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RP –HPLC METHOD DEVELOPMENT&VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMOXICILLIN, CLARITHROMYCIN AND VONOPRAZAN IN TABLETS

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ABSTRACT

The Present study represents an easy, fast, accurate and meticulous RP –HPLC method which was developed for the simultaneous estimation of Amoxicillin, Clarithromycin and Vonoprazan in tablet dosage form has been developed. The partition was performed by the Shimadzu C-18 column (250cm x 4.6 mm, 5µm) with mobile phase assemble of Buffer (0.5m potassium dihydrogen phosphate and pH was adjusted by using 1% ortho phosphoric acid, pH 4.0): methanol (50:50v/v) at a flow rate of 1.ml/min and the solvents were recognized by UV detector at the wavelength of 205nm. The method has been validated as specified by ICH guidelines. Linearity was detected for Amoxicillin 50µg/ml -300µg/ml, Clarithromycin 50µg/ml-300µg/ml and Vonoprazan 3µg/ml-15µg/ml. Developed method was discovered to be precise, accurate and rapid for simultaneous estimation of Amoxicillin, Clarithromycin and Vonoprazan in Tablets.

KEYWORDS: RP-HPLC Method, Validation, Amoxicillin, Clarithromycin and Vonoprazan

INTRODUCTON

Amoxicillin is a penicillin antibiotic and semi synthetic amino penicillin that is β-lactam antibiotic. It is active against gram positive and gram negative bacteria. Generally Amoxicillin is a bactericidal board spectrum antibiotic. Amoxicillin is well absorbed in gastrointestinal tract. The protein binding is less than 25% and moderately metabolized in liver and excreted in urine. Half life of amoxicillin is one hour, 3.7 hours in new born baby. It was taken orally or less often by injections, topical and used for respiratory infections (including pneumonia) and dental abscesses.

Clarithromycin is a board spectrum macrolide antibiotic that gives activity against gram positive and gram negative aerobic, anaerobic bacteria and mycobacterium. Clarithromycin was well absorbed in addition to the presence of food. The protein binding is 80% and it is metabolized in liver and eliminated via renal excretion (37%) and hepatic metabolism (60%). Half life of Clarithromycin is 2.9- 9.2 hours. It was taken by oral or IV .It is used to treat the Respiratory tract infections, skin and tissue infections and *Eradication of H.pylori*.

Vonoprazan is a potassium competitive acid blocker used for the treatment of acid related disorders. Vonoprazan is rapidly absorbed the food has minimal effect on its intestinal absorption. The protein binding is 80% and is metabolized in liver via inactive metabolites mainly by (CYP) 3A4 and excreted through urine (67%) and feces

(31%). The half life of Vonoprazan is 7.1 hours. It is taken orally. It is used to treat duodenal, gastric ulcer and erosive oesophagitis.

MATERIALS AND METHOD

Amoxicillin, Clarithromycin and Vonoprazan sample were buyed from Yarrow chem. Products and Dhamtec Pharma and Consultants, Mumbai, India. The HPLC graded Methanol and AR- Grade potassium dihydrogen phosphate and ortho phosphoric acid obtained from the Modern Scientific Lab.

INSTRUMENT

HPLC was done with a model: Shimadu, 1220 infinity Pump, LC-2010 CHT, Chromatographic partition was performed using Hypersil C18, (250 mm x 4.6 mm, 5 µm) column, column Injector: 20µL fixed loop and the detector-PDA Detector.

METHOD DEVELOPMENT

Preparation of standard solution

Amoxicillin, Clarithromycin and Vonoprazan were weighed accurately in an electronic balance and dissolved in methanol having concentration of 1 mg/ml. Division of standard stock solutions were trap into vials and volume was make up to mark with methanol to develop the admired concentrations ranging from 50-300 µg/ml for Amoxicillin, 50-300 µg/ml for Clarithromycin and 3-15 µg/ml for Vonoprazan. All solutions were percolated in 0.45 µm nylon filter and out gassed by sonication before analysis.

Preparation of working standard solution

A standard solution was prepared by measuring 1 ml of Amoxicillin, 1ml of Clarithromycin and 1ml of Vonoprazan stock solution and then transferred into a clean 10ml volumetric flask. The volume was adjusted to make up to the mark by using mobile phase.

Phosphate buffer preparation:

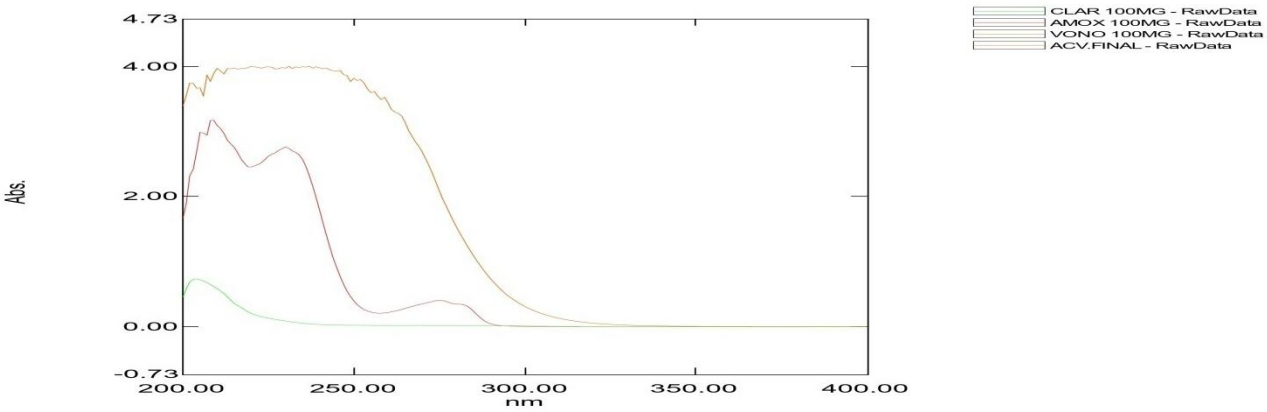
Potassium dihydrogen orthophosphate (6.8gm) were weighed and transferred into the 1000ml volumetric flask. Add 800ml of water and make up to the mark with water. Adjust the pH using 1% orthophosphoric acid solution (0.5 M potassium dihydrogen ortho phosphate).

Pharmaceutical formulation

Twenty tablets were weighed accurately and average weight was calculated. The Tablet was Powdered and an equivalent weight of 500µg/ml of Amoxicillin and Clarithromycin and 20µg/ml of Vonoprazan was taken to 100ml volumetric flask, and 60 ml of mobile phase. Then, shake for 15 minutes and sonicate for 5 minutes. Make up to the mark by using mobile phase. The solution was filtered through 0.45 nylon filter paper and first few drops of filtrate were discarded and 1 ml of this solution was diluted to 10 ml with mobile phase.

Selection of wavelength

Standard solution of 500 µg/ml of Amoxicillin and Clarithromycin and 20 µg/ml of Vonoprazan in methanol was scanned among 200-400nm using UV- Visible spectrophotometer. All the three solution were scanned among 200-400nm. Wavelength was chosen from the overlay spectra of above solution.



MOBILE PHASE SELECTION:

Mobile phase was chosen according to review of literature. Various trail of mobile phase was conducted which composed of Methanol, Acetonitrile, Water; Buffers in various ratios at different flow rate were tried. In the focus to different trials, the combination of **Potassium dihydrogen orthophosphate, pH 4.0: Methanol (50:50v/v)**

RESULT AND DISCUSSION

Optimized Chromatographic condition: 1ml/min flow rate, test to be elegant than the other in terms of resolution, peak shape and retention time.

Column **:** C 18 (25 cm x 0.46 cm) Hypersil

Mode of Elution **:** Isocratic

Mobile phase **:** Buffer (PH 4.0): Methanol (50:50 V/v)

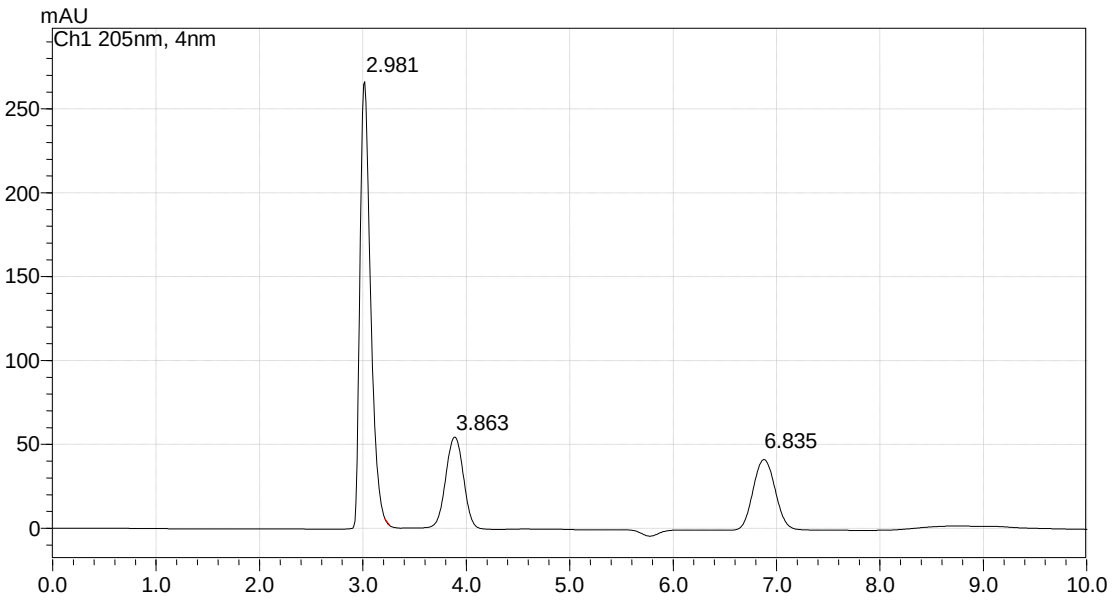
Flow Rate **:** 1.0 ml/min

Detection Wavelength : 205 nm

Run time **:** 20 min

Injection volume : 20.0µl

Optimized Chromatogram Condition of Amoxicillin, Clarithromycin and Vonoprazan



DRUG	RETENTION TIME
Amoxicillin	2.981
Clarithromycin	3.863
Vonoprazan	6.835

METHOD VALIDATION

The method was validated in terms of precision, accuracy, limit of detection, linearity, and system suitability, limit of quantification, resolution and robustness.

SYSTEM SUITABILITY

System suitability is commonly used to verify the column efficiency, resolution, and repeatability of chromatographic system for the analysis. The system suitability was measured by conducting the experiment and focuses in change of partition, retention times and asymmetry of the chromatographic peaks are calculated.

Table 1: System Suitability test

Parameters	Amoxicillin	Clarithromycin	Vonoprazan
Retention time	2.981	3.863	6.835
Theoretical plates	483053	53921	58887
Tailing factor	1.242	1.336	1.145
Resolution	-	3.13	4.15

PRECISION

Precision means measurement of Intraday and Interday precision.

1) **Intraday precision**

The sample solutions of Amoxicillin, Clarithromycin and Vonoprazan injected six times on the same day under similar conditions to calculate intraday precision and % R.S.D were calculated.

2) **Interday precision**

The sample solutions of Amoxicillin, Clarithromycin, and Vonoprazan injected six times on the different day to calculate Inter day precision and %R.S.D were calculated.

Table 2: Precision Result

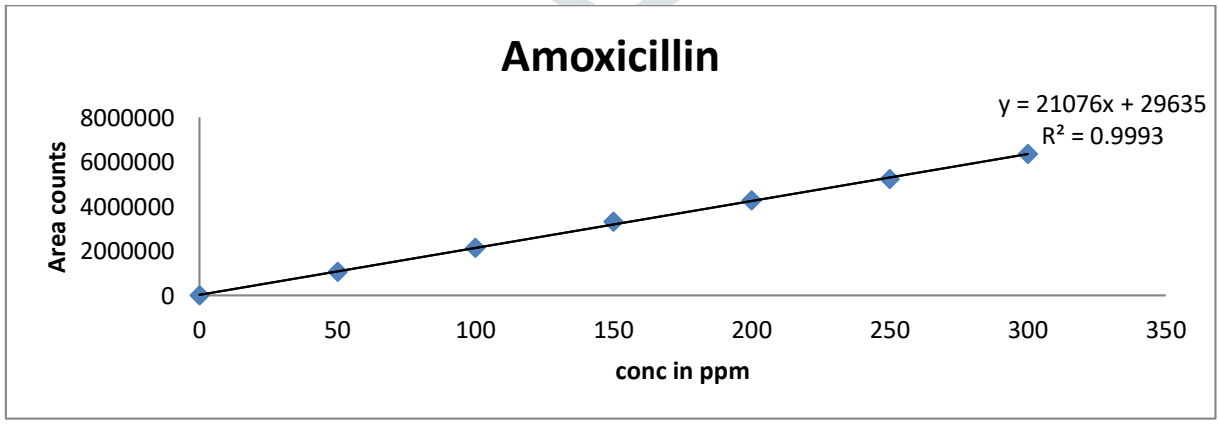
Drug	Intraday precision (%RSD)	Inter day precision(%RSD)
Amoxicillin	0.224-0.455	0.532-0.821
Clarithromycin	1.773-1.952	1.645-1.820
Vonoprazan	1.557-1.856	1.638-1.952

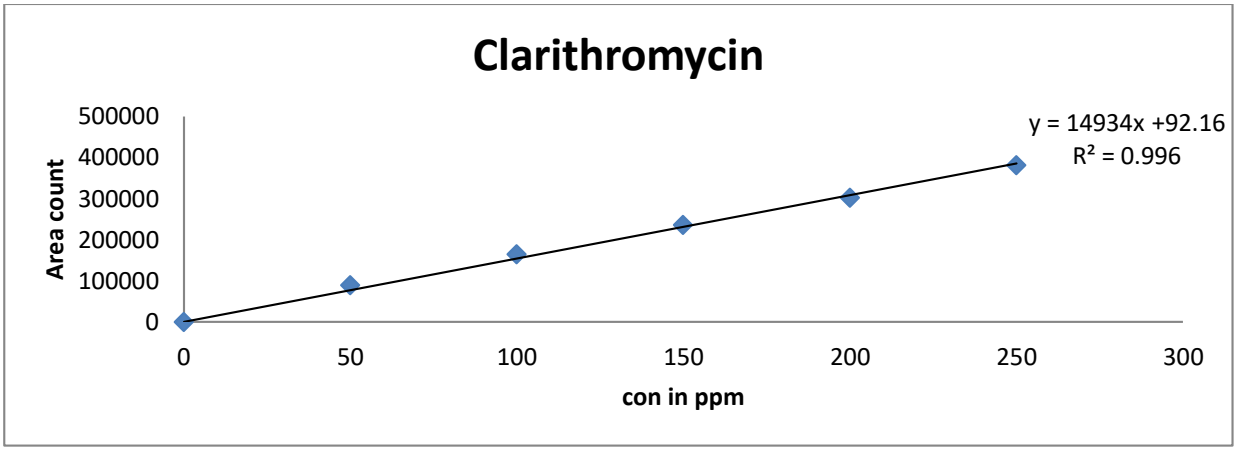
LINEARITY

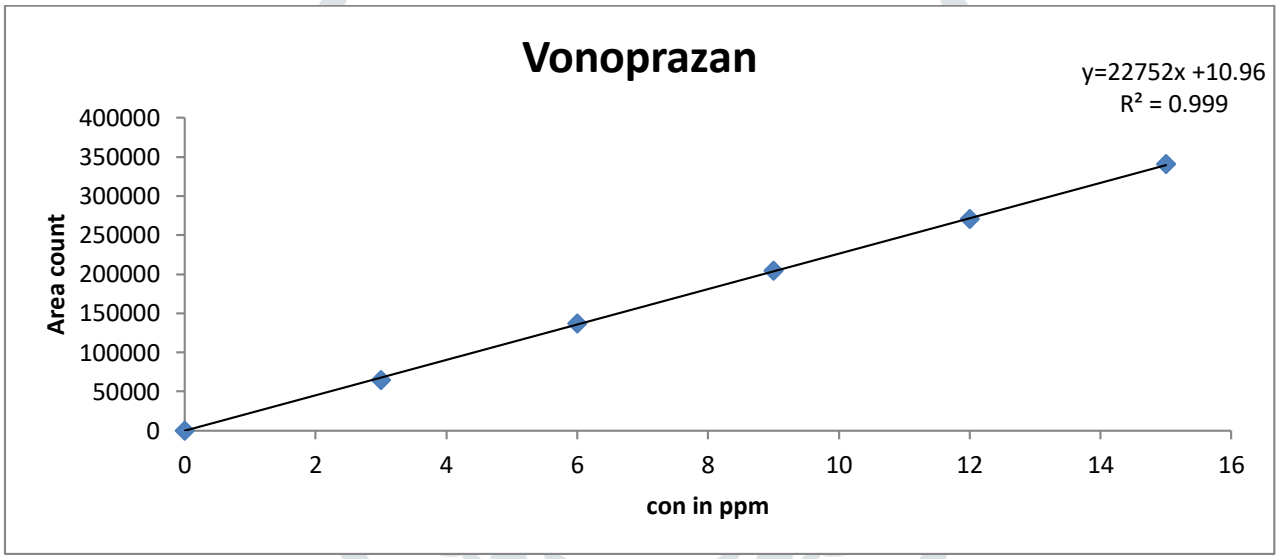
Linearity means ability to obtain test results which are directly proportional to amount of analyze in the sample. The concentration at which the calibration curve depend on linearity. Linearity range of Amoxicillin 50-300µg/ml, Clarithromycin 50-300µg/ml, and Vonoprazan 3-15µg/ml. Calibration curve of the area was plotted and detect the correlation co-efficient and regression line equation for Amoxicillin, Clarithromycin and Vonoprazan.

Table 3: Linearity Result

Drug	Linearity range	Correlation coefficient
Amoxicillin	50-300 µg/ml	0.999
Clarithromycin	50-300µg/ml	0.996
Vonoprazan	3-15µg/ml	0.999







LOD & LOQ

The LOD was evaluated from the set of 3 calibration curve used to deduction method linearity. The LOD may be intended as, LOD = 3.3 x (SD/Slope)

Where, SD= Standard deviation of intercepts. Slope = Mean of slope

The LOQ was evaluated from the set of 3 calibration curve used to deduction method linearity. The LOQ may be intended as, LOQ = 10 x (SD/Slope)

The LOD & LOQ was calculated as:

LOD = 3.3 x SD/Slope of calibration curve

LOQ = 10 x SD/Slope of calibration curve

Table 4: Result for LOD and LOQ

Parameter	Amoxicillin	Clarithromycin	Vonoprazan
LOD	3.0709µg/ml	2.5137µg/ml	1.5730µg/ml
LOQ	9.3058µg/ml	7.6175µg/ml	4.7666µg/ml

ACCURACY

Accuracy of the method was calculated by retrieval study from marketed formulation at three level of standard addition.

Table 5: Accuracy Result

Drug	Amt of drug (U=µg/ml)	Amount of drug added (µ/ml)	Amt.recovered Mean (µ/ml)	Mean % recovery ± S.D. (n=3)	Mean % RSD
Amoxicillin	50 50 50	50 100 150	50.22 100.12 150.20	100.44±0.94 100.12±1.05 100.13±1.00	0.94 1.05 1.00
Clarithromycin	50 50 50	50 100 150	50.12 100.28 150.25	100.24±1.02 100.28±1.50 100.16±2.08	1.03 1.51 2.08
Vonoprazan	3 3 3	2.0 3.0 4.0	2.15 3.11 4.06	107.05±0.52 103.66±1.00 101.05±1.64	0.53 1.00 1.65

ROBUSTNESS

Following limitations were alternate individually and their effect was noticed on system suitability for standard preparation.

1. Flow rate of mobile phase was alternate (± 0.8 ml/min)
2. pH of mobile phase was alternate (± 0.8)
3. Ratio of Mobile phase was alternate (±8) Buffer: Methanol (60:30) and Buffer: Methanol (30:60)

Table 6: Robustness data

Drug	Area at Flow Rate (+0.8 ml/min)	Area at Flow Rate (-0.8 ml/min)	Area at Mobile phase (+8)	Area at Mobile phase (-8)	Area at PH (+0.8)	Area at pH (-0.8)
Amoxicillin	1246.120	1442.297	1276.234	1290.874	1476.421	1298.736
Clarithromycin	3678.973	3474.672	3095.870	3429.576	3872.230	3498.741
Vonoprazan	8210.782	8092.422	8672.460	8698.513	8223.892	8770.652

ASSAY

Analysis of marketed formulation by commercially available tablet formulation VOQUEZNA TriplePak. The results are shown in below table.

Table 7: Assay of Marketed Formulation

Tablet	Label Claim	Assay(% of label claim Mean± S.D)
VOQUEZNNA TriplePak	Amoxicillin	98.95±7.6
	Clarithromycin	99.86±8.9
	Vonoprazan	100.15±1.7

CONCLUSION

A Reverse Phase High Performance Liquid Chromatography method was developed for the simultaneous estimation of Amoxicillin, Clarithromycin and Vonoprazan in tablets. The partition with resolution developed by C18 (250mm x 4.6mm, 5µm) column and Buffer (0.5M potassium dihydrogen ortho phosphate, pH4.0): methanol (50:50v/v) as a mobile phase at a flow rate of 1 ml/min. Detection was carried out at 205nm. The Retention time of Amoxicillin-2.981, Clarithromycin - 3.863 and Vonoprazan - 6.835 minute. Linearity viewed for Amoxicillin 50-300µg/ml, Clarithromycin 50-300µg/ml and Vonoprazan 3-15µg/ml. The comparative research paper also represent that improved method is better precise in terms of mobile phase and run time.

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