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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP- HPLC METHOD FOR THE DETERMINATION OF IRINOTECAN IN INJECTION FORMULATION

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ABSTRACT

A stability indicating RP-LC method has been developed and subsequently validated for the determination of Irinotecan hydrochloride trihydrate (IHT) in bulk and injection formulation. Separation was achieved with a C_{18} waters symmetry (250 mm x 4.6 mm, 5 µm) consisted of a UV detector and potassium dihydrogen phosphate buffer (pH 3.5): acetonitrile : methanol (60:20:20, v/v/v) as a mobile phase, at a flow a rate of 1mL/min. UV detection was performed at a wavelength of 220 nm. The method is simple, rapid, selective, accurate and more precise. The described method is linear over a range of 40-120 µg/mL. The accuracy of the method was demonstrated at 5 levels in the range of 50-150% of the specification limits and recovery of IHT was found to be in the range of 101-102.5%. The method is useful in the quality control of bulk manufacturing. The IHT sample solution in mobile phase was found to be stable for at least 24h at 25°C and at 6°C.

Keywords: Irinotecan hydrochloride trihydrate (IHT), Forced degradation, RP-LC and validation

INTRODUCTION

Irinotecan hydrochloride trihydrate[1] (IHT) is chemically known as (S) - 4, 11-diethyl - 3, 4, 12, 14 - tetrahydro - 4 - hydroxy- 3, 14 - dioxo - 1H - pyrano [3[1], 4[1], 6, 7] - indolizino [1, 2-b] quinolin - 9 - yl - [1, 4[1]bipiperidine] - 1[1]- carboxylate monohydrochloride trihydrate (Fig.I) is an anti-neoplastic agent. Irinotecan inhibits the action of topoisomerase I. Irinotecan binds 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. Only a few liquid chromato-graphic procedures have been reported for the determination of IHT and their metabolites in biological fluids[2-5] (Peter de Bruijn[2]. *et al.,*1997*;* Ziqiang Zhang[3]. *et al.* 2009; Sylvain Poujol[4]**.** *et al.,* 2003*;* Laurent P. Rivory[5]. *et al***.,** 1994). Murali Balaram V[6]. *et al.,* have reported a method, using a validated RP-LC for the determination of IHT in pharmaceutical dosage forms*;* Mohammadi . A[7]**.** *et al.,* have reported a method, using a validated HPLC using uv detection for the determination of IHT and its related compounds; Rodríguez Caceres. M. I[8]**.** *et al*, have reported a method, using a validated spectrofluorimetric determination of IHT in the presence of oxidant agents and metal ions**.** Literature survey reveals that no analytical method for the determination of stability indicating using RP-LC method for the determination of IHT in injection formulation. So it is felt worthwhile to develop a simple, rapid, selective, sensitive, highly accurate and more precise for the determination of IHT in bulk and its pharmaceutical dosage forms

MATERIALS AND METHODS

Chemicals and Reagents

Sample of Irinotecan hydrochloride trihydrate (IHT) was procured from SMS Pharma Research Centre, Hyderabad, India. Methanol (HPLC-grade), acetonitrile (HPLC-grade), Potassium dihydrogen phosphate and orthophosphoric acid were obtained from Merck, USA. All other analytical chemicals and reagents also were purchased from Merck. High purity water was prepared by using Milli-Q Elix and Milli-Q academic purification system (Millipore).

Instrumentation and Conditions

The isocratic RP-LC separation was performed on a Agilent 1200 series, column waters symmetry shield RP18 (250 mm x 4.6 mm, 5 µm) consisted of a UV detector. The colum temperature was maintained at a constant of ambient. The mobile phase comprising a mixture of potassium dihydrogen phosphate buffer (pH 3.5): acetonitrile:methanol (60:20:20, v/v/v) was pumped at a flow rate of 1.0 mL/min.The mobile phase was freshly prepared, filtered through a 0.45 µm millipore filter. The elute absorbance was monitored at 220 nm with 10 µL injection volume. To obtain good resolution between Irnotecan and the potential degradation products, the composition of buffer-acetonitrile-methanol mixtures, pH value and salt concentration of buffer were adjusted and optimized, before an optimum chromatographic condition was achieved.

Standard Preparation

A stock solution of IHT is prepared by dissolving the drug equivalent to 50 mg IHT in a 50 mL volumetric flask with diluent (mobile phase). Aliquot of this solution was suitably diluted with mobile phase to get the working standard solution of IHT in the concentration range of 40 - 120 µg/mL.

Sample Preparation

IHT containing 20 mg/mL was taken for the present research work. 1mL of the sample solution was transferred into 25 mL of volumetric flask and added 17 mL of diluents. This mixture was subjected to sonication for 2 min for complete extraction of drug and preservatives and the solution was made up to the mark with diluents. The solution was centrifuged at 3000 rpm for 5 min, the clear supernatant portion was collected and filtered through a 0.45 µm Dura pore PVDF hydrophilic membrane filter and 10 µL of this solution was injected in to the LC system.

Forced degradation of sample for specificity study

Forced degradation studies were conducted to check the ability of the method to separate IHT from its degradation products and provide the proof of the stability indicating ability and specificity of the reported method. The forced degradation was accomplished by exposing the injectable powder for reconstitution to stressed conditions of acid (0.1N Hcl for 1hr at 60^{0}C), base (0.001N NaoH for 2 min at 25^{0}C), hydrogen peroxide (1% H_2O_2 for 1hr at 60^{0}C), sunlight (for about 55hrs), UV light both wave lengths (254 – 366 nm) for about 2 days, Dry heat (for 3hrs at 105^{0}C) and Humidity (90% RH for 7 days at 25^{0}C).

METHOD DEVELOPMENT

Method Validation

The developed LC method has been extensively validated for evaluating the linearity, accuracy, precision, robustness, ruggedness and recovery.

Linearity

The calibration curve of peak area Vs concentration (µg/mL) was obtained with six standard solutions with concentration ranging from 40 to 120 µg/mL

Specificity

To confirm the specificity of the proposed method. Placebo solution containing all inactive excipients were prepared and injected in to the chromatographic system to examine the existence of the interference of excipients.

Impurity interference

Study to establish the interference of impurities was also conducted. Spiked the standard preparation with impurity blend solution in the concentration of 0.3% of Impurity A, B, C, D, E and F and injected in to HPLC system.

LOD and LOQ

The limit of detection and limit of quantification for impurities A, B, C, D, E and F were calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve for each impurity. The LOQ and LOD values were found to be 4. 28 µg/mL and 1.4127 µg/mL respectively.

Accuracy

The accuracy at five levels (50, 75, 100, 125 and 150 %) was determined by recovery experiments. Solutions of five levels were prepared in triplicate and assayed.

Precision

The method precision was evaluated by two different analysts on two different days respectively. Intra-day variations of impurities of IHT obtained are expressed in terms of RSD values. The repeatability was analysed by conducting six consecutive injection of IHT standard solutions.

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameter and provides an indication of its reliability for the routine analysis. Intentionally the optimized parameters for mobile phase were changed to examine their effect on retention time, drug recovery and peak parameters and then to evaluate the method ruggedness. The changes made included pH 3.3 to 3.7, flow rate 0.8 to1.2 mL/min and column temperature 20 to 30^{0}C.

RESULTS AND DISCUSSION

Optimized chromatographic conditions

For chromatographic development of IHT need to set up an optimum chromatographic conditions, which can elute and resolve IHT while complying with the general requirements for system suitability. Some of the main chromatographic parameters, including mobile phase, wavelength and pH of the buffer were assessed. In optimizing the composition of mobile phase conducted four trials (Trial I, II, III and IV). Trial I & II the sample mixture of acetonitrile and water (50:50 v/v), Trial III with buffer:acetonitrile (80:20 v/v) but no desirable peak shape was achieved and finally in Trial IV good separation was achieved with buffer : acetonitrile : methanol (60:20:20 v/v/v). The variation in pH from 3.0 to 3.5 resulted in disappearance of peak present in aforementioned chromatograms and the sharp increase of tailing factor of IHT peak. A desired peak was observed at pH 3.5.

Linearity

The calibration curve for IHT was constructed by plotting peak area Vs concentration. The results were listed in (Table 1**)**. It was demonstrated that an excellent correlation existed between the peak area and IHT concentration at the tested concentration range.

Specificity

No interference peak was found in the chromatogram indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed method for the determination of IHT in the pharmaceutical dosage forms. Hence this method is specific.

Impurity interferense

This study to establish the interference of impurities was conducted. The chromatogram (Fig. II) of impurity interference solution showed no impurity peaks at the retention time of IHT. This indicates that there is no impurity interference in estimation of IHT.

LOD and LOQ

The limit of detection and limit of quantification for IHT was calculated from the linearity data using RSD of the response and slope of the calibration curve. The LOD and LOQ was found to be 1.4127 and 4.28 µg/mL.

Accuracy

Accuracy of the method was demonstrated at five different concentration levels (50-150%) by spiking a known quantity of IHT in triplicate. The results of accuracy (Table 2) revealed that the method was more accurate.

Precision

Six replicate samples were injected in to the system on two different non consecutive analysts and calculated the % of the assay and RSD for six sample preparations. It has been found that the results were within the limits by both analysts. The results were depicted in (Table 3).

Robustness

System suitability parameters were verified by making deliberate changes in the chromatographic conditions. Change in flow rate by ± 0.2 mL/min, change in pH of the buffer ± 0.1 units and change in the ratio of mobile phase ±1% absolute. The method was conducted to be robust over an acceptable working range of its LC operational parameters.

Stability indicating properties

The degradation sample of IHT chromatograms by acid (Fig.III), base (Fig.IV), hydrogen peroxide (Fig.V), sunlight (Fig.VI), uvlight (Fig.VII), dryheat (Fig.VIII) and humidity (Fig.IX) were shown and demonstrating that that the degradative products could be detected and well separated from IHT with in 10 min. The retention time for IHT was as about 5.40 min.

CONCLUSIONS

The present paper describes the development of LC method for the determination of IHT and its forced degredation method. The proposed method was found to be selective, simple, fast,robust,accurate, sensitive and more precise under the present experimental conditions. Therefore the developed method can be used for routine analysis for the estimation of IHT in bulk and in injection formulation.

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