a 10 ml volumetric flask at 14 mL of Diluent and sonicate For 2 minutes to dissolve and dilute to volume with dilute and mix.

Transfer 2.5 mL of this solution into a 25 mL volumetric flask and dilute the volume with dilute and mix. Further, transfer 5 mL of the solution into a 50 mL volumetric flask and dilute the volume with dilute and mix.

Preparation of resolution solution

Precisely weigh about 20 mg of irinotecan hydrochloride trihydrate and transfer it to a 10 ml volumetric flask. Add about 3 mL of diluent and sonicate for 2 minutes to dissolve. To this solution add 2 mL of irinotecan impurity D stock solution and dilutethe volume with diluent and mix.

Preparation of sample solution (2mg/ml)

Accurately weigh about 100 mg of irinotecan hydrochloride trihydrate and transfer it to a 50 ml volumetric flask. At about 15 ml of diluent and sonicate for 2 minutes to dissolve and dilute the volume with diluent.

RESULTS

Analytical method Validation:

The validation experiments demonstrated system suitability and control sample analysis, the limit of detection and limit of quantification, Precision at LOQ and accuracy, Precision of the test method, method Precision (repeatability), intermediate precision (ruggedness- intra lab) and linearity of the detector response. The HPLC method for the determination of irinotecan Enantiomer in irinotecan hydrochloride trihydrate API by HPLC is precise (repeatability and reproducibility), accurate and linear over the range of 0.4 µg/mL -8 µg/mL.

System suitability:

Equilibrate the chromatographic system with mobile phase until a consistent baseline is seen, and then inject a blank, resolution solution, and standard solution in the appropriate order. The results were reported in Table 1 after the system's appropriateness was assessed.

Specificity

Interference from placebo and a blank

A study was conducted to determine whether placebo and blank effects were present. To determine the aforementioned chromatographic conditions and record the blank and placebo chromatograms, diluent and placebo injections were made into columns. The chromatograms of the blank samples

revealed no peak during the retention time of either the enantiomer or the analyte peak of irinotecan. This has shown that the diluent solution used to prepare the sample does not interfere with the measurement of the enantiomer of irinotecan. The Irinotecan enantiomer and Irinotecan analyte peaks did not appear on the chromatogram produced for the placebo solution. This also showed that the placebo used to make the sample solution did not affect how well irinotecan enantiomer impurities were estimated in irinotecan injection.

Precision

System precision

By making blank and standard solutions in accordance with the test procedure and chromatographing them into the HPLC system, system precision was demonstrated. For these system appropriateness injections, the analyte retention time and Pinnacle areas were noted. Retention time's percent RSD was found to be 0.2, and area response's was found to be 1.0.

Method precision

By injecting six sample solutions spiked with irinotecan enantiomer, the precision of the impurity was calculated at the specification level. The samples were made in accordance with the method. Table 2 is a summary of the precision study's findings. For irinotecan Enantiomer, the percent RSD of method precision was discovered to be 0.6 percent.

Limit of detection and quantitation:

Three injections of a solution containing 0.12 µg/ml of irinotecan enantiomer were made. The worst signal-to-noise ratio for each piece in each injection was greater than 3, and peaks were found in all three injections.

Six injections of a solution containing 0.4 µg/ml of the irinotecan enantiomer standard were made. The average deviation for each standard, the relative SD of the areas, and the deviations of each of the six replicates from the linear regression curve were computed

Limit of detection and quantitation:

Irinotecan Enantiomer limit of quantitation and limit of detection values were obtained and summarised in Tables 3 and 4 within the permissible range.

Linearity

By injecting the solutions in duplicate containing irinotecan impurity-D varying from LOQ to 200 percent of the designated limit, linearity was ascertained. It displayed a concentration versus area graphic. The peak regions were given as an entire number. Four notable figures were reported for the concentrations. The data were subjected to a linear regression analysis (without pushing via the origin). The results are tabulated in Table 5 for the correlation coefficient ®, slope, and percent y-intercept.

Accuracy

The accuracy or recovery samples were prepared by spiking the irinotecan hydrochloride enantiomer standard and hydrochloride control sample using solutions at concentrations spanning from LOQ to 200% to the specified limit. 6 preparations were made at LOQ, 100% Level and three preparations at 50%, 150% and 200%.

Once each solution was injected, it was examined. Calculating the percent recovery for each individual preparation at each level allowed for the determination of the mean percent recovery of the irinotecan hydrochloride enantiomer. The findings were compiled in Table 6.

Solution stability

Initial injection of the solution into HPLC was followed by measurements of the percent area of irinotecan impurity-D in spiked solution at each interval. The percent area difference from the initial day interval was then computed. Standard and sample solutions were consistent for 48 hours on the benchtop and in cooler (2-8^{0}C) conditions. The results are presented in tables 9 and 10. A solution stability parameter was constructed.

Table-1: System suitability results

Component name	Retention time (min)	Relative retention time	Tailing factor	Theoretical plate	Resolution
Irinotean Enantiomer	8.235	0.910	1.288	20772	NA
Irinotecan	9.043	1.00	2.273	10044	2.74

Table-2: Method precision results

Preparation No:	Assay (% Recovery)
Preparation-1	100.2
Preparation-2	100.4
Preparation-3	100.4
Preparation-4	100.2
Preparation-5	100.4
Preparation-6	100.6
Average	**100.4**
SD	**0.1410**
%RSD	**0.1**

Table-3: LOD and LOQ results

Parameter	Irinotecan Enantiomer (%) w.r.t test concentration	Irinotecan enantiomer (µg/mL)	S/N value
LOD	0.006	0.12	4.8
LOQ	0.02	0.4	13.1

Table 4: LOQ Precision results

Injection No.	Area
Injection-1	2640
Injection-2	2680
Injection-3	2656
Injection-4	2620
Injection-5	2682
Injection-6	2661
Average	**2657**
SD	**23.7802**
%RSD	**0.9**

Table-5: Results for Linearity of detector response Study

Linearity level (%)	Concentration (mg/mL)	Area
LOQ	0.00016	1728
25%	0.0014	22153
50%	0.0029	45638
75%	0.0043	69636
100%	0.0058	94018
125%	0.0072	116500
150%	0.0086	141332
175%	0.0101	165699
200%	0.0115	189362
Correlation coefficient	**0.999965**	
Slope	**16543118.8415**	
Y-intercept	**-1598.7544**	
% Y-intercept	**-1.7**	

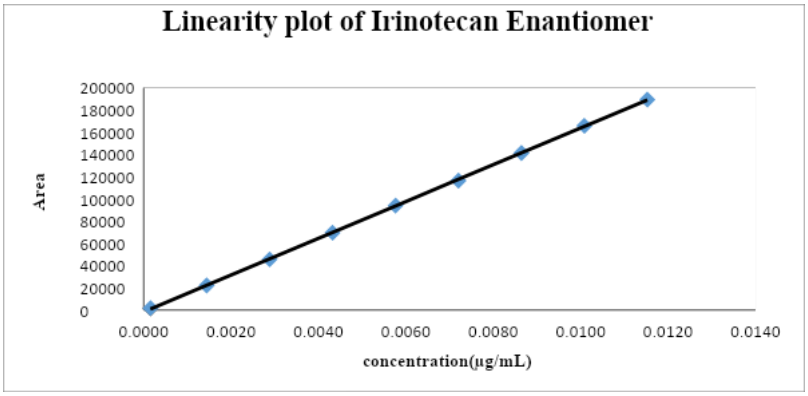
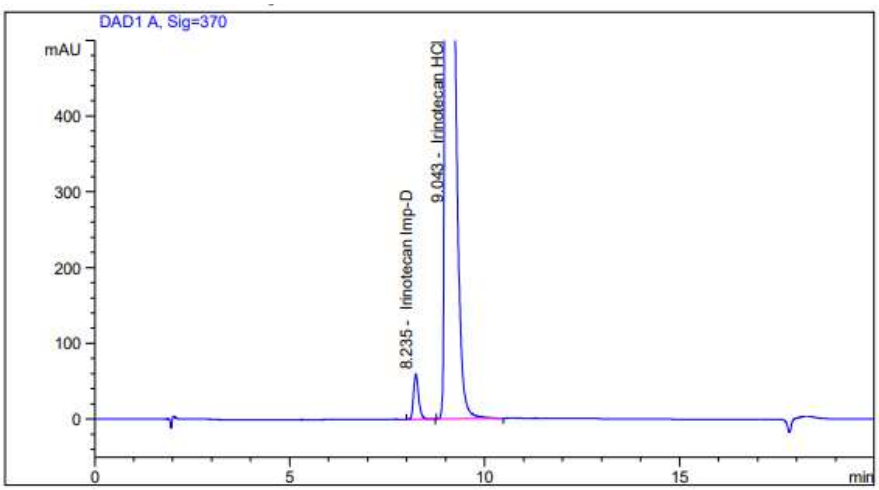


Figure 3: Linearity plot of Irinotecan Enantiomer

Table 6: Accuracy

Level (%)	% Recover	% Mean recovery	% RSD
LOQ	81.3	85.0	3.6
	84.0		
	88.3		
	83.3		
	83.8		
	89.0		
50 %	102.1	102.1	0.05
	102.1		
	102.1		
100%	100.7	100.7	0.1
	100.8		
	100.8		
	100.8		
	100.6		
	100.7		
150 %	99.0	99.1	0.1
	99.1		
	99.3		
200 %	97.3	97.2	0.03
	97.2		
	97.2		

Spiked sample Chromatogram



Blank (Diluent)

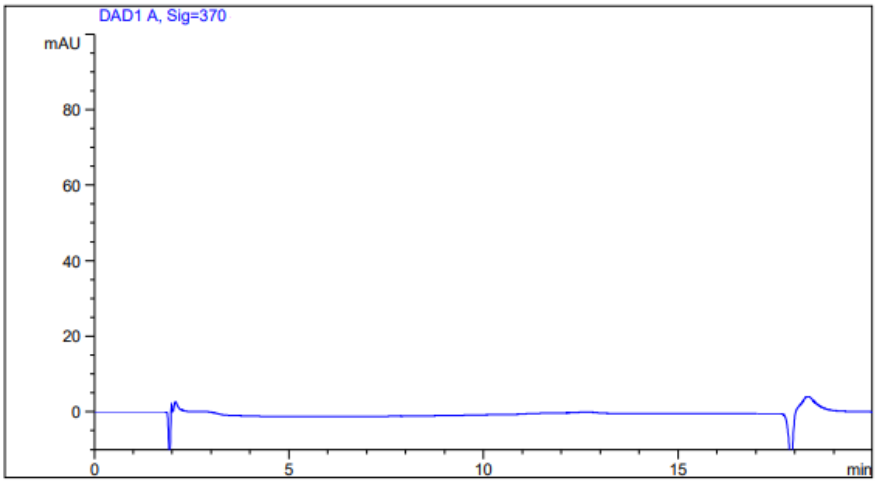


Figure 4: Spiked sample and Blank (Diluent) Chromatogram

DISCUSSION

A successful reverse phase HPLC process was devised that was simple, affordable, accurate, and precise. On a chiral Pak IC-3 (3 m, 4.6 X 150 mm) column (amylose-based chiral stationary phase), the partition was produced using formic acid concentration of 0.1 percent and an acetonitrile as a mobile phase at a flow rate of 1 ml/min. 25°C for the column temperature, 20 l for the injection volume, 5°C for the sample cooler, and 370 nm for the detection wavelength. The findings were found to be precise and repeatable. According to ICH guidelines and USP 1225>, the new technique was statically certified in terms of selectivity, accuracy, linearity precision ruggedness, and solution stability.

Chromatograms of Irinotecan enantiomer and Irinotecan standard and sample solutions were taken in order to determine the selectivity. The results of this analysis showed that the peaks were properly spaced apart. In order to determine the Irinotecan enantiomer in Irinotecan hydrochloride trihydrate API, the approach was selective. At the Irinotecan enantiomer and Irinotecan peak, there is no interference from diluent or placebo.

The method was found to be linearity over the range of 0.4 g/ml to 8 g/ml with a correlation coefficient value greater than 0.99. The LOD and LOQ for the irinotecan enantiomer standard were 0.1 and 0.4 g/ml, respectively. The result was found to be within the limitations, with the maximum percent recovered shown being between 80 and 120 percent. Consequently, the method's accuracy was determined. Additionally, the recoveries observed for the irinotecan enantiomer have relative SD values that range from 0.03 to 3.6 percent. Six replicate injections were used to determine precision studies. The peak area of irinotecan enantiomer, which was found to be 0.1 percent, was used to calculate the percent RSD. The results originated to be within the acceptance limit, and the acceptance limit should not be greater than 10.

As a result, the chromatography method created for irinotecan hydrochloride trihydrate API was quick, easy, precise, sensitive, and accurate. The suggested method is therefore helpful for the regular analysis of the pharmaceutical active components for the declaration of their quality during formulation.

CONCLUSION

The optimised method is Rapid and sensitive which is practically following the quality control a requirement of testing in Irinotecan hydrochloride.

According to ICH criteria, the suggested reverse phase HPLC technique has been sophisticated and authenticated to be able to quantify the irinotecan enantiomer in irinotecan hydrochloride trihydrate at a trace level concentration. The specificity, exactitude, linearity, and correctness of the method assured its efficacy. As a result, the method is appropriate for the task at hand, may be successfully used for routine laboratory analysis, and is acceptable for quality control.

Acknowledgement

The authors are thankful to Dr Y Srinivasa Rao, Principal, Vignan Institute of Pharmaceutical Technology for providing the necessary requirements to carry out the study.

Conflict of Interest: None

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