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Spectrofluorimetric Estimation of Rilpivirine in Bulk and Dosage Forms

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ABSTRACT: Rilpivirine was measured using a precision spectrofluorimetric technique at 363 nm λ_{ex} and 389 nm λ_{em} . The method exhibits linearity within the 2-10 ng/ml. range, with a R^2 of 0.9992. Validated according to ICH criteria, it exhibits strong linearity, precision, accuracy, detection and quantification limits, making it appropriate for routine quality monitoring of bulk medications and pharmaceutical formulations.

KEY WORDS: Rilpivirine, Spectrofluorimetry, Validation.

I. INTRODUCTION

Absorption of UV/Visible radiation causes transition of electrons from singlet ground state to singlet excited state. As this state is not stable, it emits the energy in the form of uv/visible radiation and returns to singlet ground state. This study or measurement of this emitted radiation (when electrons undergo transition from singlet excited state to singlet ground state) is the principle in Fluorimetry. Fluorescence is an emission phenomenon where an energy transition from a higher to a lower state is accompanied by radiation. Only molecules in their excited forms are able to emit fluorescence; thus, they have to be brought into a state of higher energy prior to the emission phenomenon. Rilpivirine is also known as pentyl 4-{{4-[(E)-2-cyanovinyl]-2,6-dimethylphenyl} amino} pyrimidin-2-yl} amino} benzonitrile (Figure 1). It is a non-competitive NNRTI that binds to reverse transcriptase. Binding results in the blockage of RNA and DNA- dependent DNA polymerase activities, like HIV-1 replication.

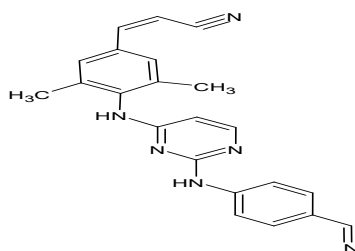


Figure 1. Structure of Rilpivirine

An extensive literature review on Rilpivirine revealed numerous analytical methods for its quantification, either as a single agent or in combination with other drugs. Reported methods include ultraviolet (UV) spectrophotometric techniques using various solvents¹³⁻¹⁶, high-performance liquid chromatography (HPLC) methods employing different combinations of stationary and mobile phases¹⁷⁻²⁶, bioanalytical techniques using HPLC^{27, 28} LC-MS/MS methods²⁹ and bioequivalence studies³⁰. Although numerous instrumental techniques were available till date, to the best of our knowledge, no spectrofluorimetric method has been reported for Rilpivirine using any fluorophore or fluorescent tagging agent. Spectrofluorimetry has gained prominence in drug analysis due to its high specificity and sensitivity. Unlike spectrophotometry, spectrofluorimetry enables analysis at both excitation and emission wavelengths^{10, 31}. Considering these advantages, a simple, extraction-free, and sensitive spectrofluorimetric method was developed for the quantification of Rilpivirine using ethyl acetoacetate and sulfuric acid. The method was validated in accordance with International Conference on Harmonisation (ICH) guidelines^{12, 32} and was successfully applied to the analysis of Rilpivirine in a marketed dosage form.

II. METHODOLOGY

2.1 Determination of λ_{\max} : DMSO was selected as solvent along with phosphate buffer for dissolving Rilpivirine. In order to prepare standard solution 10 mg of Rilpivirine were accurately weighed and transformed into 10 mL volumetric flask. And diluted upto mark with the DMSO and phosphate buffer to obtain 1000 $\mu\text{g/mL}$ concentration of Rilpivirine. 1 mL of the above stock solution was diluted to 100 mL with water (10 $\mu\text{g/mL}$). The above solution was scanned between 200 nm to 400 nm in 1 cm cell against blank in UV-Visible Spectrophotometer.

2.2. Method Optimization

2.2.1. Preparation of stock solution: To create a standard stock solution of concentration 1000 microgrammes/millilitre (1000 $\mu\text{g/mL}$), 10 milligrams of Rilpivirine were carefully weighed and then dissolved in DMSO and Phosphate buffer (3:6). To get a final volume, 1 millilitre of this standard stock solution was taken and diluted with water.

2.3. Calibration curve of Rilpivirine: Test samples of rilpivirine standard stock solution (2-10 $\mu\text{g/mL}$) were placed into volumetric flasks with a capacity of 10 mL. Each flask received 1 millilitre of standard stock solution, and the volumes were adjusted to the mark with distilled water. The absorbance of the solutions was then measured using a spectrofluorimeter, specifically between 300 and 770 nanometres.

2.4. Analytical method validation

2.4.1. Linearity studies: Stock solution containing 10 microgrammes per millilitre was accurately shifted to 10 mL volumetric flasks, yielding concentration ranges from 2 – 10 microgram/milliliter. Volumetric flasks contain standard stock solutions which are made up to the mark with water. Next, these solutions were scanned in the 300-770 nanometre spectral region, and intensities scanned at (λ_{em}) 389 nanometres and an (λ_{ex}) 363 nm.

2.4.2. Precision: Fluorometric techniques for Rilpivirine were tested for precision at concentrations of 5, 15, and 25 $\mu\text{g/mL}$. Measurements were done 3 times on the same and different days. The data were presented as relative standard deviation (% RSD).

2.4.3. Accuracy: The standard addition method was used to assess accuracy, which involved spiking 25 mg of Rilpivirine with standard solutions at 80%, 100%, and 120%. Quantification was carried out by measuring intensities at an emission wavelength of 457 nm, with three preparations for each concentration level.

2.4.4. Detection limit and Quantification limit: The Limit of Detection and Limit of Quantification of rilpivirine were calculated using the signal to noise ratio (S/N), with values of LOD and LOQ are 3.3 and 10, respectively.

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

2.4.5. Evaluation of commercially available formulations (Assay)

Take avg. wt of 5 rilpivirine 25 mg tablet dosage forms, Tablets are broken into fine powder using a mortar and pestle, 5mg of powder dosage form is weighed, 5 mg of powered tablet is placed in 25 milliliter of vol. flask, Powder is dissolved with MeOH, Solution is filtered using Whatmann filter paper, 1 mL of the filtrate is taken, 1 ml is diluted to 10mL final volume.

III. RESULTS AND DISCUSSION

3.1. Drug authentication studies

Drug authentication is done by determination of Melting Point and λ_{\max} by UV-Visible spectroscopy and comparing them with their reference values.

3.1.1. Determination of melting point:

Table 1: Determination of Melting Point

DRUG	RILPIVIRINE	
Values	Reported Value	Measured Value
Melting point	248-249 °C	248°C

3.1.2. Determination of absorption maxima: λ_{max} for Rilpivirine was found to be 306 nm which is in acceptance with the reference value.

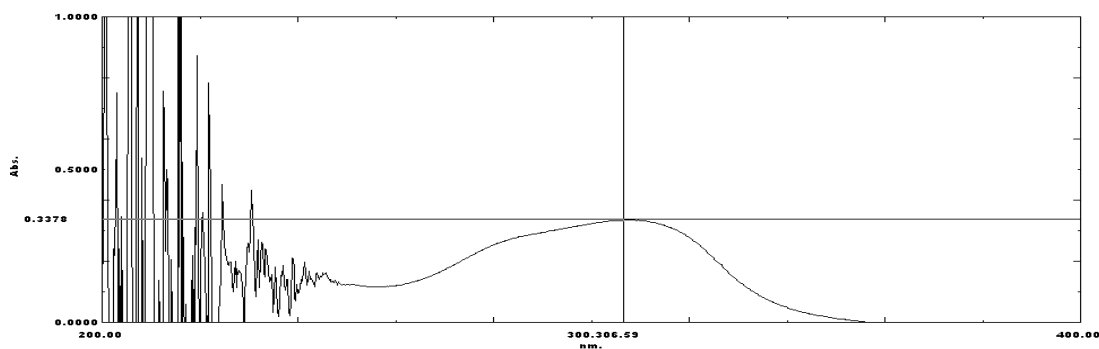


Figure.2 UV Spectra of Rilpivirine

3.1.3. Simple Method Development

The fluorescence intensity for Rilpivirine at the excitation wavelength 363 nm and emission wavelength 389 nm.

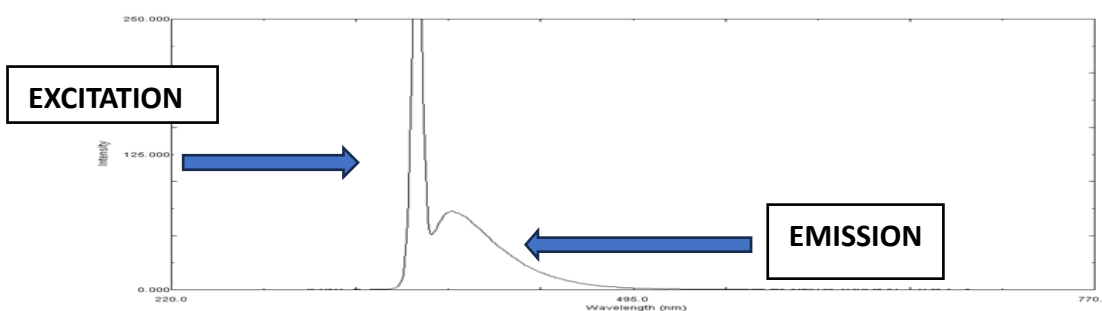


Figure.3 Excitation and Emission wavelength
(363 nm and 389 nm)

3.2. Method validation

3.2.1. Linearity: The drug shown linearity between 2-10 ng/mL for Rilpivirine at an excitation wavelength of 363 nm and emission wavelength of 389 nm. The correlation coefficient was found to be 0.9992.

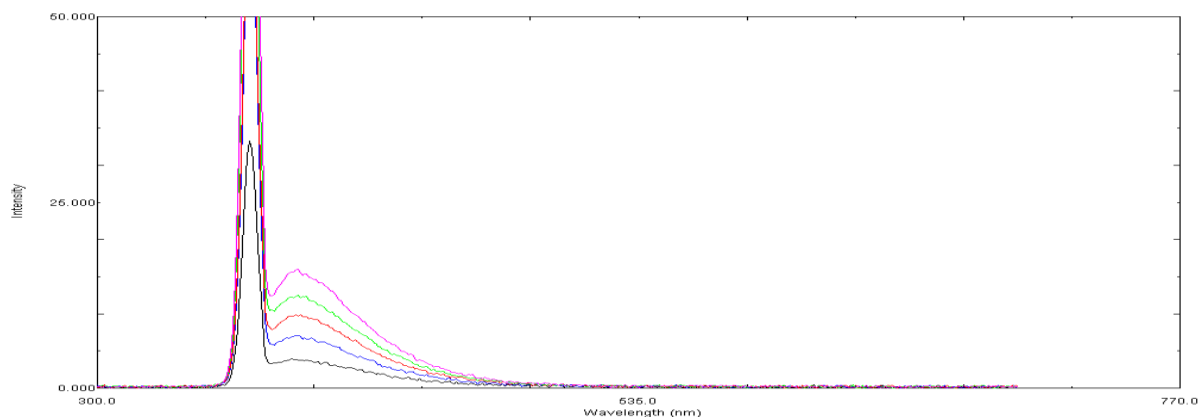


Figure 4. Linearity graph of rilpivirine

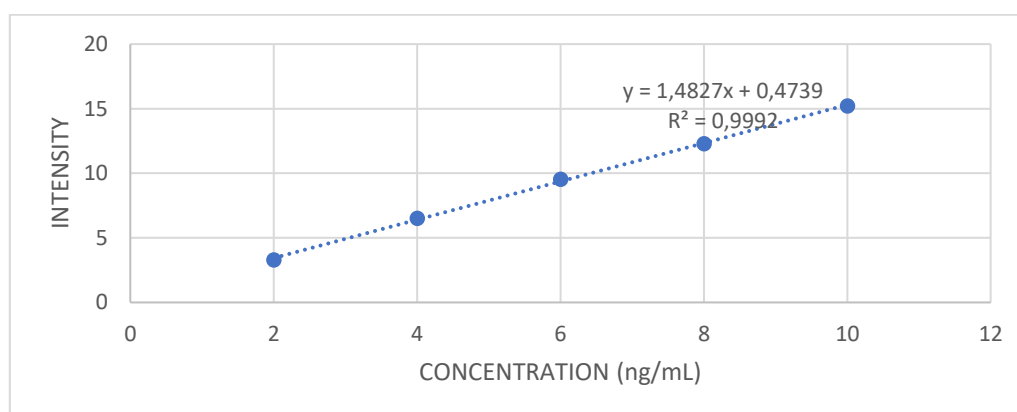


Figure 5. Calibration plot rilpivirine

3.2.2. Precision: The method's repeatability (Intra-day precision) was determined using an Intra-day (n=3) analysis of three separate Rilpivirine standard solutions at various strengths of 2, 6, and 10 ng/mL. For Rilpivirine, the % RSD of repeatability was less than 2. The inter-day (n=3) analysis of 3 Rilpivirine typical solutions at various concentrations of 2, 6, and 10 ng/mL indicated intermediate precision. For Rilpivirine, % RSD for Inter-day and Intra-day analysis was less than 2. This statistical data indicated high precision.

Table 2: Precision Data of Rilpivirine

Conc (ng/mL)	Intra Day		Inter Day	
	Conc (ng/mL) (AM±SD) (n=3)	%RSD	Conc (ng/mL) (AM±SD) (n=3)	%RSD
2	3.246 ± 0.057	1.76	3.185 ± 0.056	1.75
6	9.494 ± 0.166	1.75	9.729 ± 0.102	1.05
10	15.39 ± 0.213	1.38	15.44 ± 0.276	1.78

3.2.3. Accuracy: Accuracy of the method was determined by recovery experiments. To the sample solution containing 1000 µg/mL of rilpivirine the reference standards of the drug were added at the level of 80 %, 100 % and 120 % of test concentration. The recovery studies were carried out for three times by standard addition method. The percentage recovery and percentage relative standard deviation of the recovery for rilpivirine were calculated.

Table 1.5: Accuracy Data of Rilpivirine

% recovery level	Conc. Of sample ng/mL	Conc of Std (ng/mL)	Total conc (ng/mL)	Amt. recovered (ng/mL) AM+/- S.D (n=3)	Recovery %	P%RSD
80%	1	0.8	1.8	4.47+/-0.01	99.3	0.223
100%	1	1	1	5.08+/-0.015	101.6	0.302
120%	1	1.2	1.2	7.54 +/- 0.036	100.5	0.478

3.2.5. Limit of detection (LOD) and limit of quantification (LOQ)

Table 1.6: Data of LOD and LOQ for Rilpivirine

S.NO	DRUG	LOD (ng/mL)	LOQ (ng/mL)
1	Rilpivirine	0.325	0.987

3.2.6. Assay

Table 1.7: Analysis of Commercial Tablets (Assay)

Drug Name	Brand Name	Label Claim (mg)	Amount found \pm SD (n=3) (mg)	Assay %	% RSD
Rilpivirine	EDURANT®	25	24.9 \pm 0.655	99.6	2.3747

IV. CONCLUSION

A spectrofluorometric method for quantifying Rilpivirine has been devised, employing DMSO, phosphoric acid buffer, and distilled water. The approach produces a significant fluorescence intensity at an emission wavelength of 389 nm and an excitation wavelength 363 nm. The linear response range is 2-10 nanogrammes per millilitre. The regression equation is $Y=1.4827x+0.4739$, and the correlation coefficient (R^2) is 0.9992. The technique has been validated in accordance with ICH requirements, demonstrating crucial characteristics such as linearity, precision, accuracy, and a low limit of detection and quantification.

REFERENCES

1. Beckett AH, Stenlake JB. Practicle pharmaceutical chemistry. 4th edition. New Delhi: CBS publishers and distributors; 1997; 1:296-300.
2. Fluorescence Spectroscopy. Libre Texts chemistry. 2022.
3. Gurdeep R Chatwal, Sham K Anand. Instrumental methods of chemical analysis. New Delhi: Himalaya publishing house; 2006; 2:403-04.
4. ICH Harmonised Tripartite Guideline, (2005) Q2 (R1), validation of analytical procedures, text and methodology, ICH new publications, Geneva, P-1-13.
5. Sri KV, Jain GV, Madhuri M. UV-Visible Spectrophotometric Method for the Estimation of Rilpivirine Hydrochloride in Pharmaceutical Dosage Form by Using Multivariate Technique. Chemical Science Transactions. 2015;4(3):799-805.
6. Stellbrink H. Antiviral drugs in the treatment of AIDS: what is in the pipeline? European journal of medical research. 2007 Oct 1;12(9):483-95.
7. Miller CD, Crain J, Tran B, Patel N. Rilpivirine: a new addition to the anti-HIV-1 armamentarium. Drugs of Today (Barcelona, Spain: 1998). 2011 Jan 1;47(1):5-15.

8. Patel S, Nagappan K, Reddy GS. A new quantitative reverse phase high-performance liquid chromatographic method for the quantification of Rilpivirine hydrochloride in bulk and dosage form. *Journal of Applied Pharmaceutical Science*. 2018 Nov 30;8(11):157-62.
9. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. *Journal of pharmaceutical and biomedical analysis*. 2002 Jun 15;28(6):1011-40.
10. Kumar BM, Rajkamal B, Chandramowli B. Development and validation of Rilpivirine in pharmaceutical formulation by RP-HPLC. *Am J PharmTech Res*. 2019;9(03):345-53.
11. Else L, Watson V, Tjia J, Hughes A, Siccardi M, Khoo S, Back D. Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. *Journal of Chromatography B*. 2010 Jun 1;878(19):1455-65.
12. Mathias A, Menning M, Wiser L, Wei X, Dave A, Chuck S, Kearney BP. Bioequivalence of the emtricitabine/rilpivirine/tenofovir disoproxil fumarate single tablet regimen. *J Bioequiv Availab*. 2012;4(7):100A5.
13. Vejendla A, Talari S, Moturu R, Boddapati SM, Kola AE. Method development and validation for Cabotegravir and Rilpivirine by using HPLC and its degradants are characterized by LCMS and FTIR. *Future Journal of Pharmaceutical Sciences*. 2021 Dec;7:1-8.
14. Şanlıalp Zeyrek A, Takmak Ş, Kurban NK, Arslan S. Systematic review and meta-analysis: Physical-procedural interventions used to reduce pain during intramuscular injections in adults. *Journal of advanced nursing*. 2019 Dec;75(12):3346-61.
15. Cámara-Lemarroy CR, Rodríguez-Gutiérrez R, Monreal-Robles R, Marfil-Rivera A. Gastrointestinal disorders associated with migraine: a comprehensive review. *World journal of gastroenterology*. 2016 Sep 9;22(36):8149.
16. Wahab S, Khalid M, Ahmad S, Sweilam SH. Rilpivirine and Dolutegravir Simultaneously Measured via RP-HPLC-PDA with Box–Behnken Design Application: A Study of Forced Degradation under Various Conditions. *Separations*. 2023 Mar 8;10(3):185.
17. Noorbasha K, Nurbhasha S. A new validated stability-indicating RP-HPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form. *Future Journal of Pharmaceutical Sciences*. 2020 Dec;6:1-0.
18. Dowers E, Zamora F, Barakat LA, Ogbuagu O. Dolutegravir/rilpivirine for the treatment of HIV-1 infection. *HIV/AIDS-Research and Palliative Care*. 2018 Nov 2:215-24.
19. Rao PV, Rao NS. A new analytical method for determination of dolutegravir and rilpivirine in pharmaceutical formulations by RP-HPLC method. *International Journal of Research In Pharmaceutical Chemistry and Analysis*. 2019 Jul 28;1(3):68-74.
20. Kanchan Chauhan and Saloni Shaikh., 2021. An approach to enhance the solubility of rilpivirine hydrochloride using solid dispersion technique. *ejpmr*; ,8(3), 355-360.
21. Ghurghure SM, Dhange A, Mhetre R, Phatak AA. method development and validation of rilpivirine in bulk and solid dosage form by using uv-visible spectrophotometric method.
22. Sk M, Gottumukkala A. Development and validation of uv spectrophotometric methods for estimation of rilpivirine in bulk and pharmaceutical formulation. *International Journal of Pharmaceutical Sciences and Research*. 2014 Feb 1;5(2):483.
23. Ghurghure SM, Dhange A, Mhetre R, Phatak AA. method development and validation of rilpivirine in bulk and solid dosage form by using uv-visible spectrophotometric method.
24. Zhang T, Yang J, Zhou Z, Fu Z, Cherukupalli S, Kang D, Zhan P, Liu X. The development of an effective synthetic route of rilpivirine. *BMC chemistry*. 2021 Dec;15:1-9.
25. Kang D, Feng D, Ginex T, Zou J, Wei F, Zhao T, Huang B, Sun Y, Desta S, De Clercq E, Pannecouque C. Exploring the hydrophobic channel of NNIBP leads to the discovery of novel piperidine-substituted thiophene [3, 2-d] pyrimidine derivatives as potent HIV-1 NNRTIs. *Acta Pharmaceutica Sinica B*. 2020 May 1;10(5):878-94.
26. Wu Y, Tang C, Rui R, Yang L, Ding W, Wang J, Li Y, Lai CC, Wang Y, Luo R, Xiao W. Synthesis and biological evaluation of a series of 2-(((5-alkylaryl-1H-pyrazol-3-yl) methyl) thio)-5-alkyl-6-(cyclohexylmethyl)-pyrimidin-4 (3H)-ones as potential HIV-1 inhibitors. *Acta Pharmaceutica Sinica B*. 2020 Mar 1;10(3):512-28.
27. Venkatesan S, Kannappan N, Mannemala SS. Stability-indicating HPLC method for the simultaneous determination of HIV tablet containing emtricitabine, tenofovir disoproxil fumarate, and rilpivirine hydrochloride in pharmaceutical dosage forms. *International Scholarly Research Notices*. 2014;2014.
28. Sharma M, Saravolatz LD. Rilpivirine: a new non-nucleoside reverse transcriptase inhibitor. *Journal of Antimicrobial Chemotherapy*. 2013 Feb 1;68(2):250-6.

29. Anandakumar K, Kannan K, Vetrichelvan T. Development and validation of emtricitabine and tenofovir disoproxil fumarate in pure and in fixed dose combination by UV spectrophotometry. *Digest Journal of Nanomaterials and Biostructures*. 2011 Jul 1;6(3):1085-90.
30. Girija B. Bhavar, Sanjay S. Pekamwar, Kiran B. Aher and Sanjay R. Chaudhari., 2014. Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Rilpivirine Hydrochloride in Tablet Dosage Form. *IJAPA* Vol. 4 Issue 2 (2014) 38-43.
31. Reddy, A.P., Teja, U.C., Sultana, S.A., Vijayalakshmi, M. and Nalluri, B.N., 2014. Development and validation of RP-HPLC-PDA method for the simultaneous estimation of emtricitabine, tenofovir disoproxil fumarate and rilpivirine hydrochloride in bulk, pharmaceutical dosage forms and in dissolution samples. *Indo American Journal of Pharmaceutical Research*, 4, p.11.
32. Mathias A, Menning M, Wiser L, Wei X, Dave A, Chuck S, Kearney BP. Bioequivalence of the emtricitabine/rilpivirine/tenofovir disoproxil fumarate single tablet regimen. *J Bioequiv Availab*. 2012;4(7):100-5.
33. Sharma T, Mishra N, Moitra SS, Sankar DG. A validated RP-HPLC method for estimation of Tenofovir disoproxil fumarate in bulk and pharmaceutical formulation. *Asian J Pharm Clin Res*. 2012;5(3):108-10.
34. Karunakranth D, Midha AK, Babu RS, Kishore D. Development and Validation of HPLC Method for Simultaneous Estimation of Emtricitabine, Rilpivirine and Tenofovir Disoproxil Fumarate Tablet Dosage form. *Indian Journal of Research in Pharmacy and Biotechnology*. 2018;6(1):8-15.
35. Nalluri BN, Sushmitha K, Sunandana B, Babu DP. Development and validation of RP-HPLC-PDA method for simultaneous estimation of baclofen and tizanidine in bulk and dosage forms. *Journal of Applied Pharmaceutical Science*. 2012 Jul 30;2(7):111-6.
36. Purnima BV, Reddy TV, Rao YS, Ramu G, Ramachandran D. Stability indicating RP-UPLC method for assay of emtricitabine and tenofovir disoproxil fumarate in bulk and dosage forms. *American Journal of Analytical Chemistry*. 2015;6(10):807.
37. Kumar BR, Subrahmanyam KV. A validated stability-indicating rp-hplc method for the determination of rilpivirine. *Journal of Global Trends in Pharmaceutical Sciences*. 2014;5(3):1822-6.
38. Date AA, Shibata A, Bruck P, Destache CJ. Development and validation of a simple and isocratic reversed-phase HPLC method for the determination of rilpivirine from tablets, nanoparticles and HeLa cell lysates. *Biomedical Chromatography*. 2015 May;29(5):709-15.
39. Aouri M, Calmy A, Hirschel B, Telenti A, Buclin T, Cavassini M, Rauch A, Decosterd LA. A validated assay by liquid chromatography-tandem mass spectrometry for the simultaneous quantification of elvitegravir and rilpivirine in HIV positive patients. *Journal of mass spectrometry*. 2013 May;48(5):616-25.
40. Burugula L, Pilli NR, Makula A, Lodagala DS, Kandhagatla R. Liquid chromatography-tandem mass spectrometric assay for the non-nucleoside reverse transcriptase inhibitor rilpivirine in human plasma. *Biomedical Chromatography*. 2013 Feb;27(2):172-8.
41. Available on <https://chat.openai.com/c/59ff9373-69fc-44da-b841-1c220fbb5736> [accessed on 17 November 2023].
42. Garvey L, Winston A. Rilpivirine: a novel non-nucleoside reverse transcriptase inhibitor. *Expert opinion on investigational drugs*. 2009 Jul 1;18(7):1035-41.



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