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# Novel RP-HPLC Method for the Determination of Darunavir In Pure and Tablet Dosage Form

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## ABSTRACT

The objective of the present research work is to develop and validated analytical method for the determination of Darunavir in pure and tablet dosage form. A simple, rapid, precise, selective and accurate novel RP-HPLC method was developed and validated for separation and determination for the Darunavir in pure and tablet dosage form. Darunavir was analyzed by Zodiac $C_{18,}$ (250×4.6mm, 5µ), Shimadzu LC-20AT Prominence Liquid Chromatograph and mobile phase constituted of Triethylamine buffer: Acetonitrile (60:40 v/v). The pH of the buffer was adjusted to 4.5 with diluted ortho-phosphoric acid. The flow rate of mobile phase was 1.0 mL/min and the analysis was performed using UV-Visible detector at 260nm. The Darunavir was eluted with in 6 min and retention time was showed 2.753 min. The developed assay method was validated by the guidelines of ICH Q2R1.The method was found to be linear in the drug concentration range of 20 µg/mL -100 µg/mL. The value of correlation coefficient was found to be 0.999. Method was found good percentage recovery it indicate the method is highly accurate. The method has been successfully applied for determination of Darunavir in pharmaceutical dosage form in regular quality control analysis.

**Keywords:** Darunavir, validation, Linearity, Accuracy and Robustness.

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## INTRODUCTION

Darunavir ethanolate (Figure 1) is an antiviral drug and inhibitor of the human immuno deficiency virus protease[1]. Chemically it is [(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. Darunavir selectively inhibits the cleavage of HIV-1 encoded Gag-Pol polyproteins in infected cells, thereby preventing the formation of mature virus particles[2]. Darunavir is co-administered with ritonavir and with other antiretroviral agents, is indicated for the treatment of human immunodeficiency virus (HIV-1) infection[3]

Literature survey revealed that a few spectrophotometric[4-6], HPLC[7-16], LC-MS[17-19], HPTLC[20] and electrophoresis[21] methods were reported earlier for the determination of Darunavir in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise RP-HPLC method for the estimation of Darunavir in pure samples and in tablet dosage forms.

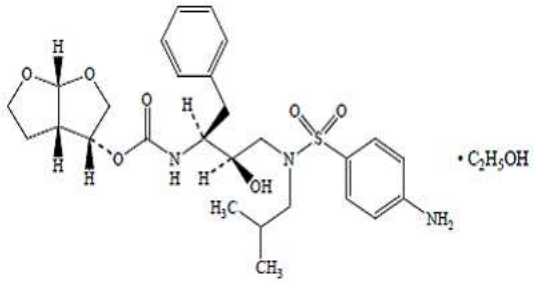

**Figure 1: Chemical structure of Darunavir monoethanolate**

## MATERIALS AND METHODS

All the chemicals and reagents used were of analytical grade, Darunavir reference standard was gifted by MARS Therapeutics and chemicals Ltd., Hyderabad, India, with the purity of 99.19% The commercial product (Daruvir) tablets containing 300mg Darunavir was procured from local marcket. HPLC Water, Methanol, Tri ethylamine and orthophosphoricacid from Merck Specialties Pvt. Ltd., Mumbai, Acetonitrile(HPLC), Sodium dihydrogen phosphate(AR), DiPotassium hydrogen orthophosphate (AR) from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, Tri ethylamine buffer and Acetonitrile in the ratio 60:40 v/v was used as mobile phase and diluents. Chromatography was performed with Shimadzu HPLC (LC 20 AT VP) equipped with UV-Visible detector. The LC separations were performed at ambient temperature and Zodiac C18 Column (250×4.6 ×5µ) and Spin Chrome Software was used for LC Peak integration. The mobile phase was degassed by sonication with an ultrasonic bath (PCI Mumbai).The Standard substance was

weighed on analytical balance (AX 200 Shimadzu). Mobile phase with flow rate of 1.0mL/min and UV detection was carried out at 260 nm for Darunavir with injection volume 20µL.

**Mobile phase:**

60:40 v/v of TEA buffer and acetonitrile was mixed to get the mobile phase. pH of the mobile phase was adjusted to 4.5 with diluted orthophosphoric acid. The mobile phase was then filtered through 0.22µm nylon membrane vacuum filtration and degassed by sonication.

**Preparation of Standard Stock Solutions:**

A standard stock solution of the drug was prepared by dissolving 100 mg of Darunavir volumetric flask containing 40 ml mobile phase, then sonicated for about 10 minutes and made up to 100 ml with mobile phase to get the primary standard stock solution containing 1000 µg/ml Darunavir. Working standard solutions were prepared by further dilution with mobile phase.

**Preparation of Sample solution:**

20 tablets of DARUVIR (Darunavir) were randomly selected and crushed them to a fine powder a quantity of 0.138g of tablet powder equivalent to 100 mg of Darunavir was transferred to 100mL volumetric flask and dissolved in diluent and the volume was adjusted up to the mark with diluent. The mixture was allowed to stand for 10 min with intermittent sonication to ensure complete dissolution. The resulting solution was filtered through a 0.22µm membrane filter. The filtrate was diluted further with mobile phase to get the working sample solution.

## RESULTS AND DISCUSSION

**Method development and optimization**

Initially method development was started with the selection of wavelength of detection. The UV spectrum of Darunavir in mobile phase was noted using UV spectrophotometer. The maximum absorbance was noticed at 260 nm. This wavelength was used for detection of Darunavir.

**Validation of the method**

The proposed method was developed by several concurrent trails in order to establish the preferred chromatographic conditions which would be helpful to conduct a complete validation study. Details of the trails for method development and optimization was shown in Table 1 and The mobile phase for consisting of TEA buffer pH4.5: Acetonitrile (60:40 v/v) at 1mL/min flow rate and detection wave length 260nm was optimized which gave sharp peak, minimum tailing factor with short run time for Darunavir. The retention time for Drunavir was found to be 2.753 minutes. The proposed method validated as per the guidelines of ICH Q2 (R1).[22] System suitability parameters and optimized chromatographic conditions are shown in Table 2.

**Table 1: Chromatographic conditions used for method development and optimization**

| Chromatographic condition | Trail 1 | Trail 2 | Trail 3 | Trail 4 | Optimized |
|---|---|---|---|---|---|
| Column | Zodiac C-18(250×4.6 ×5µ) | Zodiac C-18(250×4.6 ×5µ) | Zodiac C-18(250×4.6 ×5µ) | Zodiac C-18(250×4.6 ×5µ) | Zodiac C-18(250×4.6 ×5µ) |
| Detector wavelength(nm) | 260 | 260 | 260 | 260 | 260 |
| Column temperature($^0$C) | Ambient | Ambient | Ambient | Ambient | Ambient |
| Injection volume(µL) | 20 | 20 | 20 | 20 | 20 |
| Flow rate(mL/min) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Run time(min) | 6 | 6 | 6 | 6 | 6 |
| Asymmetry | 4.800 | 3.071 | 2.821 | 2.452 | 1.234 |
| Efficiency | 1730 | 1256 | 1499 | 1972 | 6321 |
| Retention time(min) | 3.417 | 2.710 | 2.797 | 2.607 | 2.753 |
| Mobile Phase | Methanol: Water(50:50, v/v) | Methanol: Water(60:40, v/v) | Mixed phosphate buffer: Acetonitrile (55:45, v/v) | Methanol: Acetonitrile: Water(60:20:20, v/v) | TEA Buffer(pH 4.5) :Acetonitrile (60:40, v/v) |

**Table 2: Optimized chromatographic conditions and system suitability parameters**

| Parameter | Chromatographic conditions |
|---|---|
| Instrument | SHIMADZU LC-20AT Prominence liquid chromatograph |
| Column | Zodiac $C_{18}$Column (4.6 X 250mm, 5µm) |
| Detector | SHIMADZU SPD-20A Prominence UV-Vis detector |
| Diluents | TEA Buffer(pH 4.5) : Acetonitrile (60:40, v/v) |
| Mobile phase | TEA Buffer(pH 4.5) : Acetonitrile (60:40, v/v) |
| Flow rate | 1mL/min. |
| Detection wave length | UV at 260nm. |
| Run time | 6 minutes |
| Column back pressure | 113-114 kgf |
| Temperature | Ambient temperature($25^0$C) |
| Injection Volume | 20µL |
| System suitability | Darunavir |
| Retention time ($t_R$) | 2.753 min. |
| Theoretical plates$^{\$}$ (Efficiency) | 5782 |
| Tailing factor (asymmetry)$^{\#}$ | 1.263 |

**Acceptance criteria (Limits):**$^{\$}$Theoretical Plates >2000, *Resolution >2.0, $^{\#}$Peak Asymmetry <1.5.

The specificity was studied for the examination of the presence of interfering components, while the comparison of chromatograms there was no interference from placebo shown in Figure 2 and standard chromatogram shown in Figure 3**.** The specificity results are summarized in Table 3.The chromatographic results for the calibration standards are presented in Table 4. The calibration curve for Darunavir was found to be linear over the range of 20-100µg/mL. The regression equation for Darunavir was found to be Y=131.45x - 316.47 with correlation coefficient, $R^2$=0.999 which indicates this method has good linearity. The data of the regression analysis of the calibration results is shown in Table 5 and calibration curve of Darunavir shown in Figure 5. Precision was studied to find out intra-day and inter-day variation in the test methods of Darunavir for 6 times on the same day and different days. The intra-day and inter-day precision obtained was %RSD (<2.0) indicates that the proposed method is quite precise and reproducible and results are shown in Table 6 and Table 7. Recovery studies of the drug were carried out for the accuracy parameters at three different concentration levels that are multiple level recovery studies. A known amount standard was added into pre analyzed sample and subjected them to proposed HPLC method. The % recovery was found to be within the limits as listed in Table 8. The mean percentage recovery of Darunavir at each level was not less than 98% and not more than 102%. Robustness was done by small changes in chromatographic conditions like flow rate and detection

of wave length etc. It was observed that there were no marked changes in chromatograms. In fact the parameters are within the limits which indicates that the method has robustness and suitable for routine use. The robustness results are shown in Table 9. Method validation following ICH guidelines indicated that the developed method had high sensitivity with 2.32µg/mL and 7.06µg/mL was shown in Table 10.

**Table 3: Results of specificity study**

| Name of the solution | Retention time, ($t_R$) min. |
|---|---|
| Mobile phase | No peaks |
| Placebo | No peaks |
| Solution containing a concentration of Darunavir 10µg/mL. | Peak at 2.753min for Darunavir respectively. |

**Table 4: Chromatographic results showing linearity of the proposed method for Darunavir**

| Drug | Concentration (µg/ml) | Retention time (min) | Peak area, (mV.s) | Peak Asymmetry# | Plate count$ |
|---|---|---|---|---|---|
| **Darunavir** | 20 | 2.753 | 2405.161 | 1.238 | 5782 |
| | 40 | 2.753 | 4896.964 | 1.200 | 5783 |
| | 60 | 2.753 | 7541.414 | 1.250 | 5782 |
| | 80 | 2.753 | 10021.381 | 1.200 | 5782 |
| | 100 | 2.753 | 12988.074 | 1.263 | 5781 |

**Acceptance criteria (Limits):** # Peak Asymmetry < 1.5, $ Plate count > 2000.

**Table 5: Results of linear regression analysis**

| Parameter | Darunavir |
|---|---|
| Detection wavelength($\lambda_{max}$) | UV at 260 nm |
| Linearity range (µg/mL) | 20-100µg/mL |
| Regression equation (Y = aX - b) | Y = 131.45x -316.47 |
| Slope(a) | 131.45 |
| Intercept(b) | 316 |
| Standard error of slope ($S_a$) | 0.175937 |
| Standard error of intercept ($S_b$) | 2.663371 |
| Standard error of estimation ($S_y$) | 3.679976 |
| Regression coefficient ($R^2$) | 0.999 |
| Percentage range of errors# (Confidence limits) 0.005 significance level | 0.0872 |

# Average of 6 determinations

**Table 6: Results of intra-day precision**

| **Darunavir** | | | |
|---|---|---|---|
| S. No. | RT | Area | Assay % |
| 1 | 2.753 | 10260.01 | 100.01 |
| 2 | 2.753 | 10158.72 | 100.33 |
| 3 | 2.750 | 10075.3 | 100.23 |
| 4 | 2.753 | 10304.42 | 100.21 |

| | | | |
|---|---|---|---|
| 5 | 2.750 | 10255.73 | 100.31 |
| 6 | 2.750 | 10313.540 | 100.01 |
| **AVG** | 2.753 | 10227.95 | 100.22 |
| **STDEV** | 0.001643 | 92.80981 | 0.136626 |
| **%RSD** | 0.058738 | 0.889265 | 0.136399 |

**Table 7: Results of inter-day precision**

| **Darunavir** | | | |
|---|---|---|---|
| **S. No.** | **RT** | **Area** | **Assay %** |
| 1 | 2.750 | 10313.540 | 100.01 |
| 2 | 2.750 | 10075.312 | 100.22 |
| 3 | 2.753 | 10158.720 | 100.32 |
| 4 | 2.753 | 10304.420 | 100.23 |
| 5 | 2.750 | 10255.732 | 100.31 |
| 6 | 2.753 | 10260.011 | 100.02 |
| **AVG** | 2.753 | 10227.950 | 100.20 |
| **STDEV** | 0.001643 | 92.8098 | 0.1366 |
| **%RSD** | 0.058738 | 0.8892 | 0.1363 |

**Table 8: Recovery studies**

| **Recovery level (%)** | **Amount spiked (mg)** | **Amount found (mg)** | **Average amount found (mg)*** | **% Recovery** |
|---|---|---|---|---|
| 50 | 150 | 449.63 | 449.59 | 99.90 |
| | 150 | 449.25 | | |
| | 150 | 449.89 | | |
| 75 | 225 | 524.98 | 525.05 | 100.01 |
| | 225 | 525.06 | | |
| | 225 | 525.10 | | |
| 100 | 300 | 600.10 | 598.29 | 99.71 |
| | 300 | 598.22 | | |
| | 300 | 596.56 | | |

*high %recovery indicates method accuracy.

**Table 9: Results of robustness**

| **S.No** | **Parameter** | **Optimized** | **Used** | | **Mean** | **STDEV** | **%RSD** |
|---|---|---|---|---|---|---|---|
| 1. | Flow rate(±0.2 mL/min) | 1.0 mL/min | 0.8 mL/min | Rt | 3.449 | 0.0027 | 0.0810 |
| | | | | Area | 10170.67 | 1.7070 | 0.0131 |
| | | | 1.0 mL/min | Rt | 2.765 | 0.0038 | 0.1151 |
| | | | | Area | 10207.071 | 0.9458 | 0.0092 |
| | | | 1.2 mL/min | Rt | 2.327 | 0.0023 | 0.1022 |
| | | | | Area | 10198.242 | 0.2148 | 0.0024 |
| 2. | Detection wavelength (±2 nm) | 260 nm | 258 nm | Rt | 2.755 | 0.0017 | 0.0619 |
| | | | | Area | 10458.53 | 1.2911 | 0.0123 |
| | | | 260 nm | Rt | 2.757 | 0.0021 | 0.0767 |
| | | | | Area | 10670.8 2 | 1.3431 | 0.0125 |
| | | | 262 nm | Rt | 2.755 | 0.0035 | 0.1304 |
| | | | | Area | 10330.736 | 1.9148 | 0.0201 |

**Table 10: Results of LOD and LOQ**

| Drug | LOD | LOQ |
|---|---|---|
| Darunavir | 2.32µg/ml | 7.06µg/ml |

**Table 11: Results of Assay**

| S.NO | Brand name | Label claim of Darunavir | Amount found of Darunavir | %Assay of Darunavir | %RSD* of Darunavir |
|---|---|---|---|---|---|
| **1** | Daruvir | 300mg | 297mg | 99.0 | 0.13 |

***Average of 6 determinations; RSD is Relative standard deviation

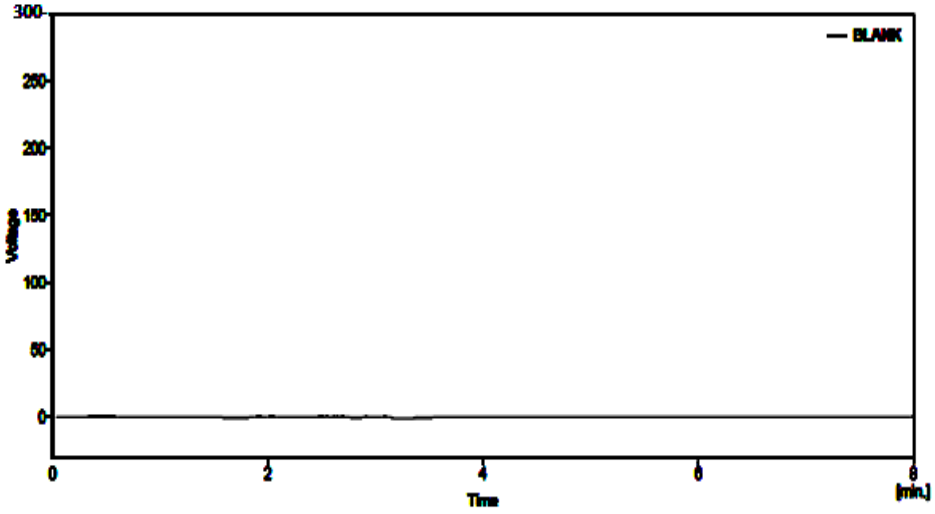


**Figure 2: Chromatogram of blank**

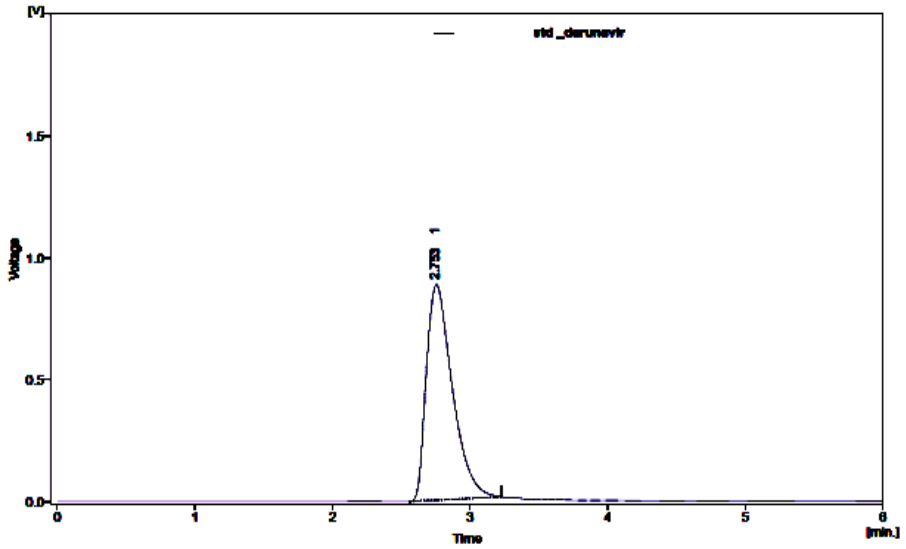


**Figure 3: Standard Chromatogram of Darunavir**

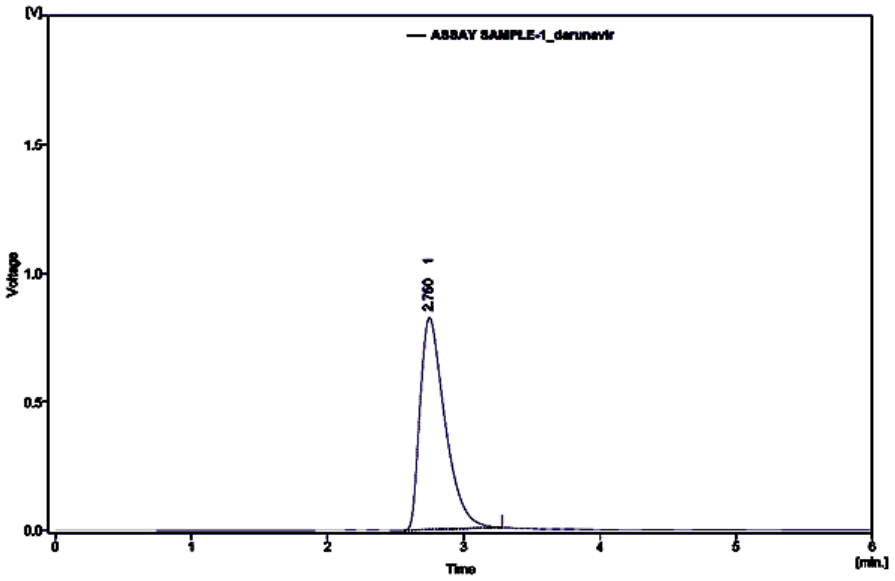


**Figure 4: Sample chromatogram of Darunavir**

**Applicability of the Developed Method to Marketed Formulations**

The assay results of Darunavir in tablet dosage form were comparable with the value claimed on the label. The obtained results, presented in Table 11 and sample chromatogram shown in figure 4 indicated the suitability of the method for routine analysis of Darunavir.

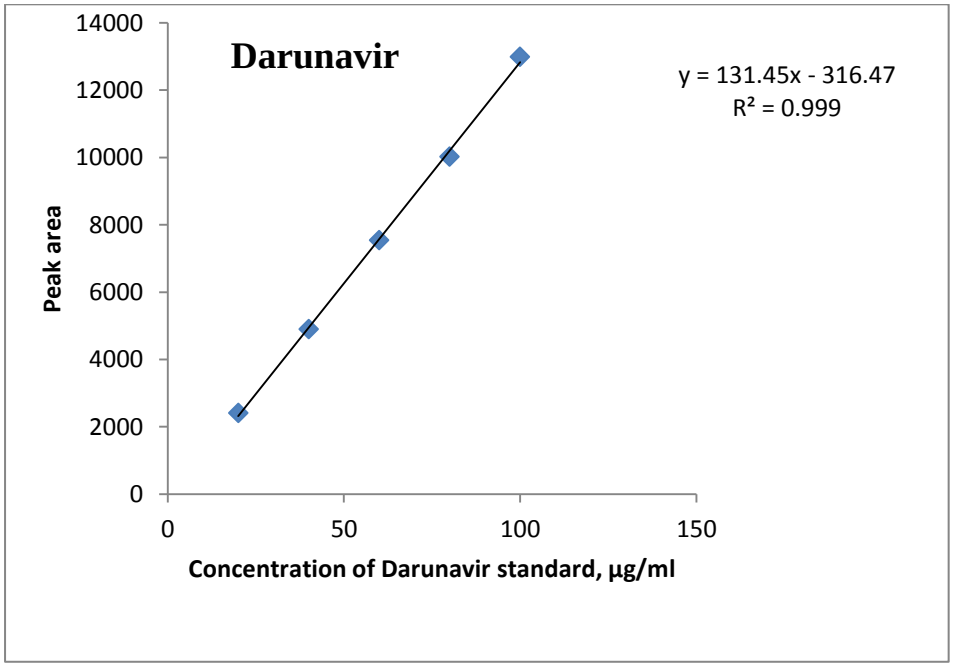


**Figure 5 : Calibration curve of Darunavir**

## CONCLUSION

A new method was developed for the quantification of Darunavir in tablet formulation. This method seems to obey the validation parameters and cost effective. This method can be routinely

employed for the analysis of this formulation. This method proven the aspects of less utilization of organic solvent, good accuracy and excellent correlation between the analyte and its response.

## REFERENCES


1. The Merck Index, 14th Edn., Merck Research Laboratories, Merck & Co., White House Station, NJ, USA, p. 477 (2006).
2. McKeage K, Perry CM and Keam SJ. Darunavir. Drugs. 2009; 69(4):477-503.
3. Ghosh AK, Dawson ZL and Mitsuya H.Darunavir, a conceptually new HIV-1 protease inhibitor for the treatment of drug-resistant HIV. Bioorg Med Chem.2007; 15(24):7576-7580.
4. Minal R. Ghante, Rohan S. Shelar, Sanjay D. Sawant, Manoj M. Kadam. Development and validation of uv spectrophotometric method for estimation of darunavir ethanolate in bulk and tablet dosage form. Int J Pharm Pharm Sci, 2014; Vol 6, Issue 7, 240-242,
5. Say Sirisha. Vanukuri1, Mastanamma.Sk, Alekhya.G. Validated UV-Spectrophotometric Methods for the Estimation of Darunavir by Absorption Maxima, First Order Derivative and Area under Curve in Bulk and Its Tablet Dosage Form. Int J Pharm Pharm Sci, 2014; Vol 6, Issue 1, 568-571.
6. Shinde VR, Gosavi SA, Pawar SS, Kasture VS, Musmade DS and Shinde VR. Development and validation of UV spectroscopic method for determination of Darunavir in bulk and tablet formulation. Inventi Rapid:Pharm Anal Qual Assur. 2013; 12:586.
7. Karthika, K. Rama and A. Chenthilnathan .RP-HPLC method development and validation for quantification of darunavir ethanolate in tablet dosage form. Der Pharmacia Lettre, 2016; Vol.8 Issue 5,291-296.
8. Hemant K. Jain, Umakant S. Jadhav. Development and validation of RP-HPLC method for estimation of darunavir ethanolate in bulk and tablets. Int J Pharm Pharm Sci, 2015; Vol 7, Issue 10, 386-389.
9. Nagendrakumar Avd, Sreenivasa Rao B and Basaveswara Rao MV. Validated RP - HPLC method for the determination of darunavir in bulk and pharmaceutical formulation. RJPBCS 2014; Vol.5 Issue 2, 64-73.
10. PG Chaudhary, BN Patel, CN Patel; A validated Stability-indicating High Performance
11. Liquid Chromatographic Method for Darunavir Ethanolate in Tablet Dosage Form; 2014; 2(9); 91-100.

12. G. Raveendra Babu, A. Lakshmana Rao and J. Venkateswara Rao. Development and validation of novel HPLC method for estimation of darunavir in pharmaceutical formulations. IJRPC 2013; Vol.3 Issue 2,438-443.
13. B.V. Rami Reddy, G. Jyothi, B.S. Reddy, N.V.V.S.S. Raman, K. Subhash Chander Reddy and C. Rambabu. Stability-indicating HPLC method for the determination of darunavir ethanolate. Journal of Chromatographic Science 2013; 51,471–476.
14. Manisha B. Mane, Pranali J. Gaikawad1, Anuja V. Patil, Aashish S. Mogale. RP-HPLC method for determination of darunavir in bulk and pharmaceutical preparations. Int. J. Pharm. Sci. Rev. Res., 2013; Vol.21 Issue 2, 20-23.
15. Bhavini N. Patel, Bhanubhai N. Suhagia, Chaganbhai N. Patel. RP-HPLC method development and validation for estimation of darunavir ethanolate in tablet dosage form. Int J Pharm Pharm Sci, 2012; Vol 4, Issue 3, 270-273.
16. L. Satyanarayana, S.V. Naidu2, M. Narasimha Rao, Alok Kumar1 and K. Suresh. The Estimation of Darunavir in Tablet dosage form by RP-HPLC., Asian J. Res. Pharm. Sci. 2011; Vol. 1: Issue 3, 74-76.
17. Raveendra B. Ganduri, Ramprasad A. Lanka, Srinivasu Pamidi, Jayachandra R. Peddareddigari , JVLNS Rao. New RP-HPLC method for the determination of Darunavir in tablet dosage form, Asian J. Pharm. Res. 2011;Vol 1, Issue 1, 10-14.
18. Gupta A, Singhal P, Shrivastav PS and Mallika S. Application of a validated ultra performance liquid chromatography-tandem mass spectrometry method for the quantification of Darunavir in human plasma for a bioequivalence study in Indian subjects. J Chromatogr B. 2011; 879(24):2443-2453.
19. Fayet A, Beguin A, Zanolari B, Cruchon S, Guignard N, Telenti A, Cavassini M, Gunthard HF, Buclin T, Biollaz J, Rochat B and Decosterd LA. A LC–tandem MS assay for the simultaneous measurement of new antiretroviral agents: Raltegravir, Maraviroc, Darunavir, and Etravirine. J Chromatogr B. 2009; 877(11-12):1057-1069.
20. Heine RT, Alderden-Los CG, Rosing H, Hillebrand MJ, Gorp ECV, Huitema AD and Beijnen JH. Fast and simultaneous determination of Darunavir and eleven other antiretroviral drugs for therapeutic drug monitoring: method development and validation for the determination of all currently approved HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom. 2007; 21(15):2505-2514.

21. Patel BN, Suhagia BN, Patel CN and Panchal HJ. A simple and sensitive HPTLC method for quantitative analysis of Darunavir ethanolate tablets. J Planar Chromatogr Mod TLC. 2011; 24(3):232-235.
22. Leonard S, Van AS, Ivanyi T, Lazar I, Rosier J, Vanstockem M, Vermeersch H and Hoogmartens J. Development of a capillary electrophoretic method for the separation of diastereoisomers of a new human immunodeficiency virus protease inhibitor. Electrophoresis. 2005; 26(3):627-632.
23. International Conference on Harmonization (ICH) harmonized tripartite guideline Validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva, Nov (2005).