Research Article

A RP HPLC- PDA Method Development and Validation of Amoxicillin, Vonoprazan Clarithromycin in Bulk and Pharmaceutical Dosage Forms - A Stability Study.

A.K.M Pawar[1], Sreelatha Gangu[2*]

[1]A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam – 530003, India.

[2]Department of Pharmaceutical Analysis, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad, Telangana – 500046, India.

***Corresponding author's E-mail:** sreelatha1801@gmail.com

Received: 12-10-2024; **Revised:** 30-12-2024; **Accepted:** 12-01-2025; **Published online:** 20-01-2025.

ABSTRACT

The objective of the project is to establish a dependable RP-HPLC-PDA method for the concurrent quantification of Amoxicillin, Vonoprazan, and Clarithromycin in bulk and mixed formulations. An efficient, cost-effective, and dependable method for quantifying Amoxicillin, Vonoprazan, and Clarithromycin has been established utilizing the RP-HPLC technique. To effectively isolate Amoxicillin, Vonoprazan, and Clarithromycin, 1.0 µl of a 100% solution was injected into a Luna phenyl hexyl column (250 x 4.6 mm, 5 µm). The mobile phase comprised 0.1% TFA: The solvent composition consisted of methanol and acetonitrile in a ratio of 60:20:20, with a flow rate maintained at 1 ml/min and a detection wavelength established at 231 nm. The column and the injection port were maintained at a constant temperature of 30 °C throughout. The retention times (RT) of Amoxicillin, Vonoprazan, and Clarithromycin were recorded at 2.54 min, 4.20 min, and 5.72 min, respectively, in accordance with established system appropriateness criteria. Linear responses were noted for Amoxicillin, Vonoprazan, and Clarithromycin within the ranges of 125 to 750 µg/ml, 5 to 30 µg/ml, and 125 to 750 µg/ml, respectively. The LOD and LOQ values were determined as 0.5 µg/ml and 2 µg/ml for Amoxicillin, 0.018 µg/ml and 0.02 µg/ml for Vonoprazan, and 0.5 µg/ml and 2 µg/ml for Clarithromycin. The % RSD values for both precisions were evaluated within the range of 0.4 to 0.8. The average recovery of Amoxicillin, Vonoprazan, and Clarithromycin ranged from 99.9% to 100.9%. The statistical analysis of the validation parameters verified that the approach was dependable for its accuracy, sensitivity, and precision, while also demonstrating a significant level of sensitivity. The examination of analytes under diverse stressful conditions ensures the stability of the substances, hence confirming their representation of the method's stability indication. The newly developed technique is highly effective in isolating Amoxicillin, Vonoprazan, and Clarithromycin from each other. The degradation products generated under stress conditions were also isolated with high resolution. The research determined that the proposed strategy has significant implementation in the pharmaceutical industry.

Keywords: Amoxicillin, Vonoprazan and Clarithromycin, RP-HPLC, Luna phenyl hexyl column C18 column, Specificity, Stability indicating.

INTRODUCTION

Clarithromycin being well tolerated than Erythromycin, Penicillin, Ampicillin/Amoxicillin to treat various upper and lower respiratory tract infections [1]. Clarithromycin on long term use is effective in treatment of COPD with less adverse effect [2]. Amoxicillin, Vonoprazan, and Clarithromycin are used to treat and prevent the recurrence of ulcer (sores in the lining of stomach or intestine) caused by a specific kind of bacterium (H. Pylori). Vonoprazan is classified as a Potassium Competitive Acid Blocker (PCAB). Clarithromycin and Amoxicillin belong to the class of medications known as antibiotics. Vonoprazan functions by reducing gastric acid production. Clarithromycin and Amoxicillin function by inhibiting the proliferation of bacteria that may induce ulcers. [3]

Vonoprazan is an innovative gastric acid suppressant utilized in Japan for the treatment of gastric diseases, including H. pylori infections. It demonstrates superiority over traditional proton pump inhibitor (PPI) therapy in eradicating clarithromycin-resistant H. pylori strains. Nonetheless, clarithromycin has been misapplied, as the combination of Vonoprazan and amoxicillin effectively cures approximately 80% of infections without the need for clarithromycin[4]. Vonoprazan (VPZ) is an innovative potassium-competitive acid blocker (PCAB). It functions by competing for potassium on the luminal side of the parietal cell, resulting in fast and reversible inhibition of H-K ATPase, hence suppressing prolonged acid secretion. Unlike PPIs, Vonoprazan is a more effective inhibitor of acid secretion. It has a rapid beginning of action, reduced anti-secretory variability, enhanced safety, and improved tolerability [5-7]. Figure 1-3 shows the chemical structure of the drugs.

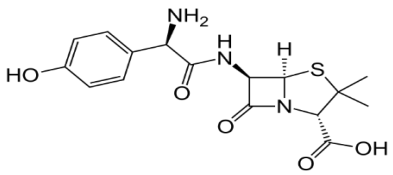

Figure 1: Amoxicillin

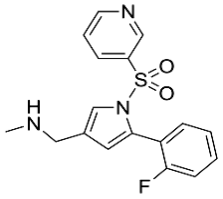

Figure 2: Vonoprazan

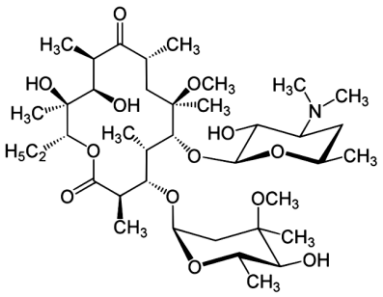

Figure 3: Clarithromycin

Literature Review

As per the literature review, we found out that there are many UPLC, HPLC, LC-MS, Bioanalytical methods either for the single drug or in combination with other drugs. When we look for the combination of Amoxicillin, Vonoprazan, and Clarithromycin there are very few UPLC and one HPLC method. Retention time in reported method is more whereas the resolution obtained was less when compared with current developed method. The mobile phase in our method consists less organic phase making it economical. System suitability parameters like USP tailing and theoretical plates were found to be better than the reported method. So, there is a need to develop a HPLC method which is more accurate, precise uses less organic phase having low retention time and high resolution for the estimation of Amoxicillin, Vonoprazan, and Clarithromycin. [8-29]

EXPERIMENTAL STUDY

Instrumentation: A Waters Alliance e-2695 chromatographic system, comprising a quaternary pump, PDA detector 2998, and Empower-2.0 chromatographic software, was utilized.

Chemicals and reagents: Acetonitrile, methanol, and HPLC-grade water were procured from Merck India Ltd, Mumbai, India. Active pharmaceutical ingredients of Amoxicillin, Vonoprazan, and Clarithromycin standards were obtained from Glenmark, Mumbai. A formulation consisting of Amoxicillin Capsules, Vonoprazan, and Clarithromycin tablets from Glenmark, with label claims of 500 mg Amoxicillin, 20 mg Vonoprazan, and 500 mg Clarithromycin, was utilized.

Mobile phase: Incorporate 0.1% TFA, methanol, and acetonitrile in a 60:20:20 ratio, mix completely, sonicate for

5 minutes, and filter through a 0.45 µm membrane filter for use as the mobile phase. Acetonitrile serves as a diluent.

Preparation of a standard solution:

Accurately measure and transfer 50 mg of Amoxicillin, 20 mg of Vonoprazan, and 50 mg of Clarithromycin working standard into a 10 ml volumetric flask. Introduce about 7 ml of diluent, sonicate for 30 minutes to guarantee full dissolution, and thereafter fill to the designated mark with diluent. Then, put 1 ml of the specified Vonoprazan solution into a 10 ml volumetric flask and dilute to the mark with the suitable solvent. This functions as the stock solution. Transfer 1 ml of the stock solution into a 10 ml volumetric flask and dilute to the mark with the appropriate diluent to achieve concentrations of 500 ppm Amoxicillin, 20 ppm Vonoprazan, and 500 ppm Clarithromycin.

Preparation of the sample solution:

Accurately measure and transfer 56 mg of Amoxicillin, 4 mg of Vonoprazan, and 57 mg of Clarithromycin into a 10 ml volumetric flask, subsequently adding 7 ml of diluent. Sonicated for dissolving, thereafter adjusted to the prescribed volume with diluents and filtered through a 0.45 µm nylon syringe filter. Then, put 1 ml of the specified sample stock solution into a 10 ml volumetric flask and dilute to the mark with the suitable diluents to achieve concentrations of 500 ppm Amoxicillin, 20 ppm Vonoprazan, and 500 ppm Clarithromycin.

Optimized chromatographic conditions:

Table 1: Optimized method chromatographic conditions

Parameters	Chromatographic conditions
Mobile phase	0.1% TFA: Methanol: Acetonitrile (60:20:20)
Column	Luna phenyl hexyl column (250 x 4.6mm, 5µm)
Flow rate	1ml/min
Column temperature	25^{0}C
Sample temperature	25^{0}C
Wave length	231 nm
Injection volume	10 µl
Run time	10 min
Retention time	2.540 min-Amoxicillin 4.209 min-Vonoprazan 5.725 min-Clarithromycin

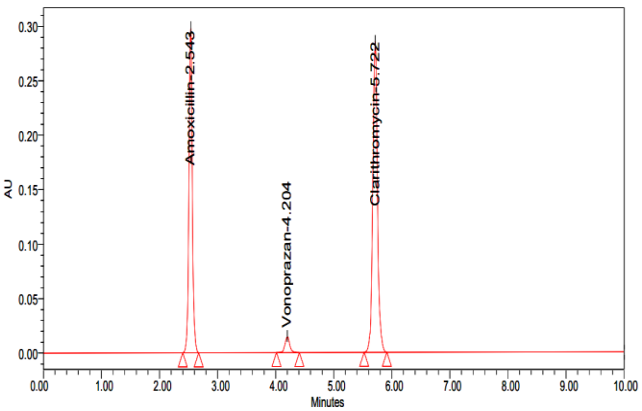

Figure 4: Typical chromatogram for standard solution

Validation of the proposed method

The method was validated for linearity, precision, accuracy, robustness, ruggedness, forced deterioration, and stability.

Specificity:

Specificity refers to the capacity to unequivocally evaluate the analytic amidst components that may be anticipated to exist. Typically, these encompass contaminants, degradants, matrix, etc. Table 2 shows the system suitability results.

Table 2: System Suitability parameters

Drug	RT	USP Resolution	USP Tailing	USP Plate Count
A	2.540		0.91	17118
V	4.209	6.71	1.16	8621
C	5.725	5.93	1.09	17845

Linearity:

Accurately weigh and transfer 50 mg of Amoxicillin, 20 mg of Vonoprazan, and 50 mg of Clarithromycin working standard into a 10 ml volumetric flask. Add roughly 7 ml of diluent, sonicate for 30 minutes to ensure complete dissolution, and then fill to the mark with diluent. Subsequently, transfer 1ml of the aforementioned Vonoprazan solution into a 10ml volumetric flask and dilute to the mark with the appropriate diluents**.** Results for Linearity are shown in Table 3.

Table 3: Results of linearity

Parameters	A	V	C
Range (µg/mL)	125-750	5-30	125-750
R^2	0.99989	0.99987	0.99983
Slope	8155.40	4103.1	7541.27
Y- intercept	28482.82	549.07	33884.54

Accuracy:

Preparation of a 50% solution relative to the target test concentration. Precisely weigh and transfer 56 mg of Amoxicillin, 4 mg of Vonoprazan, and 57 mg of Clarithromycin into a clean, dry 10 ml volumetric flask. Add diluents and sonicate to ensure full dissolution, then fill to the mark with diluents. Subsequently, transfer 0.5 ml of the aforementioned sample stock solution into a 10 ml volumetric flask and dilute to the mark using the appropriate diluent**.** Table 4 shows the accuracy values of the drug.

Table 4: Results of Accuracy

S. No	% Level	% Recovery of A	% Recovery of V	% Recovery of C
1	50	99.9	99.9	100.5
2	100	100.0	99.9	100.4
3	150	100.0	100.2	100.0
Mean		100.0	100.0	100.3
SD		0.058	0.173	0.260

Precision:

Precision refers to the extent of repeatability of an analytical process under standard operational conditions. There are three sorts of precision. Table 5 shows the Precision results.

System Precision

Method Precision

Intermediate Precision

Table 5: Results of system precision

Variable	%RSD of A	%RSD of V	%RSD of C
System Precision	0.47	0.23	0.78
Intermediate Precision	0.49	0.21	0.40
Repeatability	0.38	0.30	0.35

Limit of detection (LOD) and Limit of quantification (LOQ):

The limit of detection and limit of quantification of the substance were determined using the equation outlined in ICH recommendations. Results are shown in Table 6.

Table 6: LOD and LOQ values

Drug	LOD	LOQ
A	0.50 µg/ml	2.00µg/ml
V	0.018µg/ml	0.018µg/ml
C	0.50µg/ml	2.00µg/ml

Robustness:

To assess the method's robustness, intentional alterations were made to the flow rate, mobile phase composition, and wavelength. Table 7 shows the Robustness values.

Table 7: Robustness values

Parameter	% RSD		
	A	V	C
Flow minus (0.9 ml/min)	0.30	0.25	0.25
Flow plus (1.1 ml/min)	0.46	0.20	0.42
Organic minus (-10%)	0.36	0.20	0.21
Organic plus (+10%)	0.35	0.10	0.35

Forced Degradation:

Stock Preparation: Accurately weigh and transfer 56 mg of Amoxicillin, 4 mg of Vonoprazan, and 57 mg of Clarithromycin into a 10 ml volumetric flask, subsequently adding 7 ml of diluent. Sonicated for dissolving, thereafter adjusted to the prescribed volume with diluents and filtered through a 0.45 µm nylon syringe filter.

Acidic degradation: Transfer 1 ml of the specified stock solution into a 10 ml volumetric flask, add 3 ml of diluent, and include 1 ml of 1N HCl. Permit the mixture to rest for

15 minutes. After 15 minutes, introduce 1 ml of 1N NaOH and dilute to the designated mark using diluents. Employ a 0.45 µm syringe filter and transfer the contents to a vial.

Alkali degradation: Transfer 1 ml of the specified stock solution into a 10 ml volumetric flask, add 3 ml of diluent, and include 1 ml of 1N NaOH. Permit the mixture to rest for 15 minutes. After 15 minutes, introduce 1 ml of 1N HCl and dilute to the required volume using diluents. Employ a 0.45 µm syringe filter and transfer the contents to a vial.

Peroxide degradation: Transfer 1 ml of the specified stock solution into a 10 ml volumetric flask, add 3 ml of diluent, and incorporate 1 ml of 10% H2O2. Permit the mixture to rest for 15 minutes. After 15 minutes, modify to the required concentration using diluents. Employ a 0.45 µm syringe filter and transfer the contents to a vial.

Reduction degradation: Transfer 1 ml of the specified stock solution into a 10 ml volumetric flask, add 3 ml of diluent, and incorporate 1 ml of 10% sodium bisulfite. Permit the mixture to rest for 15 minutes. After 15 minutes, modify to the desired concentration using diluents. Employ a 0.45 µm syringe filter and transfer the contents to a vial.

Thermal degradation: 600 mg of Amoxicillin, 100 mg of Vonoprazan, and 600 mg of Clarithromycin standards were deposited on a petri dish and subjected to a hot air oven at 110°C for a duration of 3 hours. Dispense 500 mg of Amoxicillin, 20 mg of Vonoprazan, and 500 mg of Clarithromycin into a 10 ml volumetric flask and dilute to the calibration mark using diluents. Transfer 1 ml of the specified Vonoprazan solution into a 10 ml volumetric flask and dilute to the calibration line using diluents. Dilute 1 ml to a total volume of 10 ml. The sample underwent UV radiation for 72 hours, after which the exposed sample was analysed. The sample was then diluted using diluents and injected into the HPLC for analysis. Forced Degradation results are shown in Table 8.

Table 8: Results of forced degradation

Degradation Conditions	A	V	C
	% Degradation	% Degradation	% Degradation
Control degradation	0	0	0
Acid degradation	9.6	0.7	13.7
Alkali degradation	13.2	0.8	11.2
Peroxide degradation	14.6	13.6	14.4
Reduction degradation	0.6	9.5	8.5
Hydrolysis degradation	0.2	2.8	1.4
Thermal degradation	11.0	1.2	4.0
Photolytic degradation	1.1	1.9	3.2

CONCLUSION

This study presents a unique, rapid, cost-effective, sensitive, and readily accessible HPLC approach for the simultaneous quantification of Amoxicillin, Vonoprazan, and Clarithromycin in bulk and pharmaceutical formulations. The primary advantage of this method is the reporting of HPLC techniques. This approach offers reduced run time, affordability, accessibility, sensitivity, dependability, and reproducibility. These attributes are crucial for analysing a substantial quantity of samples. All characteristics, including linearity, accuracy, specificity, robustness, forced deterioration, and stability, were validated and deemed to meet acceptable criteria. The RSD values for all parameters were determined to be below 2, indicating that the method's validity and the results obtained are in reasonable concordance. The proposed method can be readily utilized for the regular analysis and pharmaceutical formulations of Amoxicillin, Vonoprazan, and Clarithromycin Sulfate in quality control laboratories without any prior separation**.**

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Sturgill MG, Rapp RP. Clarithromycin: review of a new macrolide antibiotic with improved microbiologic spectrum and favorable pharmacokinetic and adverse effect profiles. Ann Pharmacother. 1992 Sep;26(9):1099-108. doi: 10.1177/106002809202600912, PMID 1421677.
2. Vora A. Role of Clarithromycin in Acute Exacerbations of Chronic Obstructive Pulmonary Disease. J Assoc Physicians India. 2017 Sep;65(9):100-104. PMID: 29313589.
3. Vonoprazan, Clarithromycin, and Amoxicillin: MedlinePlus Drug Information [Internet]. medlineplus.gov. Available from: https://medlineplus.gov/druginfo/meds/a622051.html
4. Li M, Oshima T, Horikawa T, Tozawa K, Tomita T, Fukui H, Watari J, Miwa H. Systematic review with meta-analysis: Vonoprazan, a potent acid blocker, is superior to proton-pump inhibitors for eradication of clarithromycin-resistant strains of Helicobacter pylori. Helicobacter. 2018 Aug;23(4): e12495.
5. Zuberi BF, Ali FS, Rasheed T, Bader N, Hussain SM, Saleem A. Comparison of Vonoprazan and Amoxicillin Dual Therapy with Standard Triple Therapy with Proton Pump Inhibitor for Helicobacter Pylori eradication: A Randomized Control Trial. Pak J

Med Sci. 2022 Mar-Apr;38(4Part-II):965-969. doi: 10.12669/pjms.38.4.5436. PMID: 35634610; PMCID: PMC9121978.

6. https://www.facebook.com/Drugscom. Amoxicillin, clarithromycin, and Vonoprazan Uses, Side Effects & Warnings [Internet]. Drugs.com. 2024 [cited 2024 Nov 18]. Available from: https://www.drugs.com/mtm/amoxicillin-clarithromycin-and-vonoprazan.html.

7. Sakurai Y, Shiino M, Okamoto H, Nishimura A, Nakamura K, Hasegawa S. Pharmacokinetics and safety of triple therapy with Vonoprazan, amoxicillin, and clarithromycin or metronidazole: a phase 1, open-label, randomized, crossover study. Advances in therapy. 2016 Sep; 33:1519-35.

8. Anusha K, Sowjanya G. A Reliable Rp-UPLC-TuvX Method for Simultaneous Estimation of Clarithromycin, Amoxicillin, And Vonoprazan in Co-Packed Pharmaceutical Dosage Forms: Method Development and Validation with Stability Indicating Properties. Int J App Pharm [Internet]. 2024 Mar. 7 [cited 2024 Nov. 11];16(2):351-7.

9. Salva, C., & Galla, R. (2024). The development and validation of a stability indicating Rp-UPLC method for the simultaneous estimation of clarithromycin, amoxicillin, and Vonoprazan in a physical mixture. *Journal of Applied Pharmaceutical Science*.

10. Mustafa A, Ali H, Bibi R, Hassan A, Khan S, Khan SA. Simultaneous determination of amoxicillin, clarithromycin and esomeprazole in mice plasma after oral administration by reverse phase HPLC method. J Chromatogr Sep Tech. 2021;12(1):1–7.

11. Lal B, Jaimini M, Kapoor DU. Development and validation of a RP HPLC method for the simultaneous estimation of clarithromycin, tinidazole and lansoprazole in fixed dose combination. Trop J Pharm Life Sci. 2021;8(4):1–10.

12. Zhiqiang L, Aoxue L, Yang L, Guopeng W, Xinjing C, Hao Q, et al. Development of a stability-indicating HPLC method for simultaneous determination of ten related substances in Vonoprazan fumarate drug substance. J Pharm Anal. 2018; 149:133–142. 10.1016/j.jpba.2017.11.011

13. Smerikerova M, Bozhanov S, Maslarska V. Development and validation of a RP HPLC method to quantify amoxicillin, tinidazole, esomeprazole and lansoprazole in a mixture. Indian J Pharm Sci. 2019;81(6):1122–1127. 10.36468/pharmaceutical-sciences.611

14. Hakan Aktas A, Mine Sandag A. Liquid chromatographic—chemometric techniques for the simultaneous HPLC determination of lansoprazole, amoxicillin and clarithromycin. J Chromatogr Sci. 2017;55(8):798–804. 10.1093/chromsci/bmx039

15. Jain V, Jain NK. Analytical method development and validation for simultaneous determination of amoxicillin, omeprazole and rifabutin in bulk and in a synthetic mixture by UV spectroscopy. J Drug Deliv Ther. 2019;9(4):788–94. 10.22270/jddt. v9i4.4211

16. Gangishetty S, Surajpal V. RP-HPLC method development and validation for simultaneous estimation of clarithromycin and paracetamol. ISRN Anal Chem. 2013; 2013:1–5. 10.1155/2013/948547

17. Alam, M, Hossain S, Bhadra S, Kumar U, Rouf A.S. Development and validation of RP-HPLC method for quantitation of clarithromycin in matrix tablet dosage form. Dhaka Univ J Pharm Sci. 2017;16(1):69–75. 10.3329/dujps. v16i1.33384.

18. Tzouganaki Z, Koupparis M, Development and validation of an HPLC method for the determination of the macrolide antibiotic clarithromycin using evaporative light scattering detector in raw materials and pharmaceutical formulations. Mediterr. J. Chem. 2017;6(4):133–141. 10.13171/mjc64/01706211420-tzouganaki.

19. Osman A, Sumaiya F, Anas R. Evaluation and validation of a UPLC method for estimation of amoxiclav in oral dosage form. World J Pharm Life Sci. 2020;6(9):107–113. Available from https://www.researchgate.net/publication/344218584 Evaluation and Validation of UPLC method for estimation of Amoxiclav in oral dosage form

20. Tavakoli N, Varshosaz J, Dorkoosh F, Zargarzadeh MR. Development and validation of a simple HPLC method for simultaneous in vitro determination of amoxicillin and metronidazole at single wavelength. J Pharm Biomed Anal. 2007;43(1):325–329. 10.1016/j. jpba.2006.06.002

21. Marcel S, Tito U, Ines I, Pierre NJ. Validation of HPLC-UV method for determination of amoxicillin Trihydrate in capsule. Annals Adv Chem. 2018; 2:56–72. 10.29328/journal.aac.1001014

22. Beg S, Kohli Swain S, Hasnain MS. Development and validation of RP-HPLC method for quantitation of amoxicillin trihydrate in bulk and pharmaceutical formulations using box-Behnken Experimental design. J Liq Chromatogr Related Technol. 2012;35(3):393–406. 10.1080/10826076.2011.601493

23. Tippa DMR, Singh N. Development and validation of stability indicating HPLC method for simultaneous estimation of amoxicillin and clavulanic acid in injection. Am J Anal Chem. 2010; 1:95–101. 10.4236/ajac.2010.13013

24. Sahoo NK, Sahu M, Algarsamy V, Srividya B, Sahoo CH. Validation of assay indicating method development of amoxicillin in bulk and one of its marketed dosage forms by RP-HPLC. Annals Chromatogr Sep. 2016;2(1):1014.

25. Gebretsadik H, Kahsay G, Eticha T, Gebretsadikan T. A validated new RP-HPLC method for simultaneous determination of amoxicillin, ampicillin and cloxacillin in pharmaceutical formulations. Acta Chromatogr. 2022;1–10. 10.1556/1326.2022.01043

26. Deshmukh N, Patel R, Das PK. Development of quantitative method for analysis of meropenem and amoxycillin by RP HPLC method. Pharm Biol Eval. 2016;3(4):431–436

27. De Marco BA, Hisano Natori JS, Fanelli S, Tótoli EG, Nunes Salgado HR. Characteristics, properties and analytical methods of amoxicillin: a review with green approach. Crit Rev Anal Chem. 2017;47(3):267–277. 10.1080/10408347.2017.1281097

28. International Conference of Harmonization (ICH) of technical requirements for registration of pharmaceuticals for Human use a Use Q2:(R1), Harmonized tripartite guideline, Validation of Analytical procedures, 6th Nov 1996.

29. International Conference of Harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use Q1A:(R2), harmonized tripartite guideline, stability testing of new drug substance and products, 6th Nov 1996.