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Development of stability indicating HPLC method for the assay of irinotecan in the presence of degradents

A.K.Srivastava*, K.Pallavi, S.Shamshul, S.C.Khurana

Jiwaji University, Gwalior, (INDIA)

E-mail: mica_hplc@yahoo.co.in

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ABSTRACT

A LC method with UV detection to understand degradation path and quantification of irinotecan in injection as pharmaceuticals form has been developed and validated as per guidelines from ICH, USP and other regulatory agencies. It is to be employed in routine and stability analysis. A linear isocratic elution was employed starting with 72% A and 28% B up to 30 min. Mobile phase A was 0.005 M Heptance sulphonic acid and 0.05 M Dibasic phosphate buffer of pH 3.0. Mobile phase B was acetonitrile. UV detection was performed at 254 nm. The chromatographic column was Hypersil C18, Hyper bond (300mm X 3.9mm) 10.0µ kept at room temperature. All impurities were separated and it was possible to quantify the irinotecan in formulation with reasonable accuracy and precision. The method was validated for its specificity, precision, accuracy, linearity, ruggedness and robustness. Correlation coefficient for irinotecan peak area was found to be 1.000 for linearity ranging between 32.49 µg/mL to 48.74 µg/mL and limit of detection was 0.50 µg/mL.

INTRODUCTION

Irinotecan hydrochloride is a semi synthetic derivative of camptothecin, an alkaloid extract from plant such as Camptotheca acuminata. Irinotecan hydrochloride is a semisynthetic piperidine-containing analogue of the plant alkaloid, camptothecin. Irinotecan has the chemical name is (S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate. Although irinotecan and its active metabolite exist in alkaline solution in an equilibrium of lactone and ring-opened forms. Irinotecan, a water-soluble analoge of camptotecin, is an inhibitor of DNA topoisomerase I developed in Japan. It has clinical therapeutic efficacy against various malignancies such as cervical, colorectal, lung and ovarian cancers and non-Hodgkin's lymphoma. Although irinotecan has been reported to be inactive for salvage therapy of breast cancer in Europe. It was approved for use in breast cancer in Japan.

Its molecular formula is $C_{33}H_{38}N_4O_6HCl_3H_2O$, and its molecular weight is 677.1. Irinotecan, as the hydrochloride is a pale yellow to yellow crystalline solid. Irinotcan is soluble in methanol and water[1].

Irinotecan is a semi synthetic derivative of the plant alkaloid camptothecin[2]. Similar to the parent compound, irinotecan and its active metabolite, SN-38, blind to and stabilize the topoisomerase I-DNA complex, which leads to the formation of irreversible DNA strand breaks. Irinotecan produces cell cycle specific cytotoxicity with cells in S- phase 100-1000 fold more sensitive than cells in the G_1 or G_2 phase. The chemical structure of Irinotecan hydrochloride is shown below. It has been selected from many derivatives of podophyliotoxin that have been synthesized during the past 20 years.

Irinotecan is a derivative of camptothecin. Camptothecins interact specifically with the enzyme

topoisomerase I which relieves torsional strain in DNA by inducing reversible single-strand breaks. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent religation of these single-strand breaks. Current research suggests that the cytotoxicity of irinotecan is due to double-strand DNA damage produced during DNA synthesis when replication enzymes interact with the ternary complex formed by topoisomerase I, DNA, and either irinotecan or SN-38. Mammalian cells cannot efficiently repair these double-strand breaks.

Irinotecan serves as a water-soluble precursor of the lipophilic metabolite SN-38. SN-38 is formed from irinotecan by carboxylesterase-mediated cleavage of the carbamate bond between the camptothecin moiety and the dipiperidino side chain. SN-38 is approximately 1000 times as potent as irinotecan as an inhibitor of topoisomerase I purified from human and rodent tumor cell lines. In vitro cytotoxicity assays show that the potency of SN-38 relative to irinotecan varies from 2- to 2000-fold. However, the plasma area under the concentration versus time curve (AUC) values for SN-38 are 2% to 8% of irinotecan and SN-38 is 95% bound to plasma proteins compared to approximately 50% bound to plasma proteins for irinotecan. The precise contribution of SN-38 to the activity of irinotecan is thus unknown. Both irinotecan and SN-38 exist in an active lactone form and an inactive hydroxy acid anion form. A pH-dependent equilibrium exists between the two forms such that an acid pH promotes the formation of the lactone, while a more basic pH favors the hydroxy acid anion form.

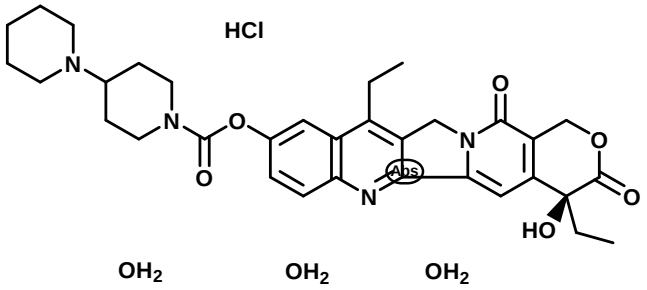

Figure 1 : Irinotecan hydrochloride

Administration of irinotecan has resulted in antitumor activity in mice bearing cancers of rodent origin and in human carcinoma of various histological types.

Irinotecan is supplied as a sterile, pale yellow, clear, aqueous solution. It is available in two single-dose sizes: 2 mL-fill vials contain 40 mg irinotecan hydrochloride and 5 mL-fill vials contain 100 mg irinotecan hydrochloride. Each milliliter of solution contains 20 mg of irinotecan hydrochloride (on the basis of the trihydrate salt), 45 mg of sorbitol NF powder, and 0.9 mg of lactic acid, USP. The pH of the solution has been adjusted to 3.5 (range, 3.0 to 3.8) with sodium hydroxide or hydrochloric acid. Irinotecan is intended for dilution with 5% Dextrose Injection, USP (D5W), or 0.9% Sodium Chloride Injection, USP, prior to intravenous infusion. The preferred diluent is 5% Dextrose Injection, USP.

Reported analytical method of irinotecan has been used to quantify lactone from irinotecan in different buffer solutions. Kramer and Thiesen[3,4] evaluated the physicochemical stability of irinotecan injection concentration and diluted infusion solution in polyvinyl chloride bags. The method was shown to be stability indicating by accelerated was about 15 min. All reported method evaluated either stability of irinotecan in different solution or checked the compatibility of the irinotecan with different infusion solution. Hence attention has been focused to developed HPLC method to understand degradation path of the irinotecan as well as for analysis of stability samples to establish the self life of the drug.

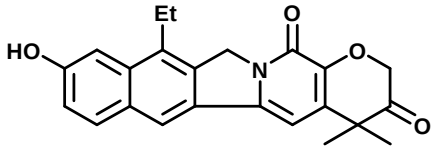

Figure 2 : 7-Ehtyl-10-hydroxy camptothecian

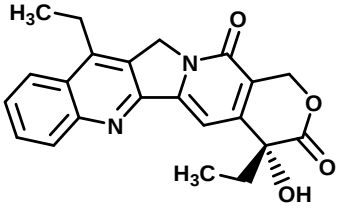

Figure 3 : 7-Ehtyl camptothecian

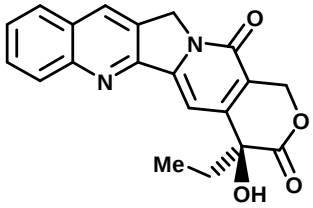

Figure 4 : Camptothecian

EXPERIMENTAL

Apparatus

A Shimadzu LC 10A HPLC system provided with

an automatic injector, a UV detector and a column oven was employed. The chromatographic analysis was performed on Hypersil C18, Hyper bond (300mm X 3.9mm) 10.0µ column with column oven temperature of 25°C.

A linear isocratic elution was employed starting with 72% A and 28% B up to 30 min. Mobile phase A was 0.005 M Heptance sulphonic acid and 0.05 M Dibasic phosphate buffer of pH 3.0. Mobile phase B was acetonitrile. The flow rate was 1.0 ml/min and the injection volume was 10µL. UV detection was performed at 254 nm and peaks were identified with retention times as compared with standards and during method development by spiking.

Chemicals

Standard of irinotecan HCl and impurities as well as injection and placebo of the specialty were provided by Cancer Hospital, Gwalior, Heptance sulphonic acid and dibasic phosphate buffer from J. T. baker Limited, Dimethylformamide, Orthophosphoric acid and methanol from Qualigens, Acetonitrile from S. D. fine chemicals limited. HPLC grade water from Merk limited.

Optimization of HPLC method

Selectivity under RP-HPLC conditions can be controlled by mobile phase composition, pH, temperature and stationary phase chemistry. The parameters were varied to achieve the separation.

Standard solution and sample preparation

Samples were dissolved in solvent A: acetonitrile 72:28 (v/v), being solvent A: 0.005 M Heptance sulphonic acid and 0.05 M Dibasic phosphate buffer and adjust pH-3.0 with ortho phosphoric acid. For quantitation Pipette 2mL of irinotecan injection in 100mL volumetric flask, dissolved and rinse with methanol and make up the volume with mobile phase. Further dilute this solution to get final concentration of 40µg/mL.

A stock solution of irinotecan was prepared with about 40mg of irinotecan was weighed in 100 mL volumetric flask, dissolved in methanol and make up the volume with mobile phase. This solution was diluted quantitatively with the same mobile phase to get final concentration of 40µg/mL of irinotecan.

System suitability check

System suitability[5] was checked with standards chromatograms. Standard solution was injected in six replicate. The tailing factor of irinotecan peak was not more than 2.0 column efficiency was not less than 4348 theoretical plates. Relative standard deviation of area of irinotecan peak for six replicate injections of standard solution was 0.10%.

Validation

Method validation is the process of proving that an analytical method is acceptable for its intended purpose. For pharmaceuticals methods, guidelines from the united state pharmacopoeia (USP)[6], International Conference on Harmonization (ICH)[7] and the Food and drug administration (FDA)[8,9] provide a framework for performing such validation.

Selectivity[10,11] was tested by injecting the placebo of the pharmaceutical specialty and checking that there was no interference peak and by spiking the drug substance and the drug product with appropriate levels of standards and demonstrating the separation of these impurities individually and other components in the sample matrix. Moreover, identification of each impurity was confirmed with migration time as compared with those of pure standards and by spiking.

For irinotecan methods, linearity[12,13] is determined by preparing standard solution at five concentration levels over a range from 32.49 µg/mL to 48.74 µg/mL. Linearity was tested by adding proportional weight of the placebo to each flask. The correlation coefficient, slope and intercept were determined. The data is provided in TABLE 1.

TABLE 1 : Linearity data

Sr. No.	Parameter	Concentration	Result's
1.	Linearity	32.49 µg/mL to 48.74 µg/mL.	R^2=1.0000
2.	Slope		10403
3.	Intercept		888.21

For pharmaceutical studies, the most widely used approach to test accuracy is the recovery study, which is performed by spiking analyte in blank matrices. It was tested in the same linearity assay which is 32.49 µg/mL and 48.74 µg/mL. The percent recovery was in the range of 99.55% to 101.04%.

LOD & LOQ of irinotecan was determined and precision was established at the predicted concentration. % Relative standard deviations was found with in Limit for LOQ at 2.50µg/mL with %RSD=0.45%. The data is provided in TABLE 2.

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Intra-assay precision data were obtained by repeatedly analyzing the samples which was independently prepared according to the method procedure. Data to evaluate intermediate precision were obtained by repeating the intra-assay experiment on a different day with a newly prepared mobile phase and samples. Relative standard deviation of results was 0.85%. The data is provided in TABLE 3.

TABLE 2 : Precision at LOQ level.

Concentration µg/mL	Injection Volume	Area Count	Mean	RSD
2.50µg/mL	20µL	54131	54392	0.45%
		54597		
		54163		
		54263		
		54807		

TABLE 3 : Intermediate precision data

Sr.No	Equipment	Analyst	Reagent	Assay	Mean	RSD
1	Instrument-1	Analyst-1	Water, HPLC grade (Milli Q) Acetonitrile, Qualigens, B.No-NL1470600H2 Na_2HPO_4:Qualigens, B.No-NL78995802 Ortho Phosph Acid, Qualigens, B.No-26825905H 1-Heptanesulphonic acid (J.T Baker), B.No-L05634	20.57mg/mL		
2	Instrument-2	Analyst-2	Water, HPLC grade (Milli Q) Acetonitrile, J.T Baker, B.No-NL1470600H2 Na_2HPO_4:Qualigens, B.No-NL78995802 Ortho Phosph Acid, Qualigens, B.No-26825905H 1-Heptanesulphonic acid (J.T Baker), B.No-L05634	20.84mg/mL	20.77mg/mL	0.85%
3	Instrument-3	Analyst-3	Water, HPLC grade (Milli Q) Acetonitrile, Qualigens, B.No-NL1470600H2 Na_2HPO_4:Qualigens, B.No-NL78995802 Ortho Phosph Acid, Qualigens, B.No-26825905H 1-Heptanesulphonic acid (J.T Baker), B.No-L05634	20.90mg/mL		

RESULT AND DISCUSSION

A typical chromatogram of irinotecan is shown in Figure 1 and peak purity of the samples was also determined by area normalization method. Throughout this study the peak purity was confirmed by photo diode array detector (PDA), which incorporates 512 diodes over UV-VIS spectral range to evaluate UV spectra across the peak of interest hence the method is specific. Presented method is sensitive enough and is found to be linear in the range of 32.49 µg/mL to 48.74 µg/mL with r^2 1.0000. Accuracy of the method was established by recovery, the recovery values are with in the acceptable limit at different concentration level (50%, 100% and 150% of specification). Over all recovery ranges from 99.55% to 101.04% and this indicates that the method is accurate. Repeatability, reproducibility and intra day precision were performed as system precision, method precision intermediate precision and ruggedness and values obtained were well within limit, the different values of validation data. Limit of quantitation of the method was 2.50µg/mL and limit of detection was 0.50µg/mL.

In addition the robustness of the method makes it easy for an operator to generate reproducible data. The method is robust under different conditions like change in mobile phase, column, flow, temperature and wavelength. The data is provided in TABLE 4.

In order to validate the stability indicating power of the analytical procedure, irinotecan standard and sample were treated with acidic, basic, oxidative media, heat and light. For thermal stressing irinotecan injection sample was kept at temperature 120°C for 110.0 Hrs in oven and analyzed at regular interval. For light stressing irinotecan injection sample was placed under white fluorescent light at the rate of 300-kilo lux per Hours at 15°C for 25 days and analyzed at regular interval of time. For Acid and Base stressing 5 mL of irinotecan injection was diluted with 0.1N HCl and 0.1N NaOH in 50 mL volumetric flask. From this stock 2 mL of sample was taken in 10 mL volumetric flask and neu-

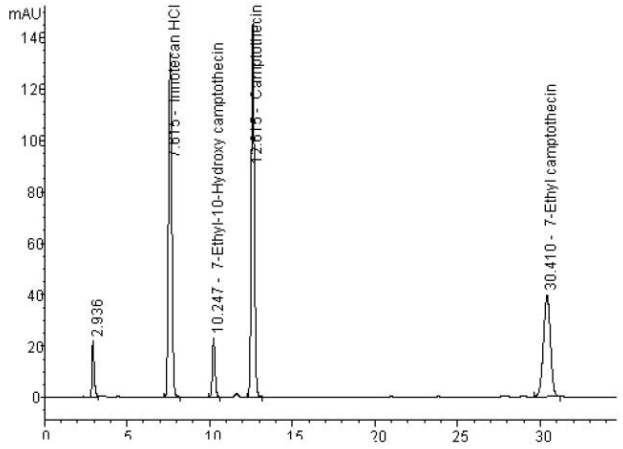

Figure 5 : Chromatogram of irinotecan, 7-Ethyl-10-hydroxy camptothecin, camptothecin and 7-Ethyl-10-Hydrocy camptothecin.

tralized with respective acid and base and make up with mobile phase so that it can be neutralized before injection in to the chromatograph. For peroxide stressing 5 mL of irinotecan injection was diluted with 3% H_2O_2 to 50 mL. From this 2 mL sample was taken in 10 mL, neutralized and injected.

The stressed samples were analyzed on HPLC. The purity of the samples was also determined by area normalization method. Throughout this study the peak purity was confirmed by photo diode array detector (PDA), which incorporates 512 diodes over UV-VIS spectral range to evaluate UV spectra across the peak of interest. Ones these data have been acquired through processing algorithms access, we can conclude whether peaks are consistent with a single compound or not.

TABLE 4 : Robustness data

Sr. No	Column	Mobile Phase	Flow rate	Temperature	Wavelength	Assay of Irinotecan
1.0	C-18, Inertsil ODS3, 5µ (250 X 4.6) mm	Buffer:ACN (70:30)	1.2 ml/min	40°C	256nm	20.86 mg/ml
2.0	Microbondapack, 10µ (300 X 3.9) mm	Buffer:ACN (72:28)	0.8 ml/min	35°C	252nm	20.76 mg/ml
3.0	C-18, Hyperbond, 10µ (300 X 3.9) mm	Buffer:ACN (73:27)	1.0 ml/min	30°C	258nm	20.91 mg/ml

CONCLUSION

The presented work describing a reverse phase HPLC method for the determination of irinotecan is specific, accurate, rugged, precise, linear and can be applied for the quantification of irinotecan in different drug products. Validated method permits the separation and identification of four impurities of irinotecan. The method was successfully applied for the determination of irinotecan in injectables and can be applied to other drug products and drug substances with small modification and optimization.

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