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Development and validation of stability indicating rp-hplc method for the estimation of amoxicillin, omeprazole and rifabutin in bulk and formulation and its applications in dissolution studies

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Abstract*:* **Aim and objective:** The purpose of this research work was to Develop and validate a sensitive and accurate stability indicating RP-HPLC method for the estimation of Amoxicillin, Omeprazole and Rifabutin in bulk and formulation and its applications in dissolution studies.

Methods: Separation of ALN, RBN & OMP was conceded using 5.0 µm C18, Waters column (at 27 °C), with 60:40 vol/vol mix ratio, NaHSO4 (0.1M, pH 4.5) & methanol like mobile phase and movement rate of 1.0 ml/min, sensed at 246 nm.

Results: Retention times of ACN, RFN and OPE were 2.122 min, 2.844 and 3.93 min, respectively. Method linearity scope was ranged from 125 – 375 µg/ml for ACN, 5 – 15 µg/ml for OPE and 6.25 – 18.75 µg/ml for RFN. The accuracy was computed in the range of 98.13–101.07% and the precision was between 0.282% and 0.569% relative standard deviation for three drugs. The method can effectively separate the degradation products from ACN, RFN and OPE.

Key words- RP-HPLC, amoxicillin, omeprazole, rifabutin, dissolution studies.

Introduction:

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. The current good manufacturing practice (CGMP) and Food Drug Administration (FDA) Guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Development of a method of analysis is usually based on prior art (or) existing literature, using the same (or) quite similar instrumentation.

It is rare today that an HPLC-based method is developed that does not in same way relate (or) compare to existing, literature based approaches. Today HPLC (High performance liquid chromatography) is the method of choice used by the pharmaceutical industry to assay the intact drug and degradation products. The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. UV spectroscopy is also a simple analytical tool widely used for routine assay of drugs. Hence for the assay of the selected drugs HPLC and UV spectroscopy has been chosen for these proposed methods.

Stomach ulcers, commonly referred as gastric ulcers, are severe lesions on the lining of the stomach Peptic ulcer illness includes stomach ulcers. Any ulcer which attacks stomach as well as the small intestine is known as a peptic ulcer.

The exact mechanism by which this bacterium seeps is unknown, but experts think it is primarily through contaminated water, food, & eating utensils. People who are infected with H. pylori can potentially transmit the bacteria by coming into close encounter of saliva. For many, this bacterial infection occurs in childhood but only in rare case it converts into peptic ulcer. In reality, the symptom are not exhibited till their old age.

Talicia is a fully - featured oral capsule that contains amoxicillin (ALN), rifabutin (RBN), plus omeprazole in a fixed-dose formulation (OMP). Antibiotics are ALN and RBN [17,18]. The proton pump blocker is OMP. ALN works by the cell wall biosynthesis inhibition which results in bacterial death. RBN hinders DNA-based RNA polymerase in liable microorganisms nonetheless in cells of mammalian. OMP blocks production of acid in stomach. Every capsule contains 250 mg ALN, 10 mg OMP, and 12.5 mg RBN.

The endorsement of Talicia by FDA was grounded, in fragment, on the fallouts of dual positive Phase 3 works in the U.S. to treat H. pylori-positive in adult suffering from epigastric pain & discomfort [19]. Talicia is particularly signposted in treating Helicobacter

pylori infection. In order to lessen drug-resistant bacteria production plus uphold the efficiency of Talicia besides supplementary antibacterial drugs, Talicia ought to be employed in preventing and treating proven infections or strongly supposed to be instigated by vulnerable bacteria.

Materials and method:

Apparatus: HPLC device set with detector photodiode array detector (make-"Waters Alliance company"),Version two empower software (make - "Waters Alliance company"),Hot air oven, Membrane filter having 0.45 µm dimension pore, Sonicator.

MATERIALS: Amoxicillin (ALN), Rifabutin (RBN), Omeprazole (OMP), NaH2PO4, NaHSO4, Phosphoric acid, Peroxide, Methanol, Hydrochloric acid, Sodium hydroxide

Columns used: Aligent C18 , Sunsil C18, Kromasil C18, Waters C18.

Conditions for ACN, OPE and RFN Combined Assay:

Waters C18 (250 × 4.6 mm, particle dimension of 5 µm) column set with 25°C temperature was used with an isocratic mobile phase having a flow at 1.0 ml/min rate. Mobile phase A was 0.1M NaHSO4 buffer. The buffer was fine-tuned to pH 4.5 units. The mobile phase B was methanol. Mobile phase A and B are mixed in 60:40 volume/volume ratio for analysis. Before using, mobile phase mixture was filtered through membrane filters of 0.45 pore size. 10µl of sample was employed for the analysis. Photodiode array detector fine-tuned to 246 nm was employed for the ACN, OPE and RFN combined analyses.

STOCK ALN, RBN & OMP SOLUTION:

Stock ALN, RBN & OMP solution was made with ALN quantity 2500 µg/ml, RBN quantity 125 µg/ml and OMP quantity 100 µg/ml.

CALIBRATION ALN, RBN & OMP SOLUTIONS:

Concentrations with five varied calibration ALN, RBN & OMP solutions were arranged:

- Solution one amounts: ALN - 125 µg/ml, RBN – 6.25 µg/ml & OMP - 5 µg/ml.
- Solution two amounts: ALN – 187.50 µg/ml, RBN – 9.375 µg/ml & OMP – 7.5 µg/ml.
- Solution three amounts: ALN - 250 µg/ml, RBN – 12.5 µg/ml & OMP - 10 µg/ml.
- Solution four amounts: ALN – 312.5 µg/ml, RBN – 15.625 µg/ml & OMP – 12.5 µg/ml.
- Solution five amounts: ALN - 375 µg/ml, RBN – 18.75 µg/ml & OMP - 15 µg/ml.

WORKING ALN, RBN & OMP SOLUTIONS:

Working ALN, RBN & OMP solution was made with ALN quantity 250 µg/ml, RBN quantity 12.5 µg/ml and OMP quantity 10.0 µg/ml from stock ALN, RBN & OMP solution (ALN - 2500 µg/ml, RBN - 125 µg/ml and OMP - 100 µg/ml) by mode of dilution correctly using diluent.

FORMULATION ALN, RBN & OMP CAPSULE SOLUTION:

Emptied 10 Talicia capsules and powder were mixed. A powder amount equal to ALN quantity 250 mg, RBN quantity 12.5 mg and OMP quantity 10.0 mg was stirred with 50 ml quantity diluent in 100 ml flask. The contents of ALN, RBN & OMP were admixed, 30 min ultrasonicated, and by mode of dilution (100 ml mark) correctly using diluent. The formulation ALN, RBN & OMP capsule solution was thoroughly agitated for 10 minutes prior being filtered using a membrane filtration. This was formulation stock ALN, RBN & OMP capsule solution with ALN quantity 2500 µg/ml, RBN quantity 125 µg/ml and OMP quantity 100 µg/ml. The formulation working ALN, RBN & OMP capsule solution was made with ALN quantity 250 µg/ml, RBN quantity 12.5 µg/ml and OMP quantity 10.0 µg/ml from formulation stock ALN, RBN & OMP solution (ALN - 250 µg/ml, RBN - 12.5 µg/ml and OMP - 10.0 µg/ml) by mode of dilution correctly using diluent.

INVESTIGATION OF ALN, RBN & OMP CONTENT IN CAPSULE FORMULATION:

The formulation working ALN, RBN & OMP capsule solution processed in above segment ("FORMULATION ALN, RBN & OMP CAPSULE SOLUTION") was assessed using the settings stipulated in segment "SETTINGS FOR ALN, RBN & OMP COMBINED HPLC MEASUREMENT". The ALN, RBN, & OMP peak responses were noted. The contents of ALN, RBN, & OMP in capsule formulation were judged reliant on the peak response reached.

METHOD VALIDATION

The proposed methodology was verified in keeping with "International Conference on Harmonization" strategies

Linearity

Combined stock solution (2500 µg/ml - ACN; 100 µg/ml - OPE; 125 µg/ ml - RFN) was diluted serially to obtain solutions in the concentration scope of 125 – 375 µg/ml for ACN, 5 – 15 µg/ml for OPE and 6.25 – 18.75 µg/ml for RFN. Each concentration solution was analysed by using the proposed method. Calibration curves of ACN, OPE and RFN were generated by determining peak area of each analyte and their respective concentrations. The regression line equations for ACN, OPE and RFN were established.

LOQ and LOD : Both the LOQ and the LOD were calculated using a signal-to - noise concept. LOQ was described as the minimal level of quantity of analyte leading to a peak height of ten times the baseline noise (i.e signal-to-noise ratio is ten). LOD was described as the minimal level of quantity of analyte leading to a peak height of three times the baseline noise (i.e signal-to-noise ratio is three).

Precision

Precision was obtained by the assessment of combined working solution (250 µg/ml - ACN; 10 µg/ml - OPE; 12.5 µg/ml -RFN) on the same day in six replicates. Determined the ACN, OPE and RFN mean peak

Accuracy

The accuracy was assessed using standard technique of addition. In this technique, previously analysed placebo solution was spiked with extra 50% (125 µg/ml - ACN; 5 µg/ml - OPE; 6.25 µg/ml - RFN), 100% (250 µg/ml - ACN; 10 µg/ml - OPE; 12.5 µg/ml - RFN) and 150% (375 µg/ ml - ACN; 15 µg/ml - OPE; 18.75 µg/ml - RFN) contents of analytes. rea values and relative standard deviation values of ACN, OPE and RFN peak areas.

ROBUSTNESS:

The working ALN, RBN & OMP capsule solution (ALN - 250 µg/ml, RBN – 12.5 µg/ml and OMP – 10.0 µg/ml) was appraised via consciously fluctuating the chromatographic settings stipulated in segment "SETTINGS FOR ALN, RBN & OMP COMBINED HPLC MEASUREMENT"

STABILITY OF ALN, RBN & OMP:

The formulation stock ALN, RBN & OMP capsule solution with ALN quantity 2500 µg/ml, RBN quantity 125 µg/ml and OMP quantity 100 µg/ml was stressed out consistent using ICH directions with situations like [50]: Acid hydrolysis, Base hydrolysis, Dry heat lysis, Oxidation, Sun light lysis

STABILITY OF ALN, RBN & OMP IN 0.1 N HCl:

Aliquot of ten ml of formulation stock ALN, RBN & OMP capsule solution (ALN - 2500 µg/ml, RBN - 125 µg/ml and OMP - 100 µg/ml) was stirred with 10 ml quantity HCl (0.1N) in 100 ml flask. The contents of ALN, RBN & OMP and HCl were admixed, 30 min ultrasonicated in room temperature, and by mode of dilution (100 ml mark) correctly using diluent. The stressed formulation ALN, RBN & OMP capsule solution was thoroughly agitated for 10 minutes prior being filtered using a membrane filtration. The stressed formulation ALN, RBN & OMP capsule solution processed in above was assessed using the settings stipulated in segment "SETTINGS FOR ALN, RBN & OMP COMBINED HPLC MEASUREMENT". The ALN, RBN, & OMP peak responses were noted. The contents of ALN, RBN, & OMP in capsule formulation remained were judged reliant on peak response reached.

STABILITY OF ALN, RBN & OMP IN 0.1 N NaOH:

Aliquot of ten ml of formulation stock ALN, RBN & OMP capsule solution (ALN - 2500 µg/ml, RBN - 125 µg/ml and OMP - 100 µg/ml) was stirred with 10 ml quantity NaOH (0.1N) in 100 ml flask. The contents of ALN, RBN & OMP and HCl were admixed, 30 min ultrasonicated in room temperature, and by mode of dilution (100 ml mark) correctly using diluent. The stressed formulation ALN, RBN & OMP capsule solution was thoroughly agitated for 10 minutes prior being filtered using a membrane filtration. The stressed formulation ALN, RBN & OMP capsule solution processed in above was assessed using the settings stipulated in segment "SETTINGS FOR ALN, RBN & OMP COMBINED HPLC MEASUREMENT". The ALN, RBN, & OMP peak responses were noted. The contents of ALN, RBN, & OMP in capsule formulation remained were judged reliant on peak response reached.

STABILITY OF ALN, RBN & OMP IN 30% PEROXIDE:

Aliquot of ten ml of formulation stock ALN, RBN & OMP capsule solution (ALN - 2500 µg/ml, RBN - 125 µg/ml and OMP - 100 µg/ml) was stirred with 10 ml quantity peroxide with 30% strength in 100 ml flask. The contents of ALN, RBN & OMP and HCl were admixed, 30 min ultrasonicated in room temperature, and by mode of dilution (100 ml mark) correctly using diluent. The stressed formulation ALN, RBN & OMP capsule solution was thoroughly agitated for 10 minutes prior being filtered using a membrane filtration. The stressed formulation ALN, RBN & OMP capsule solution processed in above was assessed using the settings stipulated in segment "SETTINGS FOR ALN, RBN & OMP COMBINED HPLC MEASUREMENT". The ALN, RBN, & OMP peak responses were noted. The contents of ALN, RBN, & OMP in capsule formulation remained were judged reliant on peak response reached.

STABILITY OF ALN, RBN & OMP IN 60 ºC:

Aliquot of ten ml of formulation stock ALN, RBN & OMP capsule solution (ALN - 2500 µg/ml, RBN - 125 µg/ml and OMP - 100 µg/ml) was uncovered to 60 ºC for ½ hr. The contents of ALN, RBN & OMP and HCl were admixed and by mode of dilution (100 ml mark) correctly using diluent. The stressed formulation ALN, RBN & OMP capsule solution was thoroughly agitated for 10 min prior being filtered using a membrane filtration. The stressed formulation ALN, RBN & OMP capsule solution processed in above was assessed using the settings stipulated in segment "SETTINGS FOR ALN, RBN & OMP COMBINED HPLC MEASUREMENT". The ALN, RBN, & OMP peak responses were noted. The contents of ALN, RBN, & OMP in capsule formulation remained were judged reliant on peak response reached.

STABILITY OF ALN, RBN & OMP IN SUN LIGHT:

Aliquot of ten ml of formulation stock ALN, RBN & OMP capsule solution (ALN - 2500 µg/ml, RBN - 125 µg/ml and OMP - 100 µg/ml) was uncovered to sun light for appro. 6 hr. The contents of ALN, RBN & OMP and HCl were admixed and by mode of dilution (100 ml mark) correctly using diluent. The stressed formulation ALN, RBN & OMP capsule solution was thoroughly agitated for 10 min prior being filtered using a membrane filtration. The stressed formulation ALN, RBN & OMP capsule solution processed in above was assessed using the settings stipulated in segment "SETTINGS FOR ALN, RBN & OMP COMBINED HPLC MEASUREMENT". The ALN, RBN, & OMP peak responses were noted. The contents of ALN, RBN, & OMP in capsule formulation remained were judged reliant on peak response reached.

DISSOLUTION STUDY:

DISSOLUTION MEDIA:

In a large beaker, weighed 12.006 gm of NaHSO4 and 0.9 gm of NaOH. Correct the pH adding HCl if required after disintegrating and diluting to volume (1000 ml) by water. Degas the material at 41 ºC for 10 min after processing to eradicate air bubbles. Then, place this medium into each of six-separate bowls, choose the appropriate schedule for the capsules, and pause on until bowl hits 37 °C. Hold the equipment and instantly release the capsules and start the tester once those bowls having reached the necessary temperature.

SPECIFICITY/STABILITY OF ALN, RBN & OMP/STABILITY INDICATING:

The formulation stock ALN, RBN & OMP capsule solution with ALN quantity 2500 µg/ml, RBN quantity 125 µg/ml and OMP quantity 100 µg/ml was stressed out consistent using ICH directions with situations like: Base hydrolysis, Dry heat lysis, Oxidation, Acid hydrolysis and Sun light lysis.

Statistical analysis

Statistical analysis during validation parameters study was performed by calculating standard deviation, relative standard deviation using Waters software Empower2 program.

RESULTS:

Optimized Method Conditions

Complete resolution between ACN, OPE and RFN were obtained by employing Waters C18 (250 × 4.6 mm, particle dimension of 5 µm) column set with 25°C temperature and with mobile phase system of 0.1M NaHSO4 buffer (fine-tuned to pH 4.5 units) – methanol (60%:40% by volume). Flow rate was 1.0 ml per min with 10 µl of sample was injected for one analysis. Quantification of ACN, OPE and RFN simultaneously was done with photodiode array detector fine-tuned to 246 nm. Typical chromatogram of ACN, OPE and RFN using optimized method conditions was displayed in Figure 1.

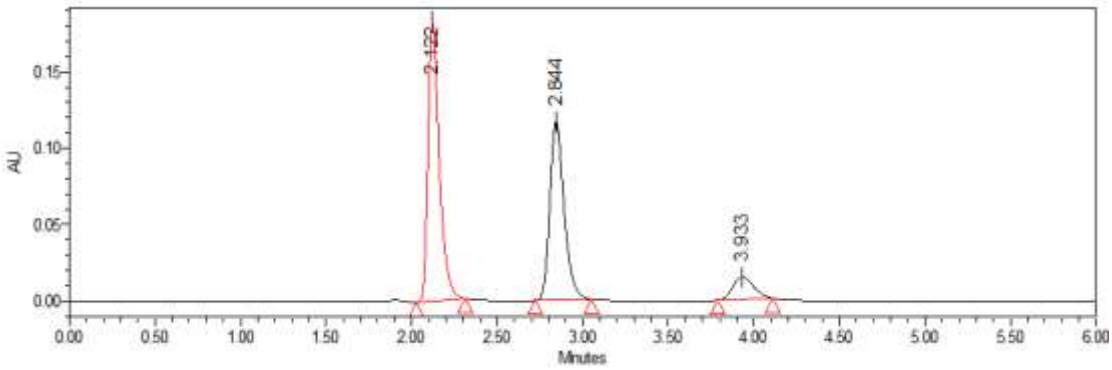


Fig-1

VALIDATION:

The chromatograms of placebo solution and blank mobile phase mixture solution and combined working solution (250 µg/ml - ACN; 10 µg/ml - OPE; 12.5 µg/ml -RFN) are presented in Figure2.

Linearity scope was 125 – 375 µg/ml for ACN, 5 – 15 µg/ml for OPE and 6.25 – 18.75 µg/ml for RFN. The obtained regression line equations along with regression coefficient were:

ALN linearity equation: $y = 3296.7 x + 10725$, $R^2 = 0.9999$

RBN linearity equation: $y = 53826x + 3867.2$, $R^2 = 0.9999$

OMP linearity equation: $y = 13072x + 1259.8$, $R^2 = 0.9998$,

DISPLAYED IN FIG 3

ALN		**RBN**		**OMP**	
Conc. (µg/ml)	**Response reached**	**Conc. (µg/ml)**	**Response reached**	**Conc. (µg/ml)**	**Response reached**
125	419996	6.25	338082	5	66395
187.50	629143	9.375	508591	7.50	99656
250.00	840780	12.5	678989	10.00	131946
312.5	1039506	15.625	848800	12.5	164549
375	1245019	18.75	1009012	15	197347

TABLE- 1 readings for linearity

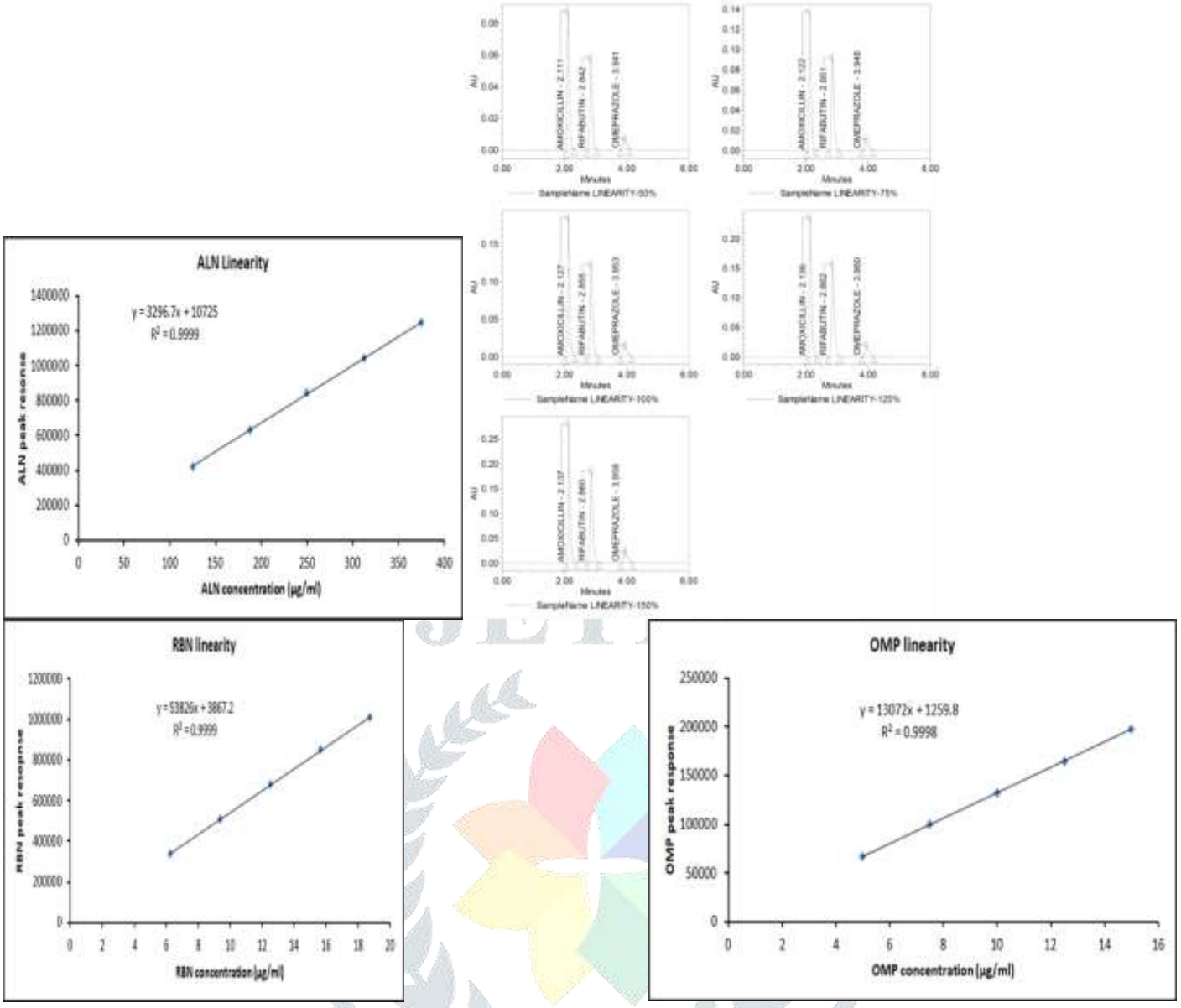


FIG-3 linearity chromatograms

(LOD)Detection limit for ALN, RBN & OMP were set on employing S/N proportion. Quantity of ALN, RBN & OMP that contributes S/N proportion estimate of ≥ 3 is ALN, RBN & OMP's detection limit.

- ALN's Detection limit – 0.300 µg/ml having S/N proportion estimate - 3.60
- RBN's Detection limit – 0.023 µg/ml having S/N proportion estimate - 3.80
- OMP's Detection limit – 0.144 µg/ml having S/N proportion estimate - 3.80 DISPLAYED IN FIG 4

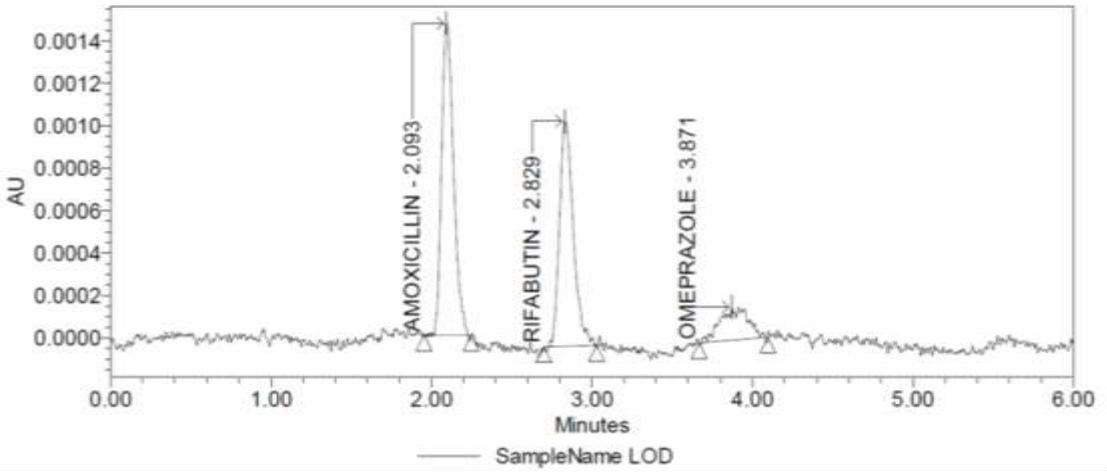


Figure 4 : LOD , ALN, RBN & OMP chromatogram

(LOQ)Quantitation limit for ALN, RBN & OMP were set on employing S/N proportion. Quantity of ALN, RBN & OMP that contributes S/N proportion estimate of ≥ 10 is ALN, RBN & OMP's quantitation limit.

- ALN's Quantitation limit – 1.00 µg/ml having S/N proportion estimate – 10.40
- RBN's Quantitation limit – 0.078 µg/ml having S/N proportion estimate – 10.20
- OMP's Quantitation limit – 0.485 µg/ml having S/N proportion estimate – 10.80

displayed in fig 5

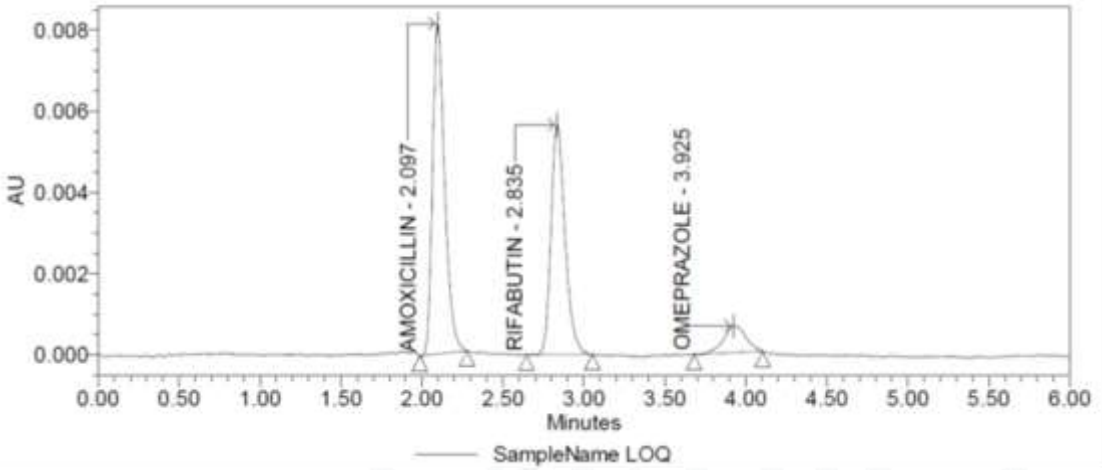


FIG-5 LOQ chromatograms

The mean peak area values were 840726 , 678839 and 131343 for ALN, RBN and OMP, respectively. The relative standard deviation values were 0.060 (ALN), 0.086 (RBN) and 0.801 (OMP). IN BELOW TABLE

ALN		**RBN**		**OMP**	
Response reached	**Statistics estimate**	**Response reached**	**Statistics estimate**	**Response reached**	**Statistics estimate**
840546	**Mean**	679755	**Mean**	131651	**Mean**
841477	840726	678275	678839	131895	131343
840355	**SD**	679279	**SD**	130007	**SD**
840889	507.1902	678804	586.88023	131643	1052.05394
841026	**RSD**	678665	**RSD**	130159	**RSD**
840066	0.060	678256	0.086	132706	0.801

table-2

The average recovery of ALN detected in the spiked placebo solution was 100.11%, 100.30% and 99.58% at 50%, 100% and 150% spiked levels, respectively (Table 1). The average recovery of RBN determined in the spiked placebo solution was 99.35% at 50% level spiked, 99.49% at 100% level spiked and 98.50% at 150% level spiked. The average recovery of OPE determined at 50%, 100% and 150% spiked levels in placebo soliton were 100.54%, 99.64% and 99.91%, respectively SHOWN BELOW,

Recovery reached for ALN

Spiked level	**Sample area**	**µg/ml added**	**µg/ml found**	**% recovery**	**% mean**
50%	**41917** **419345** **419983**	**123.750** **123.750** **123.750**	**123.78** **123.84** **124.03**	**100.02** **100.07** **100.22**	**100.11**
100%	**840186** **840065** **841497**	**247.500** **247.500** **247.500**	**248.12** **248.08** **248.51**	**100.25** **100.24** **100.41**	**100.30**

150%	**1249249** **124726** **1259141**	**371.250** **371.250** **371.250**	**368.92** **368.34** **371.84**	**98.69** **98.43** **98.39**	**99.58**	

Table 3.: Recovery readings for ALN

Table 4.: Recovery readings for RBN

Spiked level	Sample area	µg/ml added	µg/ml found	% recovery	% mean
50%	**338727** **338962** **338781**	**6.250** **6.250** **6.250**	**6.21** **6.21** **6.21**	**99.32** **99.39** **99.34**	**99.35**
100%	**678407** **678313** **679043**	**12.500** **12.500** **12.500**	**12.43** **12.43** **12.44**	**99.46** **99.45** **99.55**	**99.49**
150%	**1009702** **1007093** **1006680**	**18.750** **18.750** **18.750**	**18.50** **18.46** **18.45**	**98.69** **98.43** **98.39**	**98.50**

Table 5 : Recovery readings for OMP

Spiked level	Sample area	µg/ml added	µg/ml found	% recovery	% mean
50%	**66321** **66358** **66354**	**4.950** **4.950** **4.950**	**4.97** **4.98** **4.98**	**100.50** **100.56** **100.55**	**100.54**
100%	**131544** **131143** **131805**	**9.900** **9.900** **9.900**	**9.87** **9.84** **9.89**	**99.67** **99.37** **99.87**	**99.64**
150%	**198056** **197440** **197858**	**14.850** **14.850** **14.850**	**14.86** **14.81** **14.84**	**100.05** **99.73** **99.95**	**99.91**

The system suitability values achieved with modified conditions of assay for parameters like plate count, resolution and tailing factor for the peaks of ACN, OPE and RFN were disclosed in Table 2. In all modified conditions of assay, good segregation between ACN, OPE and RFN was achieved.

Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
ALN	2.122	837569	51.13	182812	-	1.49	5159
RBN	2.844	676070	41.27	116805	5.21	1.29	5730
OMP	3.933	124365	7.59	14655	5.61	1.14	4727

TABLE-6- system suitability parameters

Stability :

The formulation stock ALN, RBN & OMP capsule solution with ALN quantity 2500 µg/ml, RBN quantity 125 µg/ml and OMP quantity 100 µg/ml was stressed out consistent using ICH directions with situations like: Base hydrolysis, Dry heat lysis, Oxidation, Acid hydrolysis and Sun light lysis. The area, degradation percentile and assay of ALN, RBN & OMP were described in following table3 and fig:

Order of ALN's stability:

Sun > NaOH > peroxide > > 60 oC > HCl

Order of RBN's stability:

NaOH> Peroxide > HCl > sun light > 60 oC

Order of OMP's stability:

NaOH > Sun > Peroxide > HCl > 60 oC

Table: 7stability table for ALN

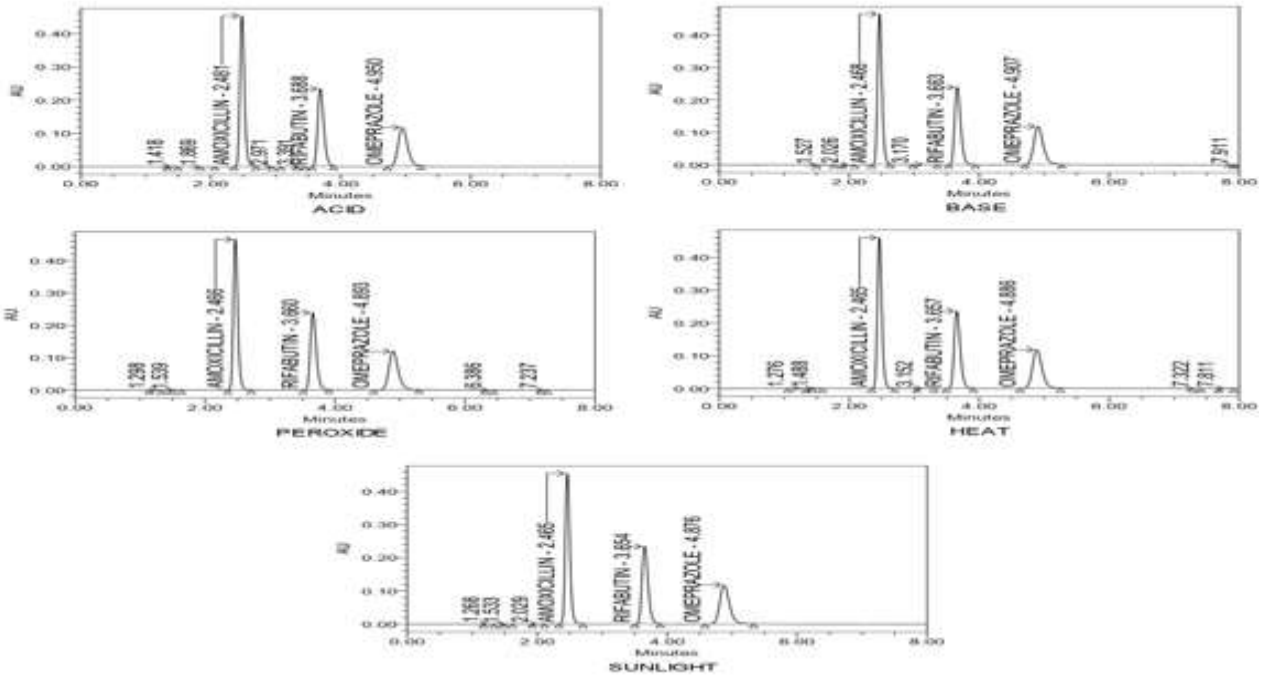
S.no	Conditions accelerated	area	% assay	% loss
1	0.1 N HCl	756921	89.41	10.59
2	0.1N NaOH	801365	94.66	5.34
3	Peroxide	770424	91.01	8.99
4	60 ℃	765071	90.38	9.62
5	Sun light	810696	95.76	4.24

Table: 8 stability table for RBN

S.no	Conditions accelerated	area	% assay	% loss
1	0.1 N HCl	630023	92.37	10.59
2	0.1N NaOH	650530	95.37	5.34
3	Peroxide	635243	93.13	8.99
4	60 oC	610351	89.48	9.62
5	Sun light	625218	91.66	4.24

Table: 9 stability table for OMP

S.no	Conditions accelerated	area	% assay	% loss
1	0.1 N HCl	120378	90.30	9.70
2	0.1N NaOH	125449	94.10	5.90
3	Peroxide	123368	92.54	7.46
4	60 oC	119718	89.80	10.20
5	Sun light	125223	93.93	6.07



Dissolution studies:

The dissolution formulation ALN, RBN & OMP capsule solution processed using the settings stipulated in segment "SETTINGS FOR DISSOLTUION TEST" was assessed using the settings stipulated in segment "SETTINGS FOR ALN, RBN & OMP COMBINED HPLC MEASUREMENT". The assay contents of ALN, RBN, & OMP in dissolution capsule formulation solution were set on BELOW TABLE -4,

Sample	ALN assay (%)	RBN assay (%)	OMP assay (%)
D1	99.29	99.66	98.76
D2	99.40	99.44	98.94
D3	99.27	99.59	97.52
D4	99.33	99.52	98.75
D5	99.35	99.50	97.64
D6	99.23	99.44	99.55

TABLE-10; DISSOLUTION READINGS

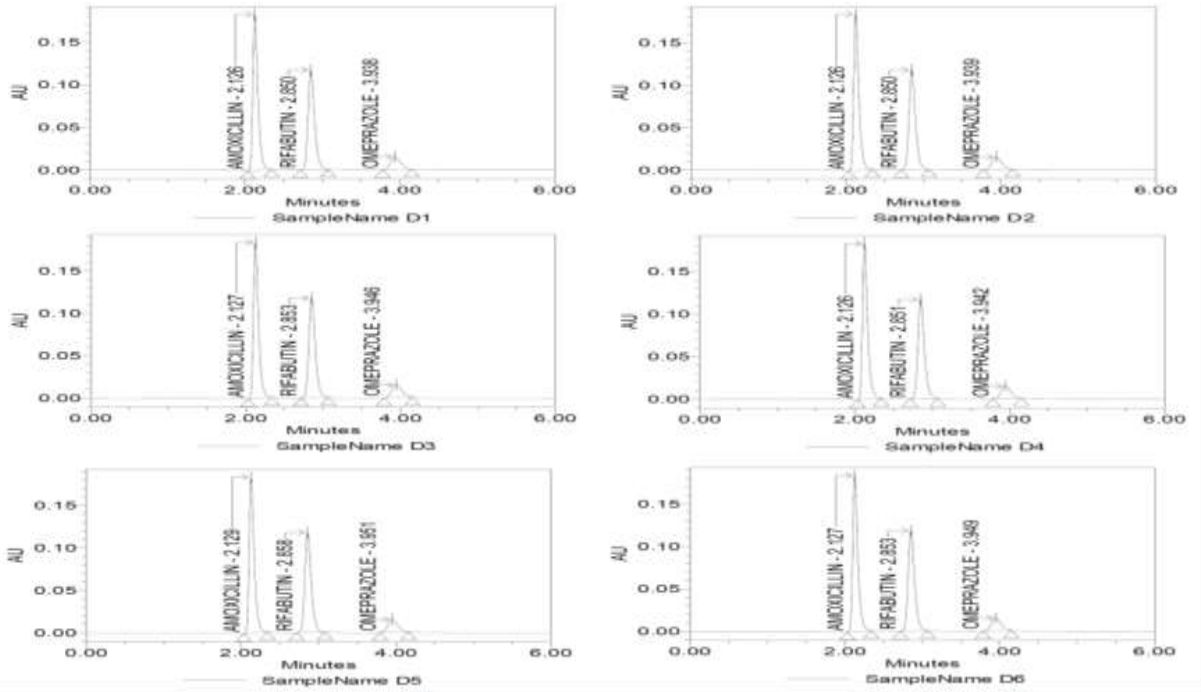


Figure: Dissolution study reached ALN, RBN & OMP chromatograms

Conclusion-

We created an RP-HPLC procedure for combined ALN, RBN, and OMP evaluations. Validated of combined ALN, RBN and OMP evaluation RP-HPLC procedure ensuing criteria of ICH. The data got met the ICH's authorization requisites. The combined ALN, RBN, and OMP evaluation RP-HPLC procedure also proved suitability for stability indicating appliance and to dissolution analyses of ALN, RBN, and OMP

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