Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DARUNAVIR ETHANOLATE IN TABLET DOSAGE FORM

BHAVINI N. PATEL[1]*, BHANUBHAI N. SUHAGIA[2], CHAGANBHAI N. PATEL[1]

[1]* Department of Pharmaceutical Chemistry, Shri Sarvajanik Pharmacy College, Mehsana-384001, [2] Department of Pharmaceutical Chemistry, L. M. College of Pharmacy, Navrangpura, Ahmedabad, India. Email: bhavi_pharma22783@yahoo.co.in

Received: 18 May 2012, Revised and Accepted: 25 Dec 2012

ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of Darunavir ethanolate in tablet dosage form. A Phenomenex Luna C18 column (250 mm i.d., 4.6mm, 5µm) with mobile phase consisting of water-acetonitrile (40: 60, v/v; pH adjusted to 3.2 ± 0.02 with formic acid) was used. The flow rate was 1.0 ml/min and the effluents were monitored at 267 nm. The retention time was 5.02 min. The detector response was linear in the concentration of 2-20 µg/ml. The limit of detection and limit of quantification was found to be 0.085 µg/ml and 0.38 µg/ml respectively. This method was simple, precise, and sensitive, and they are applicable for the determination of Darunavir ethanolate in tablet dosage form.

Keywords: Darunavir ethanolate, HPLC, Tablet formulation

INTRODUCTION

Darunavir ethanolate (DRV), [(1S,2R)-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl) propyl]-carbamic acid (3R,3aS,6aR)-hexahydrofuro[2,3-b] furan-3-yl ester monoethanolate, is a new protease inhibitor (PI) used to treat human immunodeficiency virus (HIV) type-1 Fig.1[1]. The molecular weights of darunavir base and darunavir ethanolate are 547.73 and 593.73 g/mL, respectively. According to in vitro experiments, DRV was active against HIV-1 with PI-resistance mutations and against PI-resistant clinical isolates[2–4]. This drug is expected to be effective in patients experienced in antiretroviral treatment, such as those carrying HIV-1 strains which are resistant to more than one PI[5–8]. Literature survey revealed that different analytical methods for the determination of DRV have been reported, which include high-performance liquid chromatography (HPLC) with UV detection (HPLC-UV) to determine DRV in human plasma[9]; HPLC–MS method for the simultaneous determination of DRV and 11 other antiretroviral agents in plasma of HIV infected patients[10]; validation of plasma DRV concentrations by the HPLC for PIs[11]; and electrophoretic method for the separation of DRV[12]. The aim of the study was to develop a simple, precise and accurate RP-HPLC method for the estimation of DRV in bulk drug and pharmaceutical dosage form.

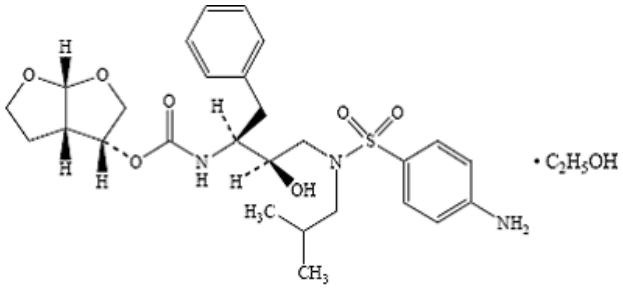

Fig. 1: Structure of Darunavir ethanolate

MATERIALS AND METHODS

Reagents and chemicals

DRV pure powder with 99.96% purity was obtained as a gift sample from Cipla Ltd (Mumbai, India). DRV tablets (300.0 mg/tablet) were procured from the market. HPLC grade acetonitrile (S.D. Fine Chemicals, Ahmedabad, India) methanol and water (Finar chemicals Ltd., Ahmedabad, India), formic acid (Spectrochem Pvt Ltd., Mumbai, India) and nylon filter (Millipore Pvt., Ltd, Bangalore, India) were used for study.

Apparatus and chromatographic conditions

HPLC method development and validation was done on a Shimadzu (Columbia, MD) RP-HPLC instrument (LC-10AT vp) equipped with a SPD-10 Avp UV detector, manual injector with 20 µL loop and Class-VP software was used. Stationary Phase used was Phenomenex (Torrance, CA) Luna C18 column (250mm × 4.6 mm id, 5 µm particle size) and the mobile phase was water-acetonitrile (40: 60, v/v; pH adjusted to 3.2 ± 0.02 with formic acid. The mobile phase was filtered through nylon 0.45 µm membrane filter (Millipore Pvt., Ltd, Bangalore, India). The mobile phase flow rate was 1 ml/min and injection volume was 20 µL. All weighing were done on analytical balance (Acculab ALC-210.4, India).

Preparation of mobile phase

The mobile phase was prepared by mixing 40.0 mL water and 60.0 mL acetonitrile, pH was adjusted to 3.2 with formic acid. The mobile phase was degassed for 15 minutes before use.

Preparation of Standard Solutions

Accurately weighed DRV standard (25.0 mg) was transferred to a 25 ml volumetric flask, dissolved and diluted to the mark with methanol to obtain a standard stock solution (1000 µg/ml). An aliquot (5.0 ml) was diluted to 25 ml with mobile phase to obtain a working standard solution of DRV (200 µg/ml).

Preparation of Sample Solutions

Twenty tablets were weighed and powdered. The accurately weighed powder equivalent to 25.0 mg DRV was transferred to 25 ml volumetric flask and methanol (15.0 ml) was added. The solution was sonicated for 15 min. The flask was allowed to stand at room temperature for 5 min, and the volume was diluted to the mark with methanol to obtain the sample stock solution (1000 µg/ml). The solution was filtered through 0.45µm-47mm membrane filter. The solution was suitably diluted with mobile phase to obtain sample solution of DRV 8 µg/ml.

RESULTS AND DISCUSSION

HPLC method

For the RP-HPLC, chromatographic conditions were optimized to get best resolution and peak shape. The selection of mobile phase was based on peak parameters; (symmetry, theoretical plates, capacity factor and tailing factor) ease of preparation and cost. A symmetrical peak with good separation (Rt of DRV 5.02 min) was obtained with C-18 column and mobile phase consisting water-acetonitrile (40: 60, v/v; pH adjusted to 3.2 ± 0.02 with formic acid) at a flow rate of 1.0

ml/min. Chromatogram of standards and formulation are given in Fig. 2 and 3 respectively. The optimum wavelength for detection and quantification was 267 nm, at which good detector response was obtained with symmetrical peak.

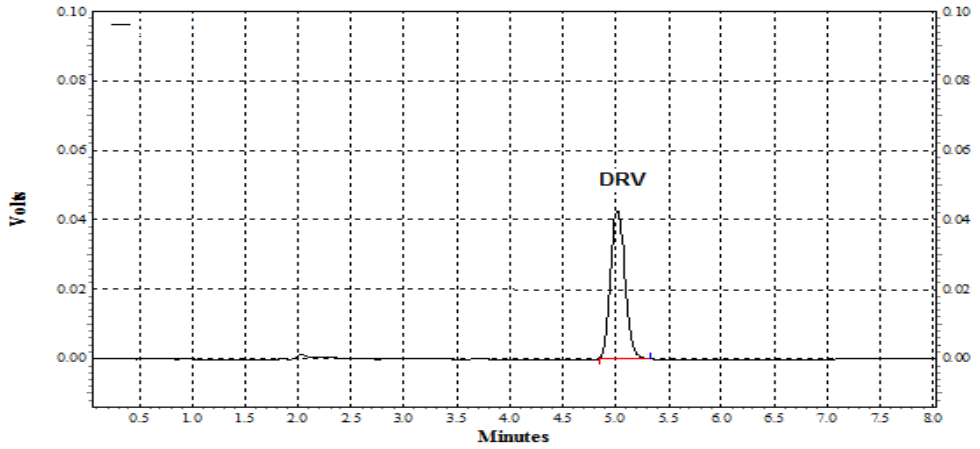

Fig. 2: Chromatogram of DRV Standard (8µg/mL)

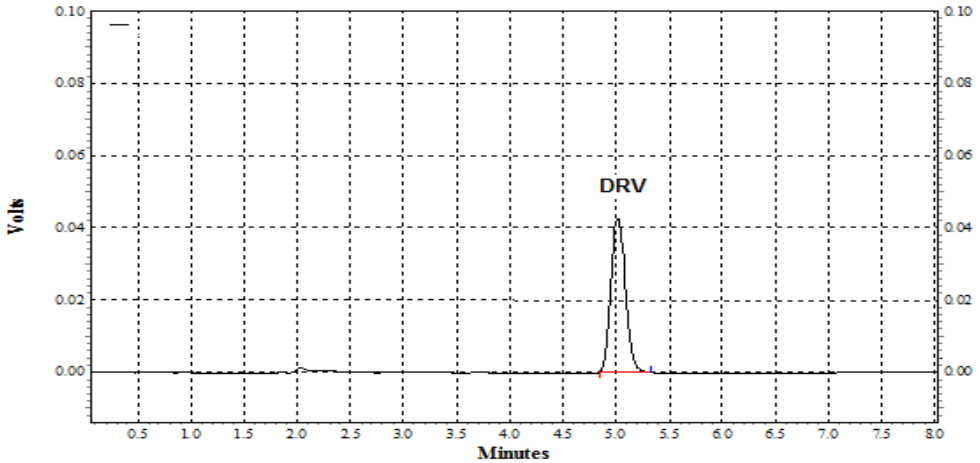

Fig. 3: Chromatogram of DRV Formulation (8µg/mL)

Method validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures[13].

Linearity and Range

The calibration curve was plotted over the concentration range of 2.0-20.0 µg/ml for DRV. Aliquots (0.1, 0.2, 0.4, 0.6, 0.8 & 1.0 ml) from working standard solution were transferred into a series of 10 ml volumetric flasks and diluted to the mark with mobile phase. Each of this drug solution (20 µL) was injected three times under the operating chromatographic conditions as described above. Calibration curve was constructed by plotting peak areas versus concentrations of DRV shown in Fig 4. The plot of the peak area of each sample against concentration of DRV was found to be linear in the range of 2-20 µg/ml with correlation coefficient of 0.9996. Linear regression least square fit data obtained from the measurement are given in Table 1. The respective linear regression equation being Y= 36260x + 5885.1.

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.04 µg/ml and 0.12 µg/ml respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

Table 1: Calibration data of DRV by RP-HPLC method

Concentration (µg/ml)	Peak area
2	81109
4	148216
8	289963
12	448417
16	587857
20	727841

Accuracy

Accuracy was determined by recovery studies of DRV, known amount of standard was added to the preanalyzed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

Precision

The repeatability was checked by repeatedly injecting [number of replicates (n) = 6] solutions of DRV (8.0 µg/ml). The RSD value for DRV was obtained 0.56 % which indicates method is repeatable. The intraday and interday precisions of the proposed methods were determined by measuring the responses 6 times on the same day and on 6 different days over a period of 1 week for 3 different concentrations of DRV 8.0, 12.0, and 16.0 µg/ml. The % R.S.D for intra- and inter-day variation is given in Table 3.

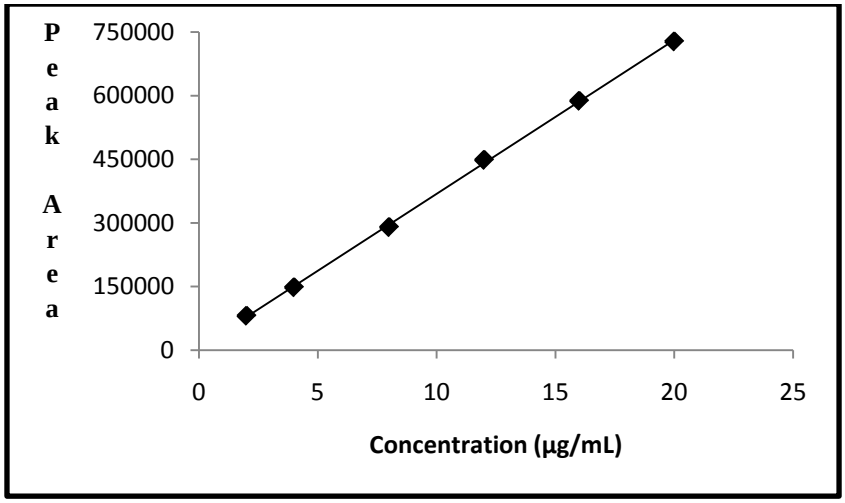

Fig. 4: Calibration curve of DRV

Table 2: Results of Recovery studies (n=6)

Drug	Amount taken (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)	% Recovery	% RSD
DRV	8	0	7.99	99.88	0.27
	8	4	11.90	99.17	0.22
	8	8	15.99	99.98	0.32
	8	12	20.08	100.14	0.56

Table 3: Results of Intra-day and Inter-day precision (n=6)

DRV	Intra-day precision		Inter-day precision	
(µg/mL)	Mean ± SD	% RSD	Mean ± SD	% RSD
2	81448 ± 457.21	0.56	82425 ± 870.82	1.06
4	146373 ± 1035.08	0.71	147490 ± 1736.55	1.18
8	288096 ± 1929.83	0.67	287405 ± 2632.63	0.92
12	447173 ± 2742.61	0.61	447644 ± 5417.46	1.21
16	582567 ± 5178.40	0.89	589806 ± 8288.02	1.41
20	728586 ± 4992.75	0.69	729405 ± 8103.17	1.11

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), number of theoretical plates (N), tailing factor (T) and resolution were evaluated for six replicate injections of the drug at a concentration of 8 µg/ml. The results are given in Table 4.

Robustness

The robustness of the proposed method was evaluated by slight modification in the organic composition and pH values of aqueous phase of the mobile phase and flow rate. During these studies it was found that there was not much change retention time, area and symmetry of peak.

The developed method was used for the assay of commercially available tablets and six replicate determinations were performed. Experimental values obtained for the determination of tablets are given in Table 5. The interference of excipients was studied by comparing the chromatography of standards and formulations. The same shape and retention times of peaks showed that there was no interference from excipients.

Table 4: System suitability test parameters (n = 6)

S. No.	Retention time, min	Tailing factor	Asymmetry	Theoretical plates
1	5.02	1.12	1.15	8881
2	5.02	1.12	1.14	8834
3	5.02	1.12	1.15	8895
4	5.03	1.13	1.15	8719
5	5.02	1.12	1.14	8846
6	5.03	1.13	1.15	8752
Mean	5.02	1.12	1.15	8821
SD	0.0052	0.0052	0.0052	70.7599
%RSD	0.10	0.46	0.45	0.80

Table 5: Analysis results of tablet dosage form (n=5)

Formulation	Labelled amount (mg)	Amount found (mg)	DRV ± SD, %
DARUVIR	300	300.54	100.18 ± 0.36

CONCLUSION

The developed RP-HPLC method was simple, sensitive, precise and accurate hence can be used in routine for the determination of DRV in bulk and pharmaceutical dosage form.

REFERENCES

1. Y. Koh, H. Nakata, K. Maeda, H. Ogata, G. Bilcer, T. Devasamudram, Novel bis-Tetrahydrofuranylurethane-Containing Nonpeptidic Protease Inhibitor (PI) UIC-94017 (TMC114) with Potent Activity against Multi-PI-Resistant Human Immunodeficiency Virus In Vitro Antmicrob. Agents Chemother. 47 (2003) 3123–3129.
2. S. Meyer, H. Azijn, D. Surleraux, D. Jochmans, A. Tahri, TMC114, a novel human immunodeficiency virus type 1 protease inhibitor active against protease inhibitor-resistant viruses, including a broad range of clinical isolates. Antmicrob.Agents Chemother. 49 (2005) 2314–2321.
3. Y. Tie, P. I. Boross, Y.F. Wang, L. Gaddis, A.K. Hussain, High resolution crystal structure of HIV-1 protease with a potent non peptide inhibitor active against multi drug resistant. J. Mol. Biol. 338 (2004) 341–352.
4. N. King, M. Jeyabalan, E. Nalivaika, P. Wigerinck, M. Bethune, C. Schiffer, Structural and Thermodynamic Basis for the Binding of TMC114, a Next-Generation Human Immunodeficiency Virus Type 1 Protease Inhibitor J. Virol. 78 (2004) 12012–12021.
5. E. Poveda, F. Blanco, P. Garcia-Gasco, A. Alcolea, V. Briz, V. Soriano, Successful rescue therapy with darunabir (TMC114) in HIV-infected patients who have failed several ritonavir-boosted protease inhibitors. AIDS 20 (2006) 1558–1560.
6. Kovalevsky, Y. Tie, F. Liu, P. Boross, Y. Wang, S. Leshchenko, A. Ghosh, R. Harrison, I. Weber. Effectiveness of Nonpeptide Clinical Inhibitor TMC-114 on HIV-1 Protease with Highly Drug Resistant Mutations D30N, I50V, and L90M J. Med. Chem. 49 (2006) 1379–1387.
7. Katlama, R. Esposito, J.M. Gatell, J.C. Goffard, B. Grinsztejn, A. Pozniak, J. Rockstroh, A. Stoehr, N. Vetter, P. Yeni, W. Parys, T. Vangeneugden. Efficacy and safety of TMC114/ritonavir in treatment-experienced HIV patients: 24-week results of power1 AIDS 21 (2007) 395–402.
8. Clotet, N. Bellos, J.M. Molina, D. Cooper, J.C. Goffard, A. Lazzarin, A. Wohrmann, C. Katlama, T. Wilkin. Efficacy and safety of darunavir-ritonavir at week 48 in treatment-experienced patients with HIV-1 infection in POWER 1 and 2: a pooled subgroup analysis of data from two randomised trials Lancet 369 (2007)1169–1178.
9. L. Goldwirt, S. Chhun, E. Rey, O. Launay, V. Jean-Paul, G. Pons, V. Jullien Quantification of darunavir (TMC114) in human plasma by high-performance liquid chromatography with ultra-violet detection J. Chromatogr. B 857 (2007) 327–331.
10. Avolio, M. Siccardi, M. Sciandra, L. Baietto HPLC–MS method for the simultaneous quantification of the new HIV protease inhibitor darunavir, and 11 other antiretroviral agents in plasma of HIV-infected patients J. Chromatogr.B 859 (2007) 234–240.
11. M. Takahashi, Y. Kudaka, N. Okumura, A. Hirano, K. Banno The Validation of Plasma Darunavir Concentrations Determined by the HPLC Method for Protease Inhibitors Biol.Pharm. Bull. 30 (2007) 1947–1949.
12. S. Leonard, A. Schepdael, T. Ivanyi, I. Lazar, J. Rosier, M. Vanstockem Development of a capillary electrophoretic method for the separation of diastereoisomers of a new human immunodeficiency virus protease inhibitor Electrophoresis 26 (2005) 627.
13. ICH Steering Committee: International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedure- Methodology, Geneva, 2006.