

Content available at: <https://www.ipinnovative.com/open-access-journals>

International Journal of Pharmaceutical Chemistry and Analysis

Journal homepage: <https://www.ijpca.org/>

Original Research Article

Method stability indicating method development and validation for Emtricitabine by UV spectroscopic and RP-HPLC methods

Pooja Surwade¹, Ashwini Shelke^{1,*}, Atul R Bendale¹, Laxmikant Borse¹, Anil G Jadhav¹¹Dept. of Pharmaceutical Chemistry, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra, India

ARTICLE INFO

Article history:

Received 17-02-2022

Accepted 23-02-2022

Available online 09-04-2022

Keywords:

UV Spectroscopy

HPLC

Validation

Emtricitabine

Force Degradation study

ABSTRACT

Emtricitabine (commonly called FTC, systematic name 2 & 3'-dideoxy-5-fluoro-3-thiacytidine) is a nucleoside reverse-transcriptase inhibitor (NRTI) for the prevention and treatment of HIV infection in adults and children. The UV analysis of the drug was carried out on the 240nm wavelength. A simple, sensitive and accurate RP-HPLC method has been developed & validated for the determination of Emtricitabine in bulk formulation. Present method shows high sensitivity with linearity 10 to 50 µg/ml ($r^2 = 0.9991$). Various parameters according to ICH guidelines are followed for validating and testing of this method. Detection limit and quantitation limit were found to be $0.1534 \mu\text{g ml}^{-1}$ and $0.4649 \mu\text{g ml}^{-1}$ respectively. The results demonstrated that the procedure is accurate, specific and reproducible and also being simple, cheap and less time consuming and appropriate for the determination of Emtricitabine in bulk formulation.

This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprint@ipinnovative.com

1. Introduction

Emtricitabine is an analogue of cytidine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. By interfering with this process, which is central to the replication of HIV, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (namely T cells/CD4+ T-cells),¹⁻³ Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness. Literature review reveals that various analytical methods like UV Spectrophotometry,^{3,4} HPLC⁵⁻⁷ have been developed. However, no stability indicating UV methods are reported but one stability indicating HPLC method was reported for the estimation of emtricitabine. Hence, an attempt was made to develop simple, accurate and

precise stability indicating UV and RP-HPLC methods for the estimation of emtricitabine in pure and their marketed formulations.⁴⁻⁷

2. Material and Method

All reagents used were of analytical reagent (AR) grade. HPLC grade methanol and water was used throughout analysis. Emtricitabine sample was kindly provided by Hetero drugs ltd.

2.1. UV-visible spectrophotometric method

2.1.1. Zero order spectrophotometric method

Standard stock solution of Emtricitabine was prepared by accurately weighing 100 mg of Emtricitabine to 100 ml volumetric flask with 100 ml of water. The drug was sonicated and volume was made up to mark with water to get the concentration of 1000 µg/ml.

* Corresponding author.

E-mail address: shelke.ashwini2@gmail.com (A. Shelke).

2.1.2. First order derivative

Standard stock solution of Emtricitabine was prepared by accurately weighing 100 mg of Emtricitabine to 100 ml volumetric flask with specific volume of water. The drug was sonicated and volume was made up to mark with water to get the concentration of 1000 µg/ml.

2.2. Validation of developed RP-HPLC method⁸⁻¹⁵

2.2.1. Linearity

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Test solutions of different concentration were injected separately and the chromatograms were recorded. A series of test preparations of Emtricitabine (10-50 µg/ml) were prepared by taking 0.1, 0.2, 0.3, 0.4, 0.5 ml from the stock solution in five 10 ml volumetric flask and final volume make up to the mark with mobile phase. A 20 µl volume of each concentration was injected into HPLC, three times under the optimized chromatographic conditions.

2.2.2. Accuracy

Samples are prepared normally covering 50 % to 150 % of the nominal sample preparation concentration. These samples are analyzed and the recoveries of each are calculated. For this study, three preparation of each 50 %, 100 % and 150 % level are prepared and injected in to the chromatography, from the data obtained individual recovery, mean recovery and %RSD is calculated.

2.2.3. Precision study procedure: Intraday and interday precision

Intraday precision study was carried out by preparing test solution of same concentration and analyzing it at two different times in a day. The same procedure was followed for two different days to determine interday precision. The result was reported as %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 2. Three concentration selected 10,30,50 µg/ml.

2.2.4. LOD and LOQ

LOD and LOQ determined by the following formula by taking the standard deviation of y-intercept and slope from the linearity curves.

$$LOD = \frac{3.0\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = the standard deviation of the y-intercept
S = slope of calibration curve of analyte

2.2.5. Robustness

The effect of small deliberate change in optimized method was studied by robustness evaluation. To evaluate robustness of the developed method, parameter was deliberately varied. This parameter included variation of flow rate and variation wavelength. Factor selected was changed at two levels, for change in the flow rate in ml/min and change in wavelength in nm. In significant differences in peak areas and less variability in retention time were observed.

1. *Effect of variation in Flow rate:* A study was conducted to determine the effect of change in flow rate. Test solution prepared as per the test method and injected 3 concentrations of test solution into HPLC system with flow rate 0.7 ml/min. and 0.9 ml/min. The system suitability parameters were evaluated as per the test method.
2. *Effect of variation in Wavelength:* A study was conducted to determine the effect of change in wavelength. Test solution prepared as per the test method and injected 3 concentrations of test solution into HPLC system with wavelength of 238 -242 nm. The system suitability parameters were evaluated as per the test method.

2.2.6. Ruggedness

It is the degree of reproducibility of the test results under variety of conditions like different analyst, different instrument.

2.2.7. Specificity

The specificity of method was determined by checking the interference of excipients with analyte. Study was done by spiking known amount of excipients stock solution to the standard solution and area was measured at 235-245 nm.

2.3. System suitability study

System suitability is an integral part of many analytical procedures. The tests are based on the concept that the equipment's, electronics, analytical operations and sample to be analysed constitute an integral system that can be evaluated as such. System Suitability test parameters to be established for particular procedure depends on the type of procedure being validated. Tests are carried out on freshly prepared solution of Emtricitabine with six replicates.

2.4. Forced degradation study¹⁶⁻²⁴

2.4.1. Acid induced-degradation

10.0 mg Emtricitabine was transferred to 100.0 mL volumetric flask, added 50.0 mL mobile phase, sonicated for 20.0 min., volume was made up to the mark with mobile phase. From this stock solution take 1 ml of solution

into 20 ml volumetric flask then add 10 ml of mobile phase and then add 1 ml of 1N HCl and stressed the samples at condition mentioned in water bath, cooled at room temperature and neutralize of acid with base of same concentration and volume. Make up the volume with mobile phase and mixed. Filter the solution through 0.45 μ membrane filter and filtrate use as such. Then these filtrates were chromatographed under optimized chromatographic system.

2.4.2. Base induced-degradation

10.0 mg Emtricitabine was transferred to 100.0 mL volumetric flask, added 50.0 mL mobile phase, sonicated for 20.0 min., volume was made up to the mark with mobile phase. From this stock solution take 1 ml of solution into 20 ml volumetric flask then add 10 ml of mobile phase and then add 1 ml of 1N NaOH and stressed the samples at condition mentioned in water bath, cooled at room temperature and neutralize of base with acid of same concentration and volume. Make up the volume with mobile phase and mixed. Filter the solution through 0.45 μ membrane filter and filtrate use as such. Then these filtrates were chromatographed under optimized chromatographic system.

2.4.3. Peroxide degradation

10.0 mg Emtricitabine was transferred to 100.0 mL volumetric flask, added 50.0 mL mobile phase, sonicated for 20.0 min., volume was made up to the mark with mobile phase. From this stock solution take 1 ml of solution into 20 ml volumetric flask then add 10 ml of mobile phase and then add 1 ml of 3% w/v H₂O₂ and stressed the samples for 4 hours at 60°C in water bath cooled at room temperature. Then this sample was chromatographed under optimized chromatographic system.

2.4.4. Thermal degradation

Thermal degradation was performed at 60°C for 24 hours. The mobile phase used was Methanol: Water (60:40) at pH 3. The concentration used was 50ppm at wavelength of 240nm.

2.4.5. Photolytic degradation

Photolytic degradation was performed at room temperature for 24 hours. The concentration used was 50ppm at 240nm. The mobile phase used was Methanol: Water (60:40) at pH 3.

3. Result and Discussion

3.1. Method development and validation by UV spectrophotometry

3.1.1. Zero order spectrophotometric method

UV Spectrophotometric method was developed for estimation of Emtricitabine in bulk form. The wavelength maxima were found to be 240 & 280 nm and are shown in Figure 1

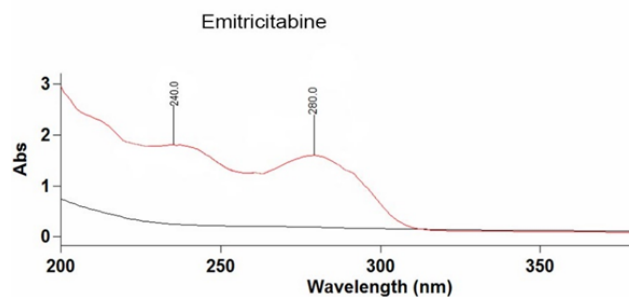


Fig. 1: Zero order spectra of Emtricitabine

3.1.2. First order derivative Spectroscopy

The wavelength maxima was found to be 228 nm and are shown in Figure 2

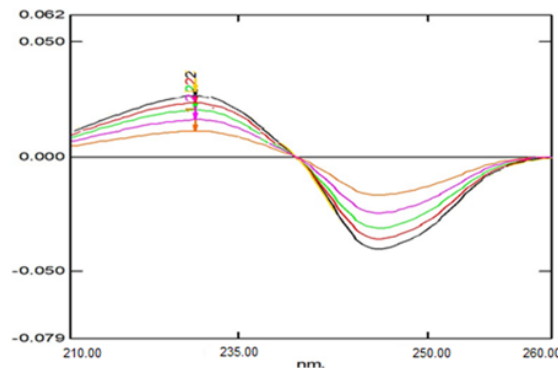


Fig. 2: First order derivative spectra of Emtricitabine

3.1.3. Area under curve method

The absorption spectra show the absorbance maxima 240 at nm and area under curve in absorption spectra was measured between 235-245 nm.

3.2. Development of HPLC method for Emtricitabine

High performance liquid chromatographic method was developed and validated for determination of Emtricitabine in bulk form. Mobile phase consists of Methanol: Water

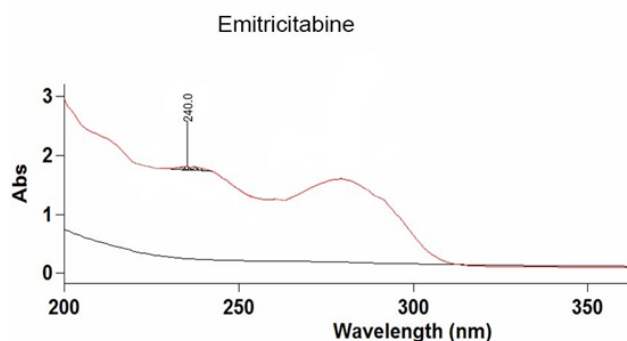


Fig. 3: Area under curve spectra of emtricitabine

(60:40) pH3. Chromatogram obtained was shows the maximum wavelength at 240 nm.

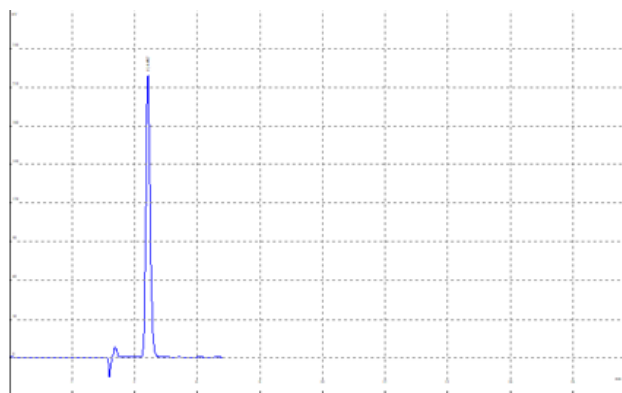


Fig. 4: Typical chromatogram of Emtricitabine

3.2.1. Linearity

Drug was found to be linear in the concentration range of 10-50 $\mu\text{g/ml}$. Results obtained are shown in Table 1 and calibration plot obtained was shown in Figure 5

Table 1: Data of calibration curve of Emtricitabine by HPLC method

Sr. No.	Conc. ($\mu\text{g/ml}$)	Area
1	1	672090
2	20	1365679
3	30	1924578
4	40	2558598
5	50	3150259

3.2.2. Optical characteristics

Optical characteristics and statistical data of linearity for Emtricitabine by HPLC method are summarized in Table 2

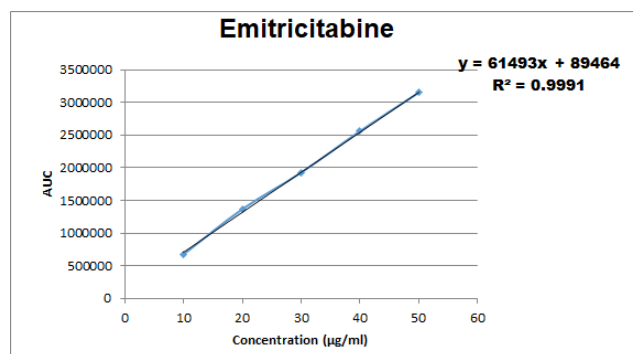


Fig. 5: Calibration curve for Emtricitabine

Table 2: Optical characteristics for emtricitabine

Sr. No	Parameters	Result
1	λ_{max} (nm)	240
2	Beer's law limit ($\mu\text{g/mL}$)	10-50
3	Regression equation[y]	y = 61493x + 89464
4	Slope[m]	61493
5	Intercept [c]	89464
6	Correlation coefficient [r^2]	0.9991
7	Limit of detection (LOD)($\mu\text{g/mL}$)	0.1534
8	Limit of quantitation (LOQ) ($\mu\text{g/mL}$)	0.4649

3.2.3. Accuracy

Accuracy was studied by standard addition method and % recovery found was within acceptable limit. Results of recovery study are shown in Table 3 and statistical validation is shown in Table 4

3.2.4. Precision

Intraday and interday precision assures the repeatability of test results. The % RSD found to be less than 2. Result of intraday and interday precision was shown in Table 5

3.2.5. Limit of detection and limit of quantitation

The LOD and LOQ are found to be 0.1534 $\mu\text{g/ml}$ and 0.4649 $\mu\text{g/ml}$ respectively

3.2.6. Robustness

Robustness was studied by different deliberate variations in the chromatographic conditions. Results are shown in Table 7

3.2.7. Ruggedness

Ruggedness was studied by different analyst. Results obtained are shown in Table 8

Table 3: Data for recovery study of Emtricitabine by HPLC method

Level of addition (%)	Conc. ($\mu\text{g/ml}$)	Standard added ($\mu\text{g/ml}$)	Total conc. ($\mu\text{g/ml}$)	Area	Drug recovered ($\mu\text{g/ml}$)	% Recovery
50	20	10	30	1925684	30.01	100.03
	20	10	30	1935698	30.17	100.56
	20	10	30	1930256	30.08	100.26
	20	20	40	2559648	40.01	100.02
100	20	20	40	2562359	40.05	100.12
	20	20	40	2574659	40.25	100.62
	20	30	50	3156294	50.09	100.18
150	20	30	50	3146598	49.94	99.88
	20	30	50	3162594	50.19	100.38

Table 4: Statistical validation of Emtricitabine by HPLC method

Level of addition	% Mean recovery*	SD	% RSD
50%	100.28	0.2657	0.2650
100%	100.25	0.3214	0.3206
150%	100.14	0.2516	0.2512

*Average of three determination

Table 5: Data for intraday precision of Emtricitabine by HPLC method

Sr. No.	Conc. ($\mu\text{g/mL}$)	Area	Mean	SD	%RSD
1	10	672531	672771	828.64	0.1231
2	10	672090			
3	10	673694			
4	30	1925684	1930546	5013.29	0.2596
5	30	1935698			
6	30	1930256			
7	50	3156294	3155162	8057.85	0.2553
8	50	3146598			
9	50	3162594			

Table 6: Data for interday precision of Emtricitabine by HPLC method

Sr. No.	Conc. ($\mu\text{g/mL}$)	Area	Mean	SD	%RSD
1	10	672869	673057.33	519.75	0.0772
2	10	673645			
3	10	672658			
4	30	1921659	1930366.66	9123.08	0.4726
5	30	1939855			
6	30	1929586			
7	50	3152659	3151525.66	8357.83	0.2651
8	50	3142659			
9	50	3159259			

Table 7: Data for robustness study of Emtricitabine by HPLC method

Sr. No	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate +/-0.1(ml/min)	0.7	1365679	1362556	4329.28	0.31
2		0.8	1364375			
3		0.9	1357614			
1	Change in Wavelength +/-0.2(nm)	238	1365679	1363715	2175.33	0.15
2		240	1361377			
3		242	1364090			

*Average of three determination

Table 8: Data for ruggedness study of Emtricitabine by HPLC method

Sr. No	Analyst	Conc. ($\mu\text{g/ml}$)	Mean area*	SD	%RSD
1	Analyst-I	30	1932659	94137	0.8322
2	Analyst-II	30	1929202	56444	0.1372

*Average of three determination

Table 9: Data for specificity study of Emtricitabine by HPLC method

Drug conc. ($\mu\text{g/ml}$)	Excipients ($\mu\text{g/ml}$)	Total conc. ($\mu\text{g/ml}$)	Area	SD	%RSD
10	20	30	672869		
10	20	30	673645	519.75	0.0772
10	20	30	672658		
20	20	40	1370589		
20	20	40	1375624	3476.42	0.2534
20	20	40	1368954		
30	20	50	1921659		
30	20	50	1939855	9123.08	0.4726
30	20	50	1929586		

Table 10: Data for system suitability study of Emtricitabine by HPLC Method

Sr. No.	conc. ($\mu\text{g/ml}$)	Retention Time/min	Theoretical plates	Asymmetry Factor
	30	4.4	8818	1.17
2	30	4.5	8823	1.17
3	30	4.4	8856	1.17
4	30	4.4	8829	1.17
5	30	4.3	8852	1.17
6	30	4.4	8789	1.17
Mean		4.4	8827	1.17
SD		0.0062	24.52	0
%RSD		0.23	0.27	0

Table 11: Results of forced degradation studies for Emtricitabine

	Acid stress	Alkali stress	Peroxide stress	Thermal stress	Photolytic stress
% Recovered	86.68%	88.51%	90.42%	94.47%	97.67%
% Degradation	13.31%	11.48%	9.57%	5.52%	2.32%

3.2.8. Specificity

Excipients and impurities were not interacting with the standard drug, hence method is specific. Results of specificity are shown in Table 9

3.2.9. System suitability

System suitability parameters were measured to verify the system, method and column performance. Standard solution of Emtricitabine was injected in to the system for five times and system suitability parameters were checked.

3.2.10. Degradation studies

Stress testing of the drug substance can help to identify the likely degradation products, the stability and specificity of the analytical procedure. Degradation studies were performed on solutions containing 50 $\mu\text{g/ml}$ of Emtricitabine. Results of the forced degradation studies are summarized in Table 11

4. Conclusion

In the present research work, a successful attempt was made for determination of Emtricitabine in Bulk form by UV-Visible Spectrophotometric, HPLC degradation study. The method was developed by experimentation, based on literature survey. The simplicity, rapidity, reproducibility and economy of the proposed method completely fulfil the objective of this research work. The HPLC method was developed and validated for estimation of Emtricitabine. The mobile phase was consisting of Methanol: water (60:40) at pH3. Detection was done at 240nm. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD was found within limit. The result showed that proposed method was

suitable for the accurate, precise and rapid determination of Emtricitabine in its bulk form. Forced degradation studies were performed on Acid, Alkali, Peroxide, Thermal and Photolytic conditions. Degradation studies were performed on solutions containing 50 µg/ml of Emtricitabine. The result showed that proposed method was suitable for the accurate and rapid determination of Emtricitabine in its bulk form.

5. Acknowledgment

I am thankful to the principal and management of Sandip Institute of Pharmaceutical Sciences, Nashik for their help, without which the work would not be completed.

6. Source of Funding

None.

7. Conflict of Interest

None.

References


- Kenkel J. Analytical Chemistry for Technicians; 2009. p. 2–4.
- Kasture AV, Wadodkar SG. Practical Pharmaceutical Chemistry - II. India; 2008. p. 28–30.
- Bassett JGH, Mendham J, Denny J. Vogel's Textbook of Quantitative Chemical Analysis; 1991. p. 3–13.
- Chatwal GR, Anand S. Instrumental methods of analysis. *J Chem Educ*. 2008;5(2):A136. doi:10.1021/ed052pA136.
- Lampman PD, Kriz GM. Introduction to Spectroscopy, vol. 356; 2001. p. 797–817.
- Beckett AH, Stenlake J. Practical Pharmaceutical Chemistry; 2004. p. 162–3.
- Furnish S. Textbook of practical organic chemistry; 1989. p. 384–6.
- Rashmin M. An introduction to analytical method development for pharmaceutical formulations. *Pharmainfo*. 2008;1(1):1–45.
- Rathee P, Rathee S, Thakur S, Kumar V. Simultaneous estimation of Amlodipine besylate and Atenolol as API and in tablet dosage forms by Vierordt's method using UV spectrophotometry. *Int J ChemTech Res*. 2010;2(1):62–8.
- Pathare VB, Tambe S, Dhole V. An update on various analytical techniques based on UV spectroscopy used in determination of dissociation constant. *Int J Pharm*. 2014;4(1):278–85.
- Choudhary J, Jain A. Simultaneous estimation of multicomponent formulations by UV-Visible spectroscopy: A review. *Int Res J Pharm*. 2011;2(12):81–3.
- Atole DM, Hrishikesh H. Ultraviolet Spectroscopy and Its Pharmaceutical Applications-A Brief Review. *Asian J Pharm Clin Res*. 2018;11(2):59–66. doi:10.22159/ajpcr.2018.v11i2.21361.
- Kalpesh N, Patel JK, Rajput NB, Ganesh C. Derivative spectrometry method for chemical analysis: A review. *Der Pharm Lettre*. 2022;2010(2):139–50.
- Tiwari G, Tiwari R, Srivastava B, Rai A, Pathak K. Simultaneous estimation of Metronidazole and Amoxicillin in synthetic mixture by ultraviolet spectroscopy. *Asian J Res Chem*. 2008;1(2):91–4.
- Hayam M, Loffy SS. Recent development in ultraviolet spectrophotometry through the last decade a review. *Int J Pharm Pharm Sci*. 2016;2016(10):40–56.
- Willard HH, Merritt LL, Settle FA. Instrumental methods of analysis; 1996. p. 598–607.
- Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis; 2007. p. 848–50.
- Snyderl, Kirkland J. Practical HPLC Method Development; 1997. p. 2–68.
- Rajput PR, Bendale A, Luhar SV. Development and Validation of Stability Indicating RP-HPLC Method for Amlodipine Besylate and Perindopril Arginine in Synthetic Formulation. *J Pharm SciBioscientific Res*. 2016;6(3):347–55.
- Sharmab K. Instrumental methods of chemical analysis; 2008. p. 80–2.
- Bendale AR, Singh RP, Vidyasagar G. Development and validation stability indicating HPTLC method for determination of vildagliptin and metformin hydrochloride in the pharmaceutical dosage forms. *Int J Appl Pharm*. 2018;10(1):36–45.
- Swadesh JK. HPLC: Practical and Industrial Applications. vol. 11; 2000. p. 146–50.
- Shinde MP, Patil A, Bendale AP, Narkhede SP, Jadhav A. Development of a UV-spectrophotometric method for study of degradation profile of tenofovir alafenamide. *Int J Pharm Chem Anal*. 2018;5(3):144–6. doi:10.18231/2394-2797.2018.0023.
- Christian DG. Analytical chemistry; 2004. p. 616.

Author biography

Pooja Surwade, Student

Ashwini Shelke, Assistant Professor  <https://orcid.org/0000-0001-8120-730X>

Atul R Bendale, Associate Professor  <https://orcid.org/0000-0002-3219-0377>

Laxmikant Borse, Associate Professor  <https://orcid.org/0000-0002-0857-1604>

Anil G Jadhav, Principal  <https://orcid.org/0000-0003-3336-6503>

Cite this article: Surwade P, Shelke A, Bendale AR, Borse L, Jadhav AG. Method stability indicating method development and validation for Emtricitabine by UV spectroscopic and RP-HPLC methods. *Int J Pharm Chem Anal* 2022;9(1):10-16.