



A Validated Chiral LC Method for the Enantiomeric Separation of Irinotecan Hydrochloride on Immobilized Cellulose Based Stationary Phase

Vitthal Dhakane, Milind Ubale*

Post Graduate Department of Chemistry, Vasantrao Naik Mahavidyalaya, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, MS, India.

*Corresponding author's E-mail: mbubale@yahoo.com

Accepted on: 15-02-2013; Finalized on: 31-03-2013.

ABSTRACT

A simple, rapid, isocratic, normal phase chiral HPLC method was developed and validated for the enantiomeric separation of irinotecan, (*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1*H*pyrano[3',4':6,7]-indolizino[1,2-*b*]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate, an antineoplastic drug substance. The enantiomers of irinotecan were resolved on a Chiralcel OD-H (immobilized cellulose based stationary phase) column using a mobile phase consisting of *n*-hexane: ethanol (45.9:50.5, v/v) at a flow rate of 0.9 mL min⁻¹. The resolution between both enantiomers was greater than 2 in the optimized method. The method validated for linearity, accuracy, precision, limit of detection, limit of quantification, enantioselective, accurate, precise, and suitable for quantitative determination of (*R*)-enantiomer in bulk drug substance and product. The calibration curve shows excellent linearity over the concentration ranges. The proposed method can be used for the quality control of bulk drugs products.

Keywords: Irinotecan, enantiomeric purity, chiral LC, method validation.

INTRODUCTION

Irinotecan hydrochloride is a semisynthetic derivative of camptothecin, an alkaloid extract from plants such as *Camptotheca acuminata* or is chemically synthesized. The chemical name is (*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1*H*pyrano[3',4':6,7]-indolizino[1,2-*b*]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate. Literature surveys reveal, high-performance liquid chromatographic methods were reported for the determination of Irinotecan Hydrochloride in bulk drugs. It felt necessary to develop a stability indicating LC method for analysis of Irinotecan hydrochloride.¹ There is no method found for Validation and stability determination of Irinotecan hydrochloride having degradation study.

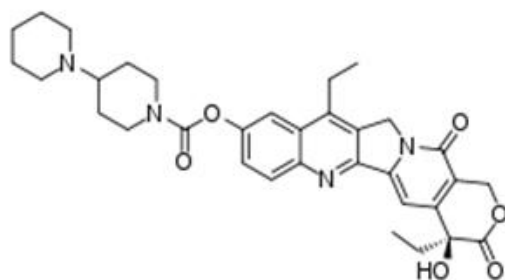


Figure 1: Chemical structure of (+)-(S)-irinotecan.

Irinotecan hydrochloride has a high affinity for dopamine. Irinotecan (Camptosar, Pfizer; Campto, Yakult Honsha) is a drug used for the treatment of cancer. Irinotecan prevents DNA from unwinding by inhibition of topoisomerase 1. In chemical terms, it is a semisynthetic analogue of the natural alkaloid camptothecin. Its main use is in colon cancer, in particular, in combination with other chemotherapy agents. This includes the regimen FOLFIRI, which consists of infusional 5-fluorouracil,

leucovorin, and irinotecan. Irinotecan received accelerated approval by the U.S. Food and Drug Administration (FDA) in 1996 and full approval in 1998. During development, it was known as CPT-11. Pharmacogenomics is the branch of pharmacology which deals with the influence of genetic variation on drug response in patients by correlating gene expression or single-nucleotide polymorphisms with a drug's efficacy or toxicity.^{2,3} By doing so, pharmacogenomics aims to develop rational means to optimize drug therapy, with respect to the patients' genotype, to ensure maximum efficacy with minimal adverse effects. Such approaches promise the advent of "personalized medicine"; in which drugs and drug combinations are optimized for each individual's unique genetic makeup.^{4,5}

MATERIALS AND METHODS

Chemicals

Samples of (*R*)-enantiomer and Irinotecan were obtained from Shilpa Medicare, Aurangabad, India. The drug product (CAMTOSAR) of Irinotecan label claims (irinotecan 40 mg) was purchased from the market. HPLC grade *n*-hexane and ethanol was purchased from Qualigens Fine chemicals, Mumbai, India. The HPLC grade diethyl amine was purchased from Merck Ltd, Mumbai, India.

Equipment

HPLC system used was a Waters, Empower (2649 series, USA), system equipped with auto sampler, quaternary pump, degasser and a UV detector. The output signal was monitored and processed using Empower software.

Sample preparation

The stock solution of (*R*) and (*S*)-enantiomers of Irinotecan (4.0 mg mL⁻¹) was prepared by dissolving appropriate amount of substance in ethanol and diethylamine (250:0.7 v/v). For quantification of (*R*)-enantiomer in irinotecan, a solution of 0.4 mg mL⁻¹ concentration was used.

Chromatographic condition

The chromatographic column used was Chiralcel, 250 x 4.6 mm, 5 μ m (Daicel Chemical Industries, Ltd., Tokyo, Japan). The mobile phase was *n*-hexane: ethanol (45.9:50.5, v/v). The flow rate of the mobile phase was 0.9 mL min⁻¹. The column temperature was maintained at 30°C and the eluent was monitored at a wavelength 255 nm. The injection volume was 20 μ L.

Method development

The racemic mixture was prepared by physical mixing of equal proportions of (*R*) and (*S*)-irinotecan (0.4 mg of each sample). A 0.2 mg mL⁻¹ solution of racemic mixture was prepared in ethanol and diethylamine (250:0.7 v/v) diluent and used for the method development. To develop the suitable chiral HPLC method for the separation of the enantiomers of irinotecan, different mobile phases were employed. Various experiments were carried out to select the best mobile phase that would give the optimum resolution and selectivity for the two enantiomers.^{6,7}

The enantiomeric separation for irinotecan was not achieved by using *n*-hexane on Chiralpak IA column. There was an indication of separation on Chiralpak ADH column using the mobile phase consisting of *n*-hexane:IPA (70:30, v/v) but the peak shapes were slightly broad. For further improvement in resolution, peak shape and column efficiency, the peak modifier diethyl amine was used. Interesting observations were found when 0.2% diethyl amine was used in the mobile phase; both the peak shape and resolution were improved.⁸ Very good separation was achieved on Chiralcel OD-H column (resolution between enantiomers was found to be > 2) using the mobile phase *n*-hexane: ethanol (45.9:50.5, v/v).

Method Validation

System suitability

The system suitability was determined by injecting racemic mixture containing equal quantity of (*R*) and (*S*)-enantiomers. Since the enantiomers form a critical pair of peaks in the chromatogram, the qualification criteria was resolution between two enantiomers, shown to be not less than 2 and tailing factor should not exceed 1.5.

Precision

Method reproducibility was determined by measuring repeatability and intermediate precision (between-day

precision) of retention times and peak areas for each enantiomer.

In order to determine the repeatability of the method, replicate injections (n = 6) of a 0.2 mg mL⁻¹ solution containing (*S*)-irinotecan spiked with (*R*)-enantiomer (0.2%) was carried out. The intermediate precision was also evaluated over three days by performing six successive injections each day.⁹

Linearity of (*R*)-enantiomer

Linearity was assessed by preparing six calibration sample solutions of (*R*)-enantiomer covering from 0.35 μ g mL⁻¹ (LOQ) to 3.75 μ g mL⁻¹ (0.35, 0.50, 0.80, 1.0, 1.20 and 1.50 μ g mL⁻¹), prepared in diluent phase from (*R*)-enantiomer stock solution.

Regression curve was obtained by plotting peak area versus concentration, using the least squares method. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve was calculated.

Quantification of (*R*)-enantiomer in bulk drug substance and product

The bulk drug substance and product did not show the presence of (*R*)-enantiomer; therefore standard addition and recovery experiment were conducted to determine the accuracy of the present method for the quantification of (*R*)-enantiomer.

The study was carried out in triplicate at 0.4, 0.5 and 0.6% of the (*S*)-irinotecan target analyte concentration. The recovery of (*R*)-enantiomer was calculated by determining recovery of the spiked amount of (*R*)-enantiomer in irinotecan.

Limit of detection and limit of quantification of (*R*)-enantiomer

Limit of detection and limit of quantification of (*R*)-enantiomer were achieved by injecting a series of dilute solutions of (*R*)-enantiomer.

The precision of the developed enantioselective method for (*R*)-enantiomer at limit of quantification was checked by analyzing six test solutions prepared at LOQ level and calculating the percentage relative standard deviation of area.

Robustness of the methods

To determine robustness of the method, experimental conditions were purposely altered, and chromatographic resolution between enantiomers was evaluated.

The flow rate of the mobile phase was 0.9 mL min⁻¹. To study the effect of flow rate on resolution of enantiomers, it was changed 0.1 units from 0.81 to 0.99 mL min⁻¹. The effect of change in percent of ethanol on resolution was studied by varying from -1 to +1% while the other mobile phase components were held constant, as stated in chromatographic condition section. The effect of column temperature on resolution was studied at 28°C and 32°C instead of 30°C while other mobile phase



components were held constant, as stated in chromatographic condition section.

Solution stability and mobile phase stability

Stability of (*S*)-irinotecan in solution at analyte concentration was studied by keeping the solution in tightly capped volumetric flask at room temperature on laboratory bench for 3 days. Content of (*R*)-enantiomer was checked at 8 h intervals up to the study period.

Mobile phase stability was carried out by evaluating the content of (*R*)-enantiomer in (*S*)-irinotecan. For 3-days same mobile phase was used during the study period.

RESULTS AND DISCUSSION

The mechanism of separation in direct chiral separation methods is the interaction of chiral stationary phase (CSP) with enantiomer that is analyte to form short-lived, transient diastereomeric complexes. The complexes are formed as a result of hydrogen bonding, dipole-dipole interactions, pi bonding, electrostatic interactions, and inclusion complexation.

The chiral stationary phase (CSP) that gave the best separation was Chiralcel which is Cellulose tris (3,5-dimethylphenylcarbamate) coated on 5 μ m silica-gel. The separation of enantiomers on Chiralcel was due to the interaction between the solute and the polar phenylcarbamate group on the CSP. In addition the dipole-dipole interaction occurs between the C=O group on the CSP and C=O group on the irinotecan.

Irinotecan is an acidic compound and to prevent peak tailing from the presence of extra high activity, small amount of diethyl amine was used in the diluent, to help block active sites and improve peak symmetry. Introduction of diethyl amine in the diluent enhanced the chromatographic efficiency, peak symmetry and resolution between the enantiomers. It played an important role for the improvement of resolution, column efficiency and peak shape.

The immobilized amylose-based stationary phase in Chiralcel OD-H column has higher selectivity than protein based (chiral AGP) and amylose based (Chiralpak IA) columns, being suitable for the enantioselective separation and accurate quantification of (*R*)-irinotecan. Another advantage of Chiralcel OD-H column is their greater stability under normal operation than other Daicel chiral columns. Immobilized column have good stability to strong solvents like THF, ethyl acetate, and chlorinated solvents. Using immobilized stationary phase columns allows a great freedom of solvent choices.¹⁰

A representative chromatogram of the enantiomeric resolution of irinotecan was shown in (Figure 2,3). An excellent resolution ($R_s = 4.68$) between two enantiomers and ideal peak shape with tailing factor 1.09 was obtained. The system suitability test results of the chiral liquid chromatographic method on Chiralcel OD-H are presented in (Table 1).

Table 1: System suitability results

Parameter	R	S
Retention time/min	10.648	15.222
Resolution (R_s)	5.44	214
USP Tailing	1.09	1.07
Theoretical plate	4780	5200
% RSD of retention time	1.22	0.77
% RSD of peak area	1.48	1.57

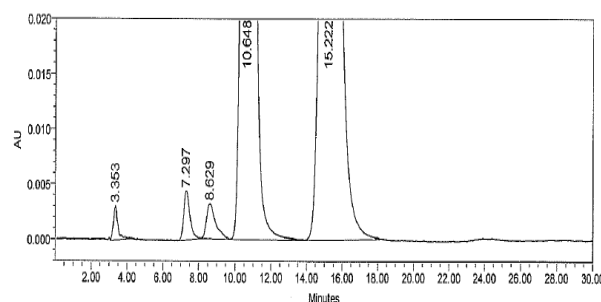


Figure 2: A chromatogram of the Irinotecan Hydrochloride system suitability solution.

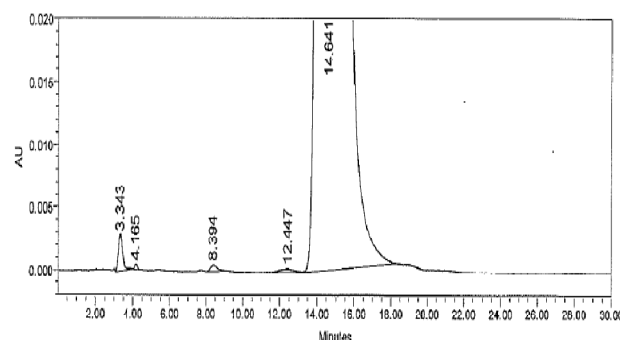


Figure 3: A chromatogram of the Irinotecan Hydrochloride Sample preparation

Validation results

In the precision study, the percentage relative standard deviation (RSD) was less than 2.0% for the retention times of the enantiomers, 0.77% for (*S*)-irinotecan peak area and 1.22% for (*R*)-enantiomer peak area. In the intermediate precision study, the results showed that R.S.D. values were in the same order of magnitude than those obtained for repeatability.

The limit of detection (LOD) and limit of quantification (LOQ) concentration were estimated to be 0.18 and 0.27 μ g mL⁻¹ for (*R*)-enantiomer, when signal-to-noise ratio of 3 and 10 were used as the criteria. The method precision for (*R*)-enantiomer at limit of quantification was less than 1.5 RSD%

The described method was linear in the range of 0.35 to 1.50 μ g mL⁻¹ for (*R*)-enantiomer in irinotecan. The calibration curve was drawn by plotting the peak area of (*R*)-enantiomer verses its corresponding concentration with correlation coefficient of 0.9999. The equation of the calibration curve for (*R*)-enantiomer was $Y = 3.9436x - 1.1654$. The percentage relative standard deviation of the

slope and Y-intercept of the calibration curve were 1.2 and 1.5 respectively.

The recovery and standard addition experiments were conducted for (*R*) enantiomer in bulk samples in triplicate at 50, 100 and 150 % of the analyte concentration. Percentage recovery ranged from 98.0 to 101.0%.

A HPLC chromatogram of (*R*)-enantiomer is shown in Figure 3, a HPLC chromatogram of irinotecan tablet is shown in Table 2.

Table 2: Robustness of the chiral LC method

Parameter	Resolution between repaglinide and (<i>R</i>)-enantiomer
Flow rate / (mL min ⁻¹)	
0.81	3.88
0.9	4.22
0.99	2.88
Column Temperature / °C	
28	3.66
30	3.78
32	3.55
Acetonitrile percentage in the mobile phase / %	
29	3.21
30	3.98
31	3.07

The chromatographic resolution of the (*S*)- irinotecan and (*R*)-enantiomer peaks was used to evaluate the method robustness under modified conditions. The resolution between (*S*)- irinotecan and (*R*)-enantiomer was greater than 2.0 under all separation conditions tested (Table 2), demonstrating sufficient robustness.

CONCLUSION

A simple, rapid and accurate normal phase chiral HPLC method has been developed and validated for the enantiomeric separation of irinotecan. Chiralcel OD-H (immobilized cellulose-based chiral stationary phase) was found to be selective for the enantiomers of the drug. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method can be conveniently used by the quality control department for the quantitative determination of chiral impurity (*R*-enantiomer) in the bulk material. The developed method is more rapid and enantioselective than reported methods. The developed

method shows right order of elution of (*R*)-enantiomer and (*S*)-enantiomer. The developed method is more suitable than the reported methods with respect to resolution (> 2), number of theoretical plates (> 5000), USP tailing (< 1.3), the percentage recovery of the (*R*)-enantiomer between 98.5 to 101.5%.

Acknowledgments: The authors are thankful to The Head, Post Graduate Department of Chemistry, Vasantrya Naik Mahavidyalaya, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, 431004, MS, India, for providing laboratory facility. We are also thankful to Shilpa Medicare (Aurangabad, India) for providing sample of irinotecan for the research work.

REFERENCES

- Notari, Stefania, Bocedi, Alessio, Ippolito, Giuseppe, et al.; Journal of Chromatography, 831, 2006, 258-266.
- Ter Heine, Rob, Alderden-Los, Carolien G, Rosing, Hilde, Hillebrand, Michel JX, Van Gorp, Eric C M, Huitema, Alwin DR, Beijnen and Jos H. Rapid Communications in Mass Spectrometry, 21, 2007, 2505-2514.
- Rebiere, Herve, Mazel, Bernard, Civade, Corinne, Bonnet and Pierre Antoine. Journal of Chromatography B, 850, 2007, 376-383.
- Raju NA and Begum S. Simultaneous RP-HPLC Method for the Estimation of the Emtricitabine, Tenofovir Disoproxil fumarate and Irinotecan in Tablet Dosage form. Research J Pharm and Tech, 1(4), 2008, 522-525.
- Shirkhedkar AA, Bhirud CH and Surana SJ. Application of UV Spectrophotometric Methods for Estimation of Irinotecan hydrochloride. Pak J Pharm Sci, 22, 2009, 27-29.
- Sparidans RW, Crommentuyn KM, Schellens JH and Beijnen JH. Liquid- Chromatography assay for the antiviral nucleotide analogue irinotecan in plasma using derivitization with chloroacetaldehyde. J Chromatogr B, 791, 2003, 227-33.
- Persiani C and Cukor P. J. Chromatograph, 109, 1975, 413-417.
- Venkataramanan R, Burckart GJ and Ptachcinski RJ. Am. J. Hosp. Pharm, 43, 1986, 2800-2802.
- International Conference on Harmonisation, Draft Guideline on Validation Procedures, Definitions and Terminology, Federal Register, Fed, 60, 1995, 112-60.
- Kearney A, Patel K and Palepu N. Int. J. Pharm, 127, 1996, 229-237.

Source of Support: Nil, Conflict of Interest: None.

