Chapter 17

STABILITY HPLC METHOD DEVELOPMENT AND VALIDATION FOR VOQUEZNA COMBINATION AMOXICILLIN, CLARITHROMYCIN AND VONOPRAZAN

17.1 EXPERIMENTALS

17.1.1 Instruments Utilised

The Shimadzu-HPLC system LC-20-AT-system with LC-Solution and Peak chrom software with both PDA & UV detector. Stationary phase coloumn in reverse phase has been used C-18-Hypersil-BDS and Hypersil-ODS-250 x 4.6 mm, 5 micron size has been selected.

Systronics UV-visible spectrophotometer was used along with other Shimadzu UV 1800 spectrophotometer & Systronics UV for the wavelength maxima estimation. FTIR Spectrometer Shimadzu 8400 series has been utilised for identification of drugs standard samples. Melting point apparatus Labtronics was used for melting point determinations.

Wist Temperature Chamber was used for drying the drug samples and thermal degradation study. Ultra-sonicator Lab Branson ultrasonic's corporation was utilised. Digital pH meter labtronics was utilised. Photostability Test Chamber Sanwood SM-LHH-GSD-UV Series was utilised. Electronic analytical balance AUX-220 Shimadzu has been used. Borosil glass-wares volumetric flasks measuring cylinder pipettes of analytical were used. 0.22 and 0.45 µm nylon Millipore filters caps were used.

17.1.2 Materials and Reagents Utilised

The chemicals used working reference standard drugs Amoxicillin AMOX, Clarithromycin CLAR, Vonoprazan VONO drugs samples of solisom & upcare pharma has been utilised. Acetonitrile, Methanol, potassium dihydrogen ortho phosphate, orthophosphoric acid, TEA-Tri Ethyl amine used analytical HPLC Merck grade. H_2O_2, HCl , NaOH analytical grade of Rankem used. Milli-Q pure water is utilized.

17.1.3 Identification of Standard Drug Samples

17.1.3.1 Melting Point Determination

The working standard drugs Amoxicillin AMOX, Clarithromycin CLAR & Von oprazan VONO were identified by melting point determination. Melting point apparatus used was made of Labtronics™ Melting Point Apparatus. The melting points observed for the standard drug samples are shown in the Table 14.1.

Drug	Observed Melting Range	Standard Melting Range
AMOX	193-195.6 ^{0}C	194 ^{0}C
CLAR	218-221.7 ^{0}C	217-220 ^{0}C
VONO	193-197 ^{0}C	192-195 ^{0}C

Table 14.1: Melting Points of AMOX, CLAR & VONO

17.1.3.2 FTIR Spectral Determination for Identification Standard drug samples AMOX, CLAR & VONO

The pure active pharmaceutical working standard drug substances AMOX, CLAR & VONO were scanned between 400-4000cm^{-1} in FTIR Spectrometer Shimadzu 8400 series. The drug dry powder samples were made die pressed pellets with KBr and the FRIR spectra were determined shown in Fig 14.1 for AMOX, Fig 14.2 for CLAR & Fig 14.3 for VONO. The principal IR peaks recorded and observed for the drugs are shown in Table 14.2, 14.3 & 14.4 for AMOX, CLAR & VONO respectively.

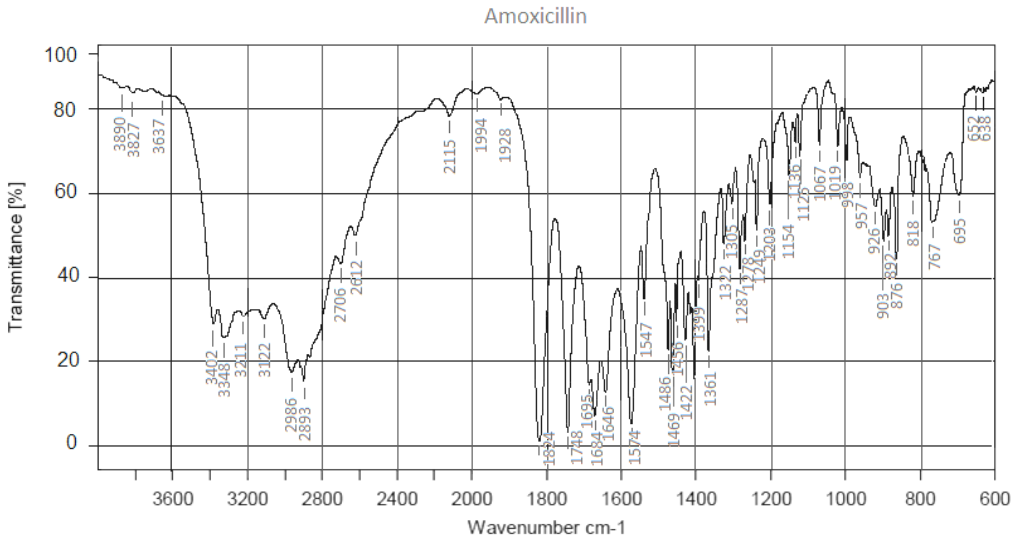

Figure 14.1: FTIR Spectra of Amoxicillin AMOX

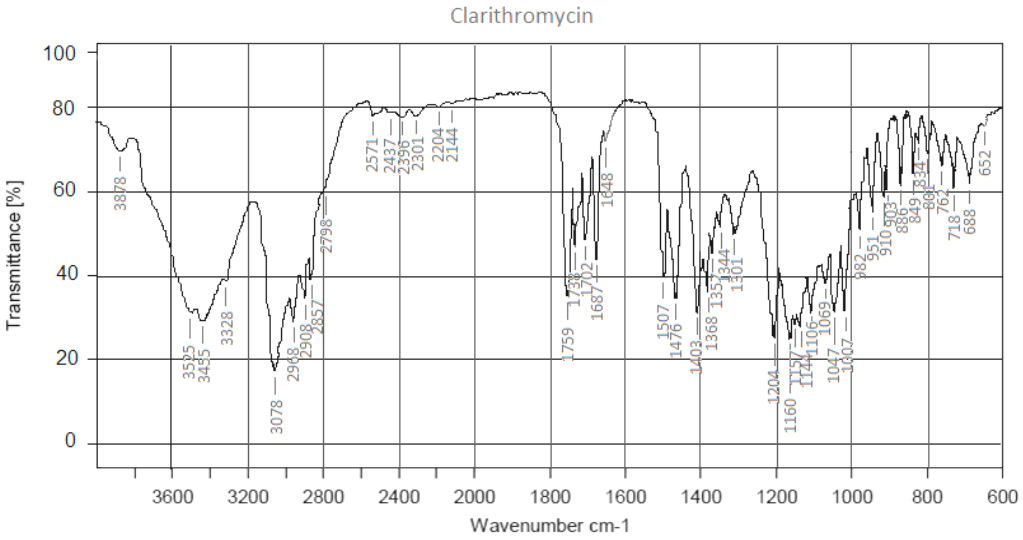

Figure 14.2: FTIR Spectra of Clarithromycin CLAR

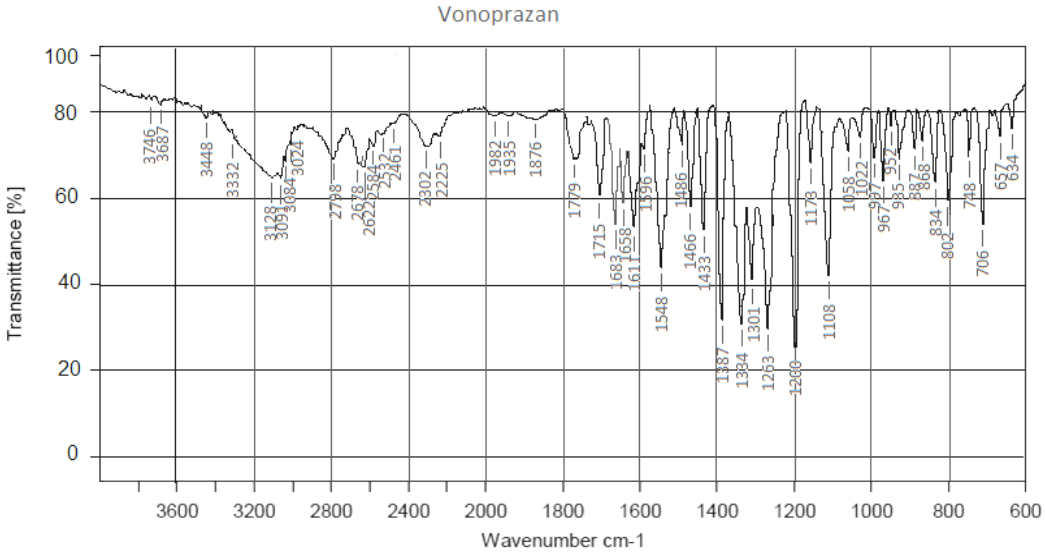

Figure 14.3: FTIR Spectra of Vonoprazan VONO

AMOXICILLIN					
Energy (Cm^{-1})	**Band Assignment**	**Peak Intensity**	**Energy (Cm^{-1})**	**Band Assignment**	**Peak Intensity**
1650	C=O lactam	16.79	3000-2800 1450	C-H Methyl group	18.72 16.47 29.47
1680-1690	C=O Amide	9.76	1250-1020 3300-3400	C-N C-N	64.52 66.47 29.86

1390-1310	OH Phenol	22.56 47.67 58.92	705-570 725-655	C-S	60.07
3400-3300	N-H 1^0 Amine	29.86	3350-3310	N-H 2^0 Amine	25.68
1647 1600-1553	C=C (Aromatic)	14.79 36.57	1250-1020	C-N	64.52 66.47
1500-1700 1650-1580	N-H	8.97 7.26	1760- 1720-1706	C=O Acid	5.64
2000-1650	C-H Aromatic	2.14 14.79 83.64	3300-2500 1440-1395	O-H Acid	42.72 17.74

Table 14.2: FTIR Interpretation of Amoxicillin AMOX

CLARITHROMYCIN					
Energy (Cm^{-1})	**Band Assignment**	**Peak Intensity**	**Energy (Cm^{-1})**	**Band Assignment**	**Peak Intensity**
2700-3200 3550-3200 3700-3584	O-H Alcohol	59.87 32.48	1750-1735 1770-1780	C=O Ester	47.61
3000-2800 1450	C-H Aliphatic	28.56 36.49	1250-1020 3300-3400	C-N C-N	29.75
1200-1350	N-C (3^0 Amine)	53.69 50.74	1210-1163	C-O Ester	24.68 23.17
1124-1087	C-O Alcohol	31.07	1450	C-H	28.56
1749-1792 1275-1200 1150-1085	C-O (Ether)	38.31 24.68	1725-1705 1685-1666	C=O Ketone	43.57 49.54

Table 14.3: FTIR Interpretation of Clarithromycin CLAR

VONOPRAZAN					
Energy (Cm^{-1})	**Band Assignment**	**Peak Intensity**	**Energy (Cm^{-1})**	**Band Assignment**	**Peak Intensity**
3000-2800 1450	C-H Methyl group	68.84	1500-1700 1650-1580	N-H	44.89 76.29 56.74
1415-1380 1370- 1335-1300 1070-1030	S=O	33.47 31.04 41.28	2000-1650	C-H Aromatic	79.24 60.72
1250-1020 3300-3400	C-N	24.84 74.27 78.56	3350-3310	N-H 2^0 Amine	74.27
1690-1640	C=N	54.63 59.23	1647 1600-1553	C=C (Aromatic)	75.27
1400-1000	-CF	42.75 68.72	1415-1380	S-N	33.52

Table 14.4: FTIR Interpretation of Vonoprazan VONO

17.1.4 Preparation of Solutions

17.1.4.1 Preparation of standard solutions of Vonoprazan VONO, Amoxicill in AMOX and Clarithromycin CLAR

The standard stock soln. individual drugs prepared in 50:50 Methanol : Water solvent mixture. 50mg of AMOX & 50mg of CLAR were individually dissolved in solvent mixture and made upto 100ml with same solvent to give 500 µg/ml standard stock solutions of AMOX & CLAR were prepared and for VONO, 20mg VONO was dissolved in solvent mixture and made upto 100ml to give 200 µg/ml standard stock solution. From the above stock solutions of individual drugs AMOX, CLAR & VONO each, 10ml from each was taken individually and diluted upto 100ml in individual volumetric flasks to give AMOX 50 µg/ml , CLAR 50 µg/ml , VONO 20 µg/ml individual drug standard Final solutions.

17.1.4.2 Preparation of Sample Solutions

Sample solution from the VOQUEZNA™ containing combination of AMOX CAPS 500mg, CLAR TAB 500mg & VONO TAB 20mg made. Accurately the avg. wt. of 10 tablets was done and crushed triturated, the tablet powder was taken weighing equivalent wt of VONO 20mg dissolved in 10ml 50:50 Methanol:Water to give VONO 2000ug/ml soln V1, and capsule powder of AMOX 500mg CLAR 500mg was taken and dissolved individually in 50:50 Methanol : ACN 50ml solution in a volumetric flask and then sonicated, filtered and made upto 100ml to give AMOX 5000 µg/ml soln A1, and CLAR 5000 µg/ml soln C1.

From these stock solutions A, 10ml was taken aliquots from A1, C1, V1 and made upto 100ml in a volumetric flask to give combined solution B containing AMOX 500 µg/ml , CLAR 500 µg/ml & VONO 200 µg/ml. From this combined soln B 1ml was taken and made upto 10ml in mobile phase to give combined final solution C containing AMOX 50 µg/ml , CLAR 50 µg/ml & VONO 20 µg/ml which was used for analysis.

17.1.4.3 Preparation of Optimized Mobile Phase

The mobile phase made by taking 80:15:5 ratio, 0.05M Phosphate buffer : ACN : Methanol with 0.1%TEA of pH 3.5. The phosphate buffer was prepared by accurately weighing 6.8gm KH_2PO_4 (MW. 136), & 1ml of TEA Triethylamine (99.98%) in 1000ml HPLC grade milli-Q system purified water. The pH adjusted by 1% OPA Ortho-phosphoric acid. After filtration it was sonicated and the 1% OPA was prepared by taking (1.176ml) of 85%w/v orthophosphoric acid (MW 98) in 100ml HPLC grade water.

17.1.5 Selection of Wavelength for Detection

The Final standard solns of AMOX 50 µg/ml , CLAR 50 µg/ml & VONO 20 µg/ml scanned in 200 - 400 nm in UV-visible double beam spectrophotometr spectrophotometer at a medium scanning speed. The overlain spectraashown in Fig. 14.4 of AMOX 50 µg/ml , CLAR 50 µg/ml & VONO 20 µg/ml were taken in 50:50 Methanol : Water and the 229nm wavelength was selected for estimation in the detection during the HPLC analysis.

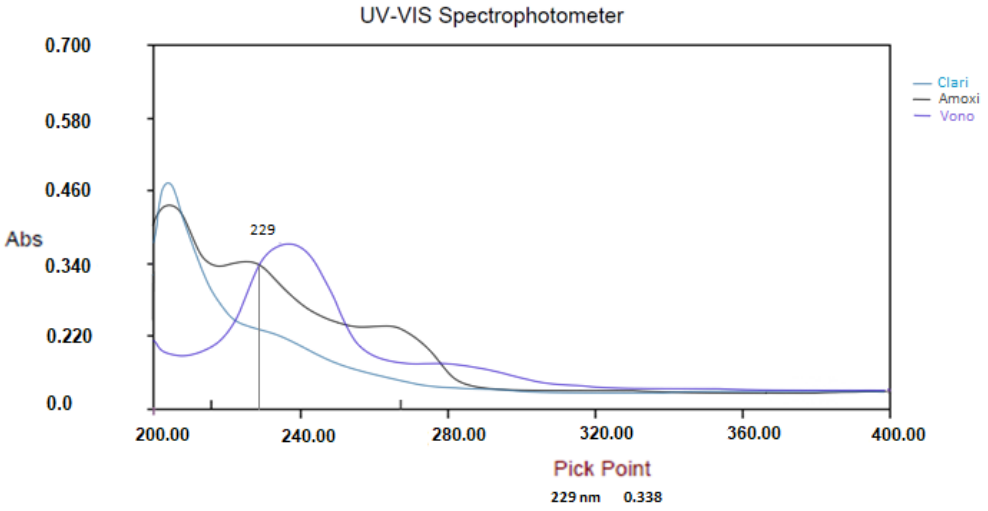

Figure 14.4: UV Spectra Overlay of AMOX, CLAR & VONO

17.1.6 Selection and Optimization of Mobile phase

For the detection analysis of the AMOX, CLAR & VONO drugs in the combined form in the working standard solutions by the HPLC method had been carried out in reverse phase by using polar solvents in mobile phase. The various trials with different mobile phase's has been carried out for the detection and seperation of the drugs was carried out shown in Table 14.5

Sr No	Mobile Phase	pH	Ratio v/v/v	Retention Time (min)			REMARK
				AMOX	CLAR	VONO	
1	H_2O:MeOH	-	50:50	-	-	-	No peak detected
2	H_2O:MeOH	-	80:20	-	-	-	No peak detected
3	H_2O:MeOH	-	20:80	-	-	-	No peak detected
4	0.05 M - Phosphate - buffer : ACN	7	50:50	-	-	-	No peak detected

5	0.05 M - Phosphate - buffer : ACN	7	20:80	-	-	-	No peak detected
6	0.05 M - Phosphate - buffer : ACN	6.5	20:80	12.63	14.1	-	No peak of VONO detected, Peak tailing in AMOX & CLAR
7	0.05 M - Phosphate - buffer : ACN	5	30:70	9.86	10.09	13.67	Peak merging of AMOX & CLAR, Tailing and asymmetric Peaks
8	0.05 M - Phosphate - buffer : ACN	4.5	50:50	6.24	6.48	8.53	Peak merging of AMOX & CLAR, Tailing and asymmetric Peaks
9	0.05 M Phosphate buffer :ACN with 0.1%TEA	4	60:40	5.31	5.53	6.27	Peak merging of AMOX & CLAR
10	0.05 M Phosphate buffer :ACN with 0.1%TEA	3	70:30	2.75	2.93	5.43	Peak merging of AMOX & CLAR
11	0.05 M Phosphate buffer :ACN : Methanol with 0.1%TEA	3	80:10 :10	2.53	2.61	4.01	Peak merging of AMOX & CLAR
12	**0.05 M Phosphate buffer :ACN : Methanol with 0.1%TEA**	**3.5**	**80:15 :5**	**2.75**	**4.23**	**5.57**	**Good seperation, No tailing, No peak merging**

Table 14.5: Trials for Selection of Mobile Phase for AMOX, CLAR & VONO

17.1.7 Optimized Chromatographic Conditions

Optimized chromatographic conditions for developed HLPC analytical method are shown below-

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer : ACN : Methanol 80:15:5 pH- 3.5 with 0.1%TEA
Flow rate	1ml/minl
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	229nm
Retention Times (min)	AMOX-2.75, CLAR-4.23, VONO-5.57

Table 14.6: Optimized Chromatographic Conditions for AMOX, CLAR & VONO

17.2 STABILITY STUDIES BY FORCED DEGRADATIONS

The stability studies for the pure working standard drugs AMOX, CLAR & VONO as well as for the pharmaceutical marketed formulation VOQUEZNA^{TM} containing the triple combined drugs has been carried out by performing the forced-degradations stress testing method has been utilised in method. Developed- HPLC-analytical method is been applied in stability study as well as in the assay analysis and dissolution profile study. The stability study has been performed on the pure drug and marketed formulation samples under different types of stress conditions which helps in the forced degradations of the drug substances, under the conditions like thermal, acid, base-alkali, photo, & oxidative degradations were performed in accordance with the guideline ICH - guidelines and are effectively analysed by the developed HPLC method as well as validated.

17.2.1 Acid Degradation

For the acid degradation study, was performed in 0.1N HCl solution. The working standard drug solution of 1ml of AMOX (500ug/ml) std stock soln, 1ml of CLAR (500ug/ml) std stock soln, and 1ml of VONO (200ug/ml) std stock were taken and 2ml of 0.1N HCl added and kept for 2hrs for degradation and then neutralized with 2ml of 0.1N NaOH soln, then it was made up soln to 10ml final volume with

mobile phase solvent to give AMOX 50ug/ml, CLAR 50ug/ml and VONO 20ug/ml. And the analysed this sample by developed HPLC method. In the similar manner the combined drug sample of marketed VOQUEZNA^{TM} formulation was prepared stock soln containing 500ug/ml AMOX, 500ug/ml CLAR and 200ug/ml VONO. 1ml from this stock soln was taken and 2ml of 0.1N HCl was added and kept for 2hrs for degradation and then neutralized with 2ml 0.1N NaOH, and the made up soln to 10ml final volume with mobile phase to give AMOX 50ug/ml, CLAR 50ug/ml and VONO 20ug/ml. And the analysed this sample by developed HPLC method.

17.2.2 Base Degradation

The Base degradation study, performed in 0.1N NaOH solution. The working standard drug solution of 1ml of AMOX (500ug/ml) std stock soln, 1ml of CLAR (500ug/ml) std stock soln, and 1ml of VONO (200ug/ml) std stock were taken and 2ml of 0.1N NaOH added and kept for 2hrs for degradation and then neutralized with 2ml of 0.1N HCl soln, was made up soln to 10ml final volume with mobile phase to give AMOX 50ug/ml, CLAR 50ug/ml and VONO 20ug/ml. And the analysed this sample by developed HPLC method. In the similar manner the combined drug sample of marketed VOQUEZNA^{TM} formulation was prepared stock soln containing 500ug/ml AMOX, 500ug/ml CLAR and 200ug/ml VONO. 1ml from this stock soln was taken and 2ml of 0.1N NaOH was added and it has been, kept for 2hrs for degradation and then neutralized with 2ml 0.1N HCl, and the made up soln to 10ml final made volume with mobile phase to give AMOX 50ug/ml, CLAR 50ug/ml and VONO 20ug/ml. And the analysed this sample by developed HPLC method.

17.2.3 Oxidative Degradation

The oxidative degradation study, was has been performed in 3% H_2O_2 solution as a oxidizing agent. The working standard drug solution of 1ml of AMOX (500ug/ml) std stock soln, 1ml of CLAR (500ug/ml) std stock soln, and 1ml of VONO (200ug/ml) std stock were taken and 2ml of 3% H_2O_2 solution added and kept for 2hrs for degradation and then made up soln to 10ml final volume with mobile phase to give AMOX 50ug/ml, CLAR 50ug/ml and VONO 20ug/ml. And the analysed this sample by developed HPLC method. In the similar manner the combined drug sample of marketed VOQUEZNA^{TM} formulation was prepared stock soln containing 500ug/ml AMOX, 500ug/ml CLAR and 200ug/ml VONO. 1ml from this stock soln was taken and 2ml of 3% H_2O_2 solution was added and kept for 2hrs

for degradation and then made up soln to 10ml final volume with mobile phase to give AMOX 50ug/ml, CLAR 50ug/ml and VONO 50ug/ml. And the analysed this sample by developed HPLC method.

17.2.4 Thermal Degradation

It has carried out for the working standard drug powders AMOX, CLAR & VONO individually in Wist Temperature chamber oven at 60 ^{0}C for 24hrs. After thermal degradation, the drug powder AMOX 50mg, CLAR 50mg and VONO 20mg were taken in flask dissolved in 50ml of 50:50 Methanol : Water solvent, dissolved, sonicated , filtered and final volume made upto 100ml to give stock soln of 500ug/ml of AMOX, 500ug/ml CLAR & 200ug/ml VONO. From this stock soln, 1ml taken n diluted to 10ml with mobile phase to give final soln containing AMOX 50ug/ml, CLAR 50ug/ml and VONO 20ug/ml. This final solution was subjected to be analysed by developed HPLC method. In similar manner marketed formulation VOQUEZNA^{TM} sample was powdered and kept in Wist Temperature chamber oven at 60 ^{0}C for 24hrs. After thermal degradation, the powder weighing equivalent to AMOX 50mg, CLAR 50mg and VONO 20mg were taken in flask dissolved in 50ml of 50:50 Methanol : Water solvent, dissolved by heating on water-bath at 60 ^{0}C 20mins, shaken & sonicated , filtered and final volume made upto 100ml to give stock soln of 500ug/ml of AMOX, 500ug/ml CLAR & 200ug/ml VONO. From this stock soln, 1ml, taken and then it has been, diluted to 10ml with mobile phase to give final soln containing AMOX 50ug/ml, CLAR 50ug/ml and VONO 20ug/ml. This final solution was subjected to be analysed by the developed HPLC method.

17.2.5 Photo Degradation

The photo degradation has been carried out in UV chamber 1.2million-lux-hrs and 200-watt-hrs in a photo stability test chamber Sanwood SM-LHH-UV series. The standard drug powder of AMOX, CLAR and VONO were kept into UV chamber for 24hrs.

After photo degradation, drug powder AMOX 50mg, CLAR 50mg and VONO 20mg were taken in flask dissolved in 50ml of 50:50 Methanol : Water solvent, dissolved, sonicated , filtered and final volume made upto 100ml to give stock soln of 500ug/ml of AMOX, 500ug/ml CLAR & 200ug/ml VONO. From this stock soln, 1ml taken n diluted to 10ml with mobile phase to give final soln containing AMOX 50ug/ml, CLAR 50ug/ml and VONO 20ug/ml. This final solution was subjected to be analysed by developed HPLC method. In similar manner marketed formulation

VOQUEZNA^{TM} sample was powdered and kept into UV chamber for 24hrs. After degradation, the powder weighing equivalent to AMOX 50mg, CLAR 50mg and VONO 20mg were taken in flask dissolved in 50ml of 50:50 Methanol : Water solvent, dissolved by heating on water bath at 60 ^{0}C, 20mins, shaken & sonicated , filtered and final volume made upto 100ml to give stock soln of 500ug/ml of AMOX, 500ug/ml CLAR & 200ug/ml VONO. From this stock soln, 1ml taken and diluted to 10ml with mobile phase to give final soln containing AM OX 50ug/ml, CLAR 50ug/ml and VONO 20ug/ml. This final solution was subjected to be analysed by the developed HPLC -method .

17.3 METHOD VALIDATION

17.3.1 Linearity (Calibration Curve)

The working standard and sample solutions of Amoxicillin AMOX & Clarithromycin CLAR were 12.5, 25, 37.5, 50, 62.5,75ug/ml, prepared in the serial dilutions for both drugs individually, while 5, 10. 15, 20, 25, 30ug/ml of Vonoprazan VONO, for conc. range, linearity, validation parameters and same con. ranges were used for the stability forced degradation studies. The calibration curves has been generated by plotting graph of peak area vs conc. for the drugs, and the regression equations, correlation coefficient R^2 value and the, Limit of Detection (LOD) & Limit of Quantification (LOQ) had been calculated .

17.3.2 Specificity and Selectivity

The selectivity and specificity parameters are utilised in selective detection particular analyte which are in the matrix or along with other substances without any interventions. 50ug/ml of AMOX & CLAR and 20ug/ml of VONO were injected individually, and blank mobile phase as well as sample solutions from dosage form were compared to check the specificity & selectivity. Selectivity is a type of a qualitative determination of analytes, while the specificity is applied for both qualitative as well as quantitative estimations. The developed method must be selective and highly specific for the analyte for which the method is intended to use, even in presence of impurities or any other degraded products, additives, excipients, reagents or other substances.

17.3.3 Accuracy (Recovery Studies)

Accuracy is one of the important validation parameter which describes the trueness-exactness of the test results in accordance with the true values. The accuracy studies has been performed by doing the drug recovery studies of deliberately added working standard drugs from the sample, n=3 samples taken for each drug AMOX, CLAR, VONO at 50%, 100% & 150% had performed at each level to the pre-analysed samples. The amount of drug-substance added and amount of drug-substance recovered were calculated from the sample peak area and total peak area and the % Recovery had been calculated.

17.3.4 Precision

17.3.4.1 Repeatability (n=6)

The repeatability study has been performed by repeatedly n=6 sample standards injected 50ug/ml of AMOX & CLAR and 20ug/ml of VONO and the area response of drugs was obtained and the %RSD had been calculated

17.3.4.2 Intraday Precision (n=3)

The intraday precision was performed by using the 12.5, 50, 75 ug/ml of AMOX & CLAR, while 5, 20, 30 ug/ml for VONO was used, and the solutions were repeatedly injected analysed by HPLC three times on same day, obtained results calculated into the terms of %RSD.

17.3.4.3 Interday Precision (n=3)

The interday precision was performed by using the 12.5, 50, 75 ug/ml of AMOX & CLAR, while 5, 20, 30 ug/ml for VONO was used, and the solutions were repeatedly injected analysed by HPLC three times in different days obtained results calculated into the terms of %RSD.

17.3.5 LOD and LOQ

The LOD Limit of Detection has been obtained from 5 set of the calibration curves performed in the linearity-range studies, the LOD is calculated as LOD = 3.3 x SD/Slope

LOQ Limit of Quantitation has been obtained from the same 5 set of the calibration curves performed as per the linearity-range studies, the LOD is calculated as LOD = 10 x SD/slope

17.4 APPLICATION OF DEVELOPED ANALYTICAL METHOD AS A ASSAY METHOD FOR MARKETED FORMULATION

The developed analytical HPLC method is applied in the estimation-analysis of AMOX, CLAR, VONO in the VOQUEZNA™ marketed dosage form. VOQUEZNA™ contains triple pack AMOX 500mg CLAR 500mg and VONO 20mg. VOQUEZNA™ containing combination of AMOX CAPS 500mg, CLAR TAB 500mg & VONO TAB 20mg made. Accurately the avg. wt. of 10 tablets was done and crushed triturated, the tablet powder was taken weighing equivalent wt of VONO 20mg dissolved in 10ml 50:50 Methanol:Water to give VONO 2000ug/m l soln V1, and capsule powder of AMOX 500mg CLAR 500mg was taken and dissolved individually in 50:50 Methanol : ACN 50ml solution in a volumetric flask and then sonicated, filtered and made upto 100ml to give AMOX 5000 µg/ml soln A1, and CLAR 5000 µg/ml soln C1.

From these stock solutions A, 10ml was taken aliquots from A1, C1, V1 and made upto 100ml in a volumetric flask to give combined solution B containing AMOX 500 µg/ml , CLAR 500 µg/ml & VONO 200 µg/ml. From this combined soln B 1ml was taken and made upto 10ml in mobile phase to give combined final solution C containing AMOX 50 µg/ml , CLAR 50 µg/ml & VONO 20 µg/ml which was used for analysis, and were prepared n=3 samples, analysed by the developed HPLC method. The working standard drugs AMOX 30ug/ml, CLAR 30ug/ml and VONO 10ug/ml were prepared and analysed by HPLC and the % purity or % label claim was estimated by comparing the area & calculating from regression equation, for working standard drug and marketed formulation.

17.5 RESULTS & DISCUSSIONS

17.5.1 Method Development

The developed analytical HPLC method found to be reliable, accurate.,- precise for analysis and quality control testing for AMOX, CLAR and VONO in pure form, in marketed dosage form's. The method is advantageous as the low cost solvents are used, good resolution and seperation has been achieved, as well as the peak symmetry tailing factor are in greater acceptable limits. The isocratic mode adds the advantage of simplicity of the developed method. Method consists of the optimized mobile phase Phosphate buffer:ACN:Methanol (80:15:5) pH 3.5, flow rate 1ml /

min , detection wavelength at 229nm. Excipients in the marketed formulation does not affect in the resolution, separations as well do not have any interfering peaks. The average retention times were found to be AMOX-2.75, CLAR-4.23 and VONO-5.57 minutes. The chromatogram of the drugs are shown below.

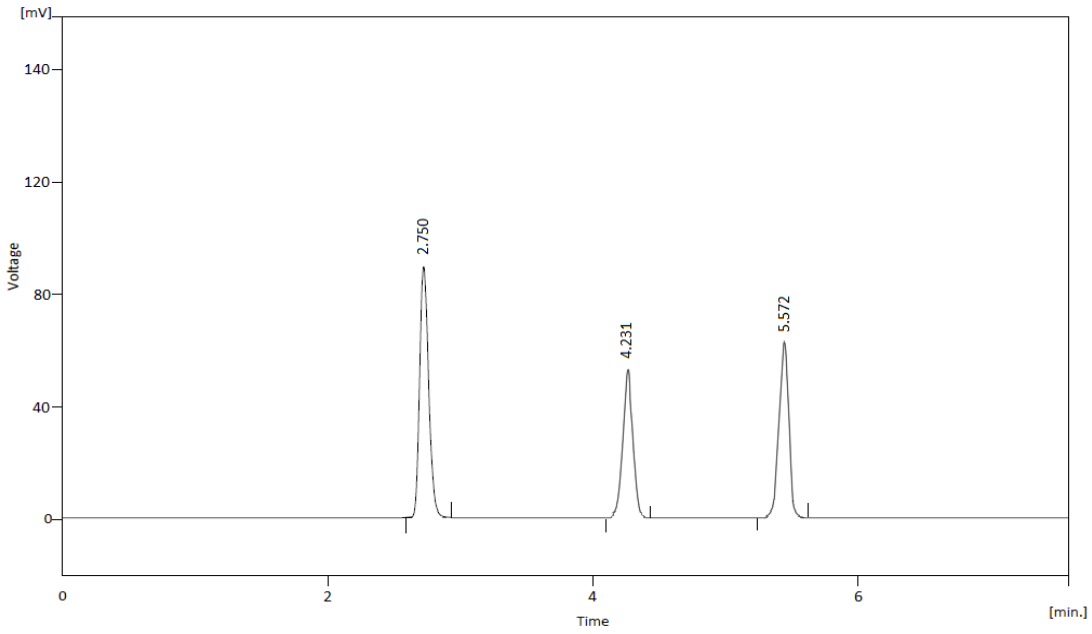

Figure 14.5: Chromatogram of Standard AMOX, CLAR & VONO

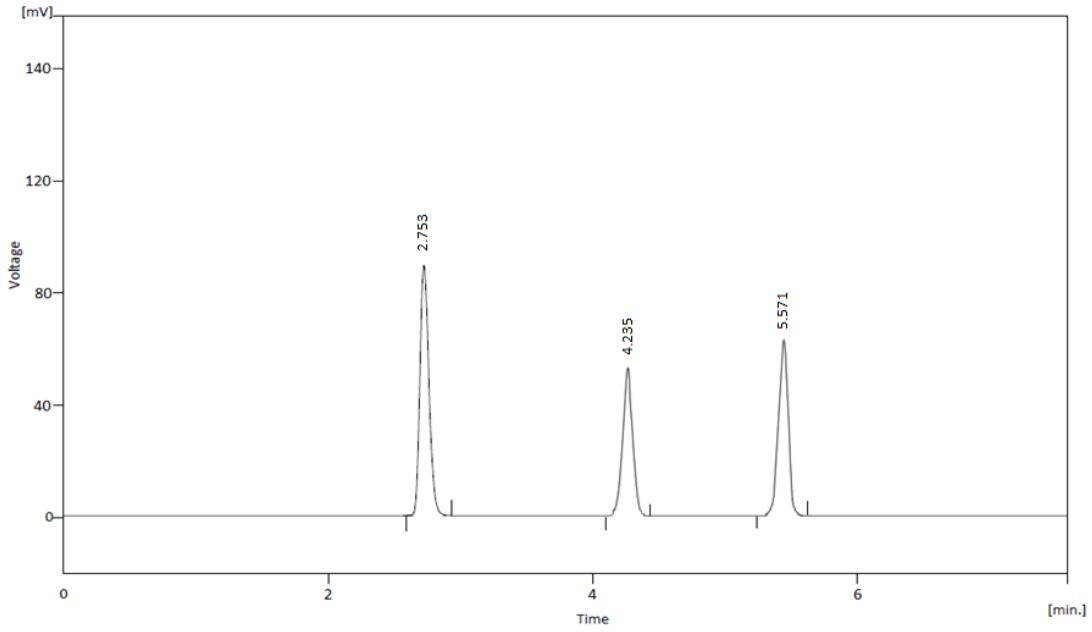

Figure 14.6: Chromatogram of Sample AMOX, CLAR & VONO

17.5.2 Stability & Forced Degradation Studies

Stability studies of drug substances under forced degradation by acid, base, thermal, oxidative and photo degradation has been successively carried out for the working standard drugs AMOX, CLAR & VONO and for the marketed formulation sample VOQUEZNA^{TM}. Developed analytical HPLC method is competent to detect and quantify main peaks of the drugs, along with impurities, degraded products effectively without any interference or overlapping of other peaks. The chromatograms of drugs in different degradation conditions are shown below.

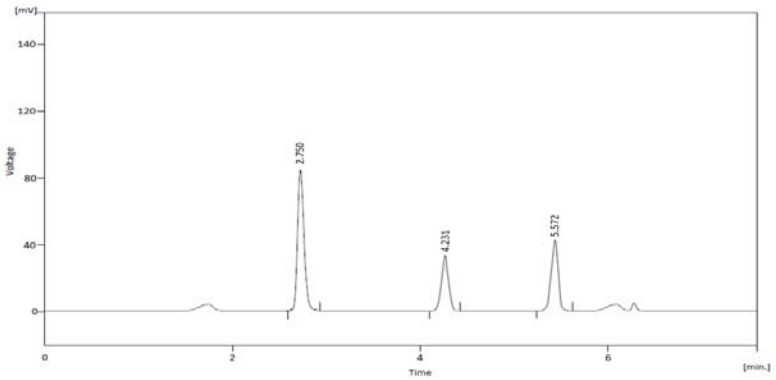

Figure 14.7: Chromatogram of Acid Degradation Standard AMOX, CLAR & VONO

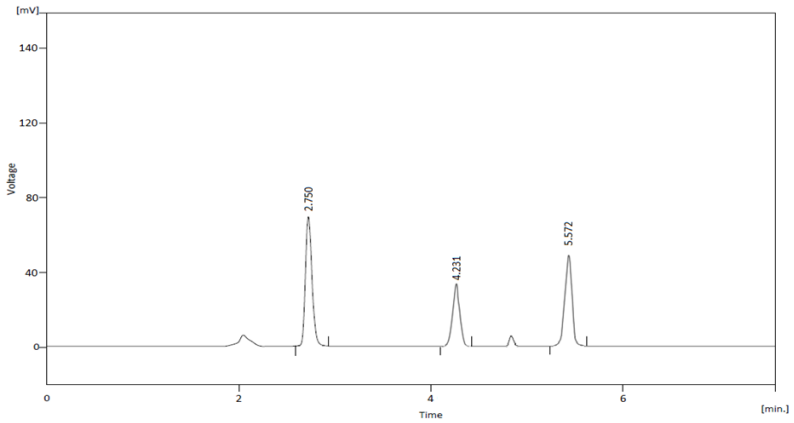

Figure 14.8: Chromatogram of Base Degradation Standard AMOX, CLAR & VONO

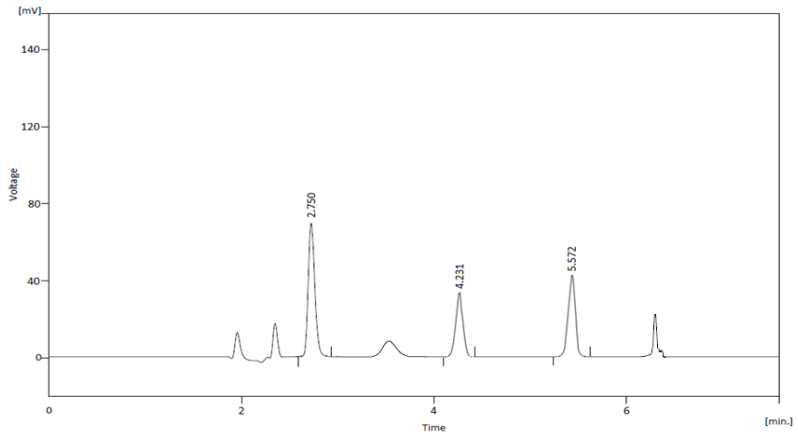

Figure 14.9: Chromatogram of Oxidative Degradation Standard AMOX, CLAR & VONO

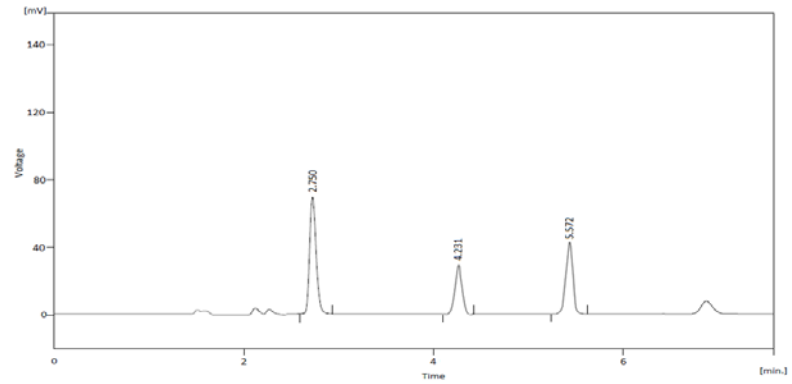

Figure 14.10: Chromatogram of Thermal Degradation Standard AMOX, CLAR & VONO

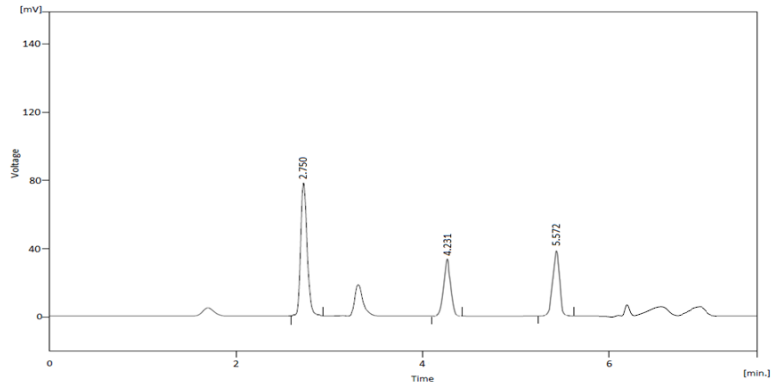

Figure 14.11: Chromatogram of Photo Degradation Standard AMOX, CLAR & VONO

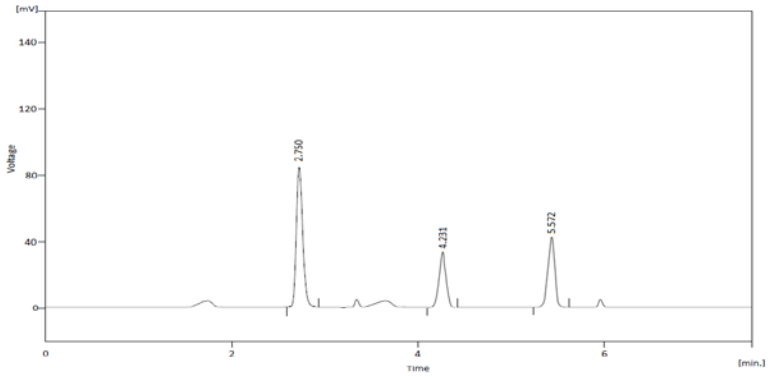

Figure 14.12: Chromatogram of Acid Degradation Sample AMOX, CLAR & VONO

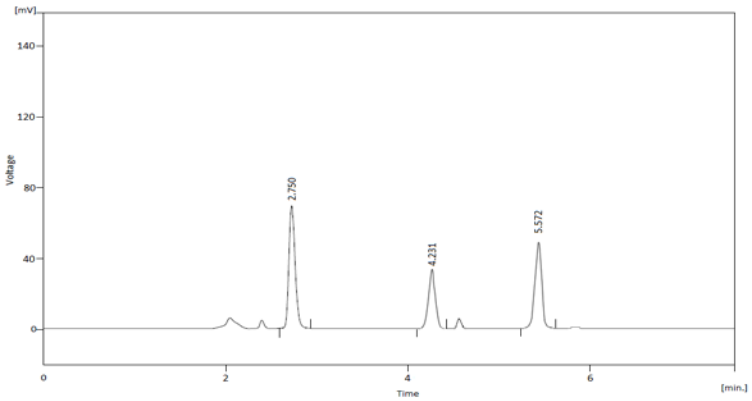

Figure 14.13: Chromatogram of Base Degradation Sample AMOX, CLAR & VONO

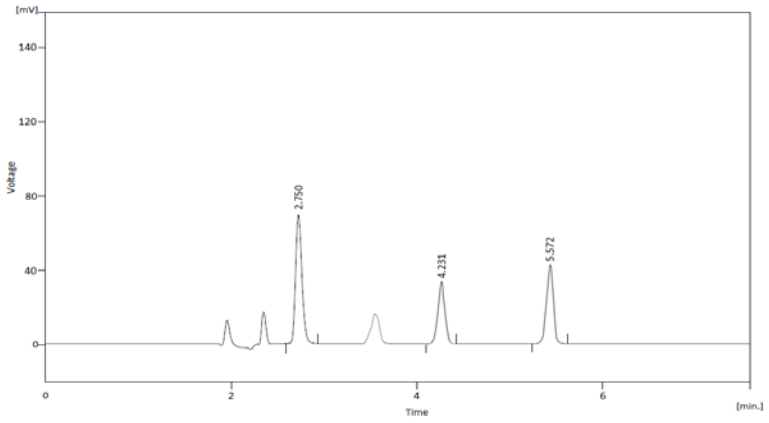

Figure 14.14: Chromatogram of Oxidative Degradation Sample AMOX, CLAR & VONO

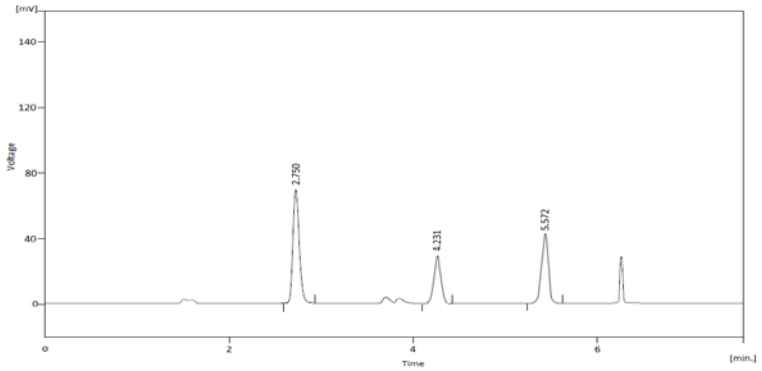

Figure 14.15: Chromatogram of Thermal Degradation Sample AMOX, CLAR & VONO

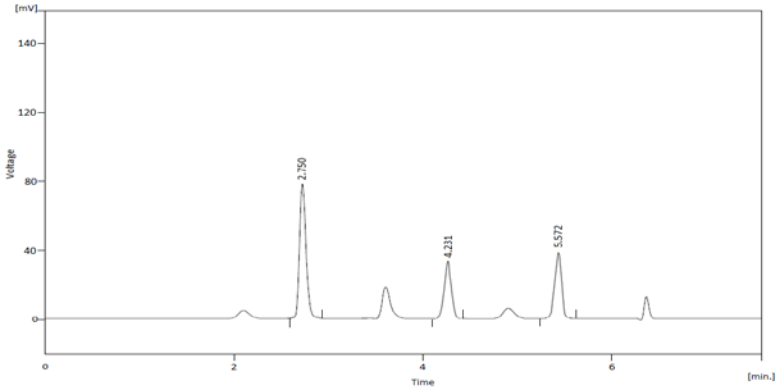

Figure 14.16: Chromatogram of Photo Degradation Sample AMOX, CLAR & VONO

Degradation Condition	**Peak Area**			**% Drug Recovered**			**% Degraded**		
	AMOX	CLAR	VONO	AMOX	CLAR	VONO	AMOX	CLAR	VONO
Acid	1129.63	511.46	613.46	98.55	78.05	75.05	1.44	21.94	24.94
Base	986.36	639.33	791.21	86.05	97.57	96.79	13.94	2.42	3.20
Oxidative	998.26	534.29	658.19	87.09	81.54	80.52	12.90	18.45	19.47
Thermal	1068.28	451.73	712.09	93.19	68.94	87.11	6.80	31.05	12.88
Photo	1133.55	551.07	678.25	98.89	84.10	82.97	1.10	15.89	17.02

Table 14.7: % Drug Degraded & % Drug Recovered AMOX, CLAR & VONO

PEAK PURITY				
Drug	**Stress Type**	**Peak Purity Angle**	**Peak Purity Threshold**	**Peak Purity**
AMOX	Untreated Sample	0.101	0.397	0.999
	Acid	0.128	0.256	0.999
	Base	0.263	0.374	0.996
	Oxidative	0.234	0.383	0.997
	Photo	0.117	0.317	0.999
	Thermal	0.149	0.324	0.997
CLAR	Untreated Sample	0.214	0.416	0.999
	Acid	0.236	0.371	0.997
	Base	0.226	0.399	0.998
	Oxidative	0.224	0.369	0.996
	Photo	0.231	0.402	0.998
	Thermal	0.237	0.381	0.996
VONO	Untreated Sample	0.113	0.348	0.999
	Acid	0.233	0.319	0.997
	Base	0.142	0.283	0.999
	Oxidative	0.232	0.311	0.997
	Photo	0.218	0.337	0.998
	Thermal	0.212	0.324	0.998

Table 14.8: Peak Purity for AMOX, CLAR & VONO

17.5.3 Method Validation

17.5.3.1 Specificity

Developed method is specific and selective as the no other peaks of, mobile phase or any excipients impurities were interfering or overlapping in the chromatograms.

The method effectively analyses the drugs in pure form as well as in the marketed formulations with accuracy, and has reproducible results for individual drugs as well as for the combined formulation analysis.

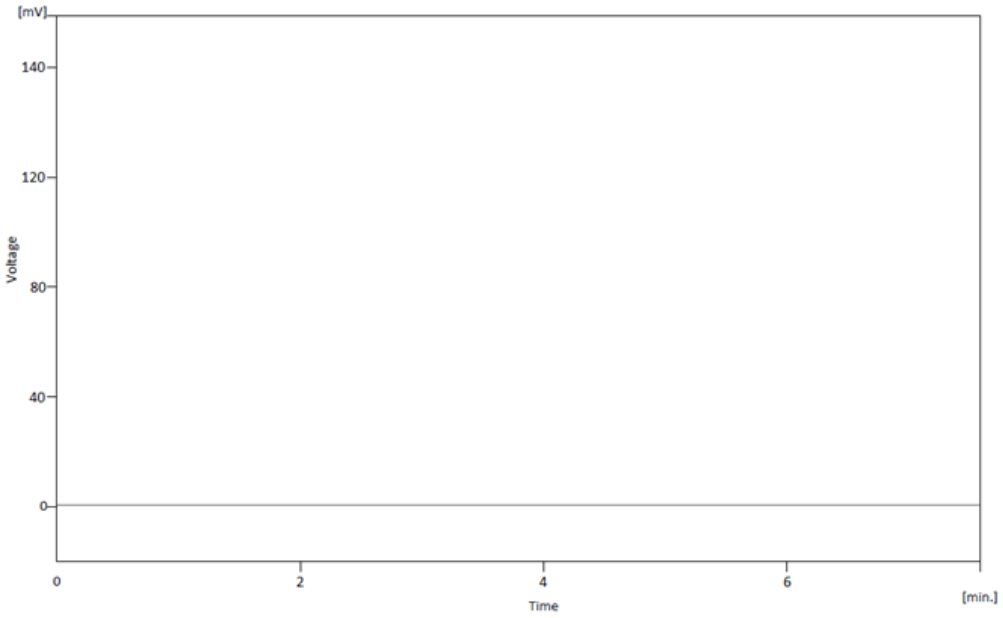

Figure 14.17: Blank Chromatogram

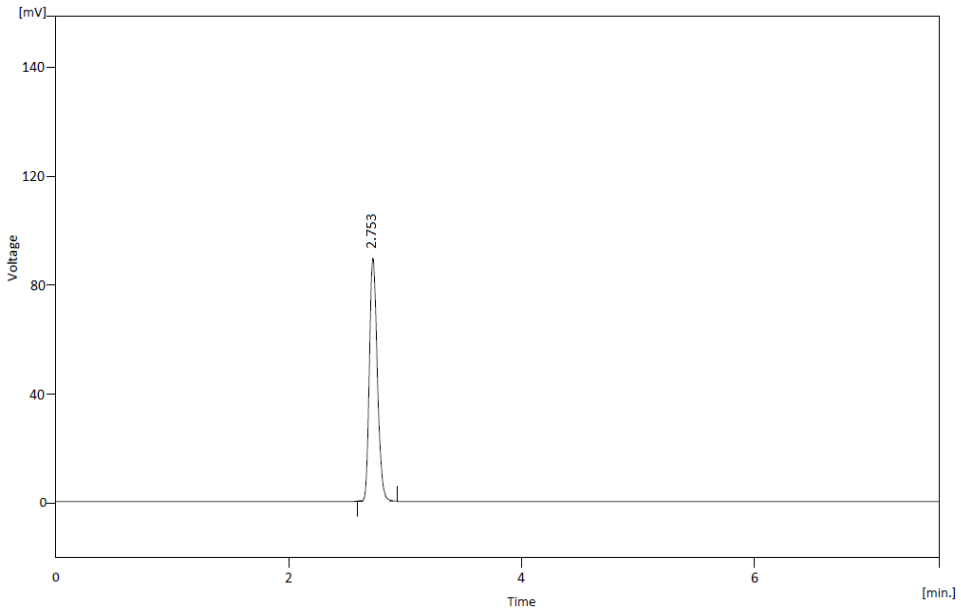

Figure 14.18: Chromatogram of AMOX

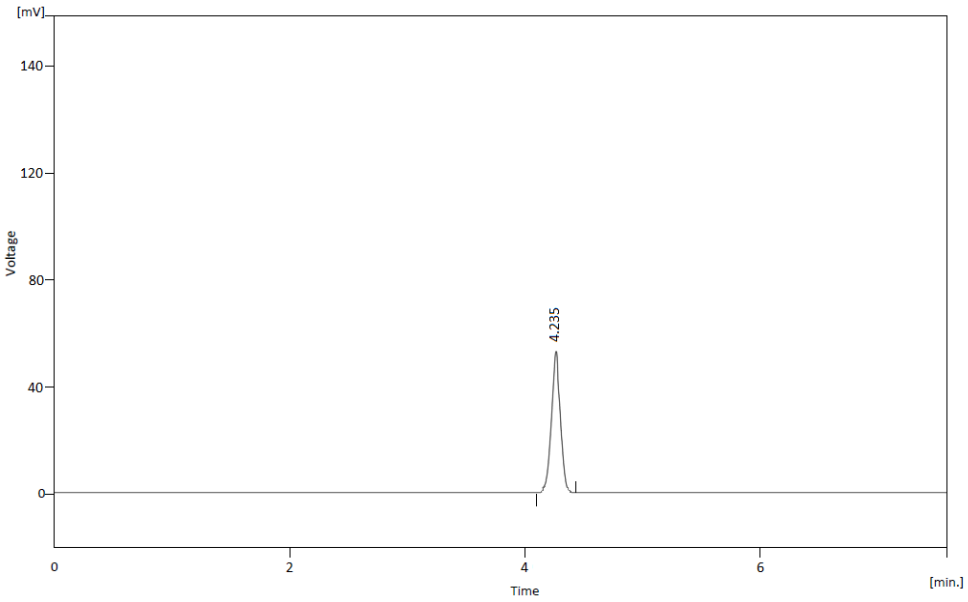

Figure 14.19: Chromatogram of CLAR

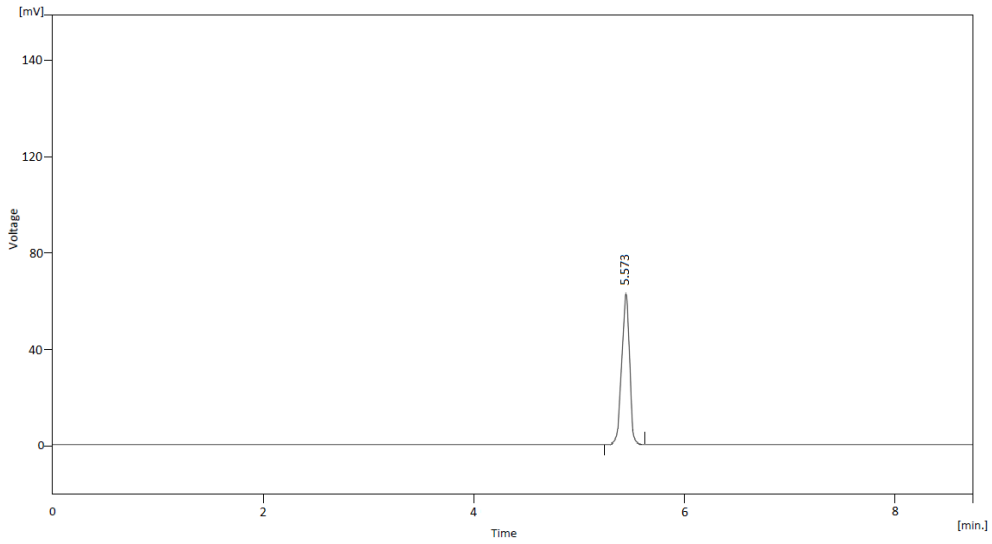

Figure 14.20: Chromatogram of VONO

17.5.3.2 Linearity and Range (n = 5)

Drugs AMOX, CLAR and VONO Linearity has been followed in a particular concentration ranges of 12.5-75ug/ml for AMOX, CLAR both drugs, and 5-30ug/ml for VONO. The linearity showing overlain chromatogram had been generated and the calibration curve been plotted of peak area vs conc. and straight line eqn. and correlation coefficient had been calculated.

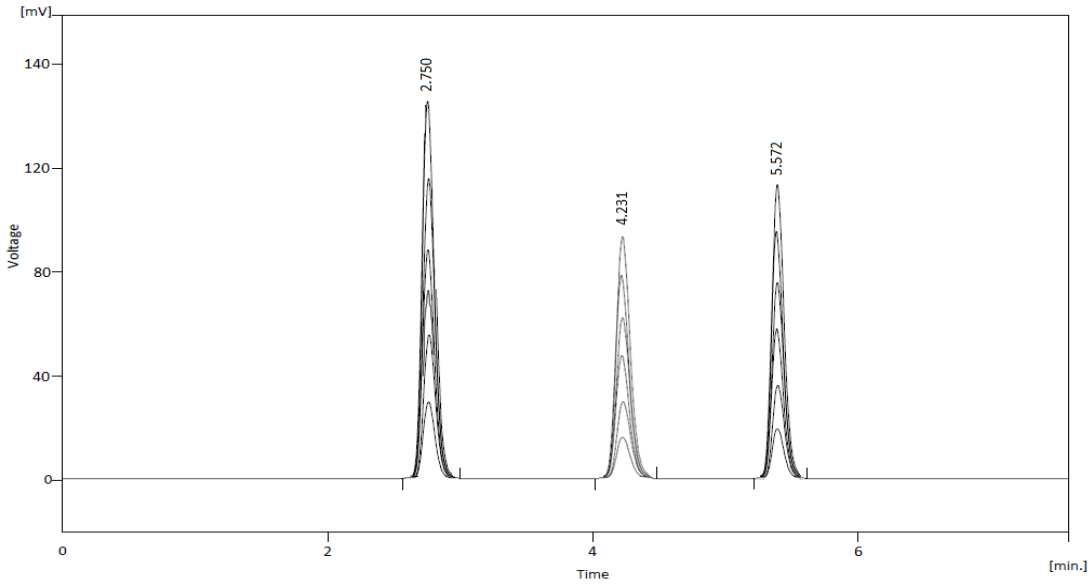

Figure 14.21: Overlain Chromatogram of Linearity for AMOX, CLAR & VONO

(x) Conc. µg/ml	(y) Area
12.5	285.64
25	565.89
37.5	845.18
50	1148.91
62.5	1423.67
75	1689.92
STD ERROR	10.04
Slope	22.62
LOD	1.46
LOQ	4.44

Table 14.9: Linearity data of AMOX

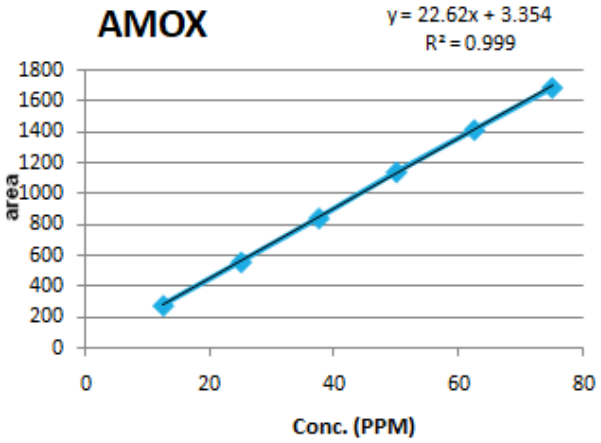

Figure 14.22: Calibration Curve for AMOX

(x) Conc. µg/ml	(y) Area
12.5	165.29
25	339.41
37.5	488.25
50	659.73
62.5	826.17
75	989.07
STD ERROR	5.62
Slope	13.14
LOD	1.41
LOQ	4.28

Table 14.10: Linearity data of CLAR

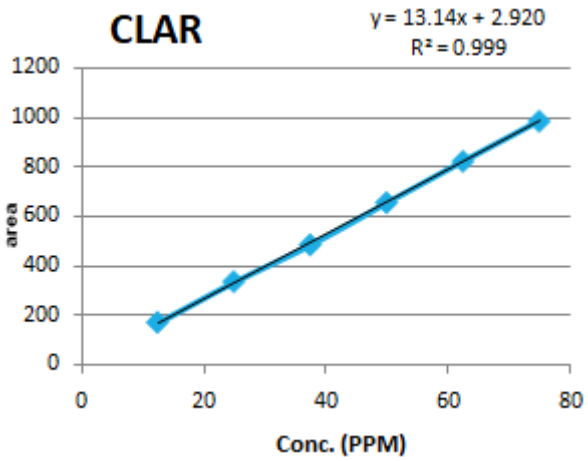

Figure 14.23: Calibration Curve for CLAR

(x) Conc. µg/ml	(y) Area
5	224.36
10	419.76
15	619.17
20	821.58
25	1026.47
30	1223.05
STD ERROR	2.96
Slope	40.09
LOD	0.244
LOQ	0.740

Table 14.11: Linearity data of VONO

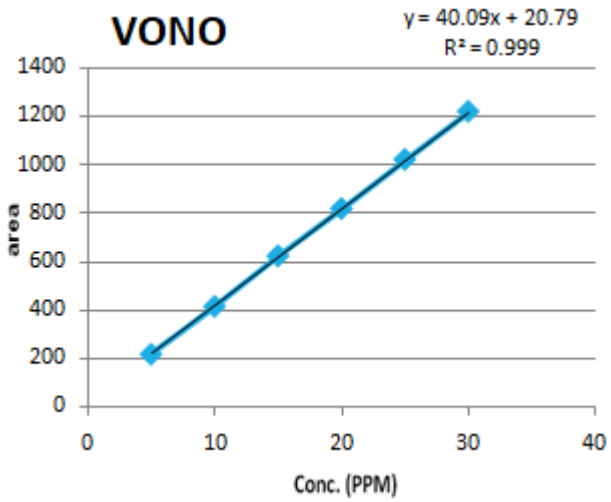

Figure 14.24: Calibration Curve for VONO

17.5.3.3 Accuracy (Recovery Studies) (n = 3)

The accuracy has been done by performing the recovery studies of the working standard drug from the pre-analysed sample of the drugs AMOX, CLAR and VONO. The recovered drug from the samples has been calculated as % Recovery is been reported in the table below.

Drug	Amt of Sample Taken (µg)	% Amt of Std Added	Spiked Std Drug Amount (µg)	Spiked Amt Recovered Mean (µg)	% Recovery	% Mean Recovery
AMOX	50	50	25	25.48	101.92	101.49
	50	100	50	50.54	101.09	
	50	150	75	76.09	101.45	
CLAR	50	50	25	24.47	97.88	99.03
	50	100	50	49.84	99.68	
	50	150	75	74.65	99.53	
VONO	20	50	10	10.08	100.84	100.40
	20	100	20	20.10	100.50	
	20	150	30	29.96	99.87	

Table 14.12: Accuracy Study of AMOX, CLAR & VONO (n = 3)

17.5.3.4 Precision

17.5.3.4.1 Repeatability (n = 6)

The repeatability study of AMOX, CLAR & VONO have been performed by multiple injections of the samples of the drugs (n = 6). The repeatability data for the AMOX, CLAR & VONO is shown in the table below.

Conc. of AMOX (µg/ml)	Area	Conc. of CLAR (µg/ml)	Area	Conc. of VONO (µg/ml)	Area
50	1146.51	50	658.93	20	819.78
	1154.98		655.87		822.53
	1148.19		650.45		816.72
	1143.87		651.85		819.51
	1153.71		653.27		828.34
	1145.68		658.19		817.56
Mean	1148.82	Mean	654.76	Mean	820.74
SD	4.51	SD	3.45	SD	4.23
% RSD	0.39	% RSD	0.52	% RSD	0.51

Table 14.13: Repeatability Study of AMOX, CLAR & VONO (n = 6)

17.5.3.4.2 Intraday Precision (n = 3)

The Intraday precision for the AMOX, CLAR & VONO has been performed by taking multiple injections (n = 3) in a same day at different 25, 100, 150 % Levels. The data for the intraday precision is shown in table below.

AMOX			CLAR			VONO		
Conc. (µg/ml)	Mean area ± SD	% RSD	Conc. (µg/ml)	Mean area ± SD	% RSD	Conc. (µg/ml)	Mean area ± SD	% RSD
12.5	285.2 ± 2.7	0.97	12.5	166.9 ± 2.8	1.68	5	224.3 ± 1.78	1.78
50	1149.5 ± 3.1	0.27	50	657.0 ± 3.6	0.56	20	814.9 ± 2.6	0.32
75	1692.4 ± 3.2	0.19	75	992.8 ± 3.7	0.37	30	1217.6 ± 5.0	0.41

Table 14.14: Intraday Precision of AMOX, CLAR & VONO (n = 3)

17.5.3.4.3 Interday Precision (n = 3)

The Interday precision for the AMOX, CLAR & VONO has been performed by taking multiple injections (n = 3) in different day at different 25, 100, 150 % Levels. The data for the intraday precision is shown in table below.

AMOX			CLAR			VONO		
Conc. (µg/ml)	**Mean area ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean area ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean area ± SD**	**% RSD**
12.5	284.0 ± 3.4	1.20	12.5	169.3 ± 2.5	1.48	5	221.3 ± 2.0	0.84
50	1151.7 ± 2.3	0.20	50	658.0 ± 5.8	0.89	20	813.8 ± 2.9	0.36
75	1688.0 ± 3.6	0.21	75	990.4 ± 3.9	0.40	30	1221.6 ± 3.6	0.29

Table 14.15: Interday Precision of AMOX, CLAR & VONO (n = 3)

17.5.3.5 LOD and LOQ

It has been calculated from the n=5 samples from the calibration curve slope and standard deviation. The LOD value are found to be 1.46 1.41, and 0.244 ug respectively for AMOX, CLAR and VONO, and the LOQ values are found to be 4.44, 4.28 and 0.740 ug respectively for AMOX, CLAR and VONO.

17.5.4 Application of the Developed Analytical Method to Formulation

The proposed analytical method been tested in assay analysis % Assay of the Label claim on the VOQUEZNA^{TM} triple pack containing AMOX 500mg, CLAR 500mg & VONO 20mg. Analytical method successfully applied to the estimation of drugs i n marketed product by comparing with the standard and the sample formulation. The assay result are shown in the table below.

	Serial no	Label claim (mg)	Result (mg)	% Label Claim	Avg % Assay	SD	% RSD
AMOX	1	500	490.58	98.12	98.02	0.33	0.34
	2	500	488.22	97.64			
	3	500	491.44	98.29			
CLAR	1	500	489.73	97.95	97.93	0.24	0.24
	2	500	488.41	97.68			
	3	500	490.80	98.16			
VONO	1	20	19.90	99.51	99.94	0.44	0.44
	2	20	19.99	99.94			
	3	20	20.08	100.39			

Table 14.16: Assay of Formulation VOQUEZNA^{TM} (n = 3)

17.5.5 Summary of Results

Sr No	**Parameters**	**Results**		
		AMOX	**CLAR**	**VONO**
1	System Suitability:			
	Theoretical plates-	4136	6371	7289
	Tailing Factor-	1.123	1.314	1.227
	Retention time min-	2.751	4.230	5.573
2	Precision (%RSD)	0.27	0.56	0.32
3	Linearity (R^2)	0.9998	0.9996	0.9999
4	Accuracy (% Recovery)	101.49	99.03	100.40
5	LOD (ug/ml)	1.46	1.41	0.244
6	LOQ (ug/ml)	4.44	4.28	0.740
7	% Assay	99.08	97.93	99.94

17.6 CONCLUSIONS

The Stability Indicating HPLC method for AMOX, CLAR & VONO combinational tripple pack drugs has been successfully developed and validated. The analytical method is optimized for the testing even in degraded conditions and analysis for AMOX, CLAR & VONO in individual as well in combined forms and all the validation parameters are performed in the acceptance criteria as per ICH regulatory guideline. Developed method is accurate., & precise to detect the main drug peaks without any interference or overlap of degraded impurities & products produced during forced degradation stress conditions. The accurate precise metho d can be used for analysis of AMOX, CLAR & VONO combination as well as individual in as Assay method and dissolution testing procedures in academics, research, analytical laboratories and in pharmaceutical industries.