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OPTIMIZED AND VALIDATED ANALYTICAL METHODS FOR THE DETERMINATION OF ROXITHROMYCIN IN PHARMACEUTICAL FORMULATION

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Abstract: In present research, method development and validation of Roxithromycin in pure and pharmaceutical formulation by using UV Visible spectrophotometric and RP-HPLC methods. The developed spectrophotometric method shows maximum absorbance at 238 nm and linearity calibration curve was obtained in a concentration range of 0.1-0.7µg/ml for Roxithromycin. The result of analysis shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation and %RSD will be less than 2 for all the validation parameters. Recoveries studies revealed that results within the specified limits. RP-HPLC method based on UV detection for the determination of Roxithromycin was also developed. Best results were obtained with the mobile phase composition consisting of a mixture of Acetonitrile-water (80:20, v/v) with a retention time of 1.301. Acceptance criteria for system suitability, tailing factor not more than 2.0, Theoretical plate not less than 5000 and %RSD of peak area not more than 2.0 were fulfilled during all validation parameter. Hence the proposed methods were found to be satisfactory and could be used for the routine analysis of roxithromycin in their marketed formulation.

***Index Terms*:** Roxithromycin, UV-Visible Spectrophotometer, RP-HPLC, Validation parameter

1. INTRODUCTION

Roxithromycin (RXM) is a semi-synthetic long-acting macrolide antibiotic derived from erythromycin. Chemically, it is a (3*R*, 4S, 5S, 6R, 7R, 9R, 11S, 12R, 13S, 14R)-6-[(2*S*,3*R*,4*S*,6*R*)-4-d-3-hydroxy-6methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2*R*, 4*R*, 5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan2yl]oxy-10-(2-methoxy ethoxy methoxy imino)-3,5,7,9,11,13-hexamethyl-1-oxacyclo tetradecan-2-one and its molecular formula $C_{41}H_{76}N_2O_{15}$ .RXM prevents bacterial growth by interfering with protein synthesis. It binds to the 50S subunit of bacterial ribosomes and inhibits the translocation of peptides. It is used to treat respiratory tract, urinary and soft tissue infection. Lung Infection, Recurrent Upper and Lower Respiratory Tract Infections (RTIs), SkinInfections Ear, nose, and throat infection.[1-2]

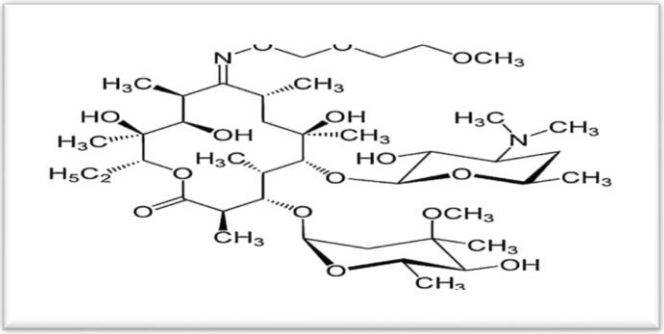

Fig. 1: Structure of Roxithromycin

The literature review [3-9] revealed that a very few analytical methods were appeared for the estimation of roxithromycin in pure and dosage forms. In view of the above facts, the present studies aim to develop a validated UV Spectrophotometric and RP-HPLC methods for the estimation of roxithromycin in pure and dosage forms.

2. METERIALS AND METHODS

2.1. Instrument Used:

Spectrophotometric system: The spectrophotometer system used to perform development and validation of this assay method was Systronics-2203 UV Visible double beam spectrophotometer (10mm optical path length matched quartz cell,50 nm min^{-1} scan speed) and 5 nm fixed slit width) was used for recording spectra and absorbance measurements.

Chromatographic system: Chromatographic system used to perform development and validation for the estimation of Roxithromycin was Analytical C_{18} Column (250mm x 4.6mm i.d, 5µm) was used for separation at a flow rate of 1 mL/min.

Other instruments: pH-Meter (MKV1, Systronics), Digital Balance and Ultrasonicator

2.2.Chemicals

All chemicals were analytical HPLC grade:Pure drugs [Roxithromycin (99.8% pure)], Acetonitrile (HPLC grade), double distilled water (HPLC grade)

2.3.Experimental

Method A: Spectrophotometric Method

Method Development

Preparation of Standard Solution: 10mg of Roxithromycin was weighed and transferred in to 10 ml volumetric flask. The drug was dissolved in acetonitrile and the volume was made up to the mark with water to obtain final concentration of 1000 µg/ml (Stock -A solution). From the above stock -A solution, 1ml of aliquot was pipette out in 100 ml volumetric flask and the volume was made up to the mark with water to obtain the final concentration of 10 µg/ml (stock –B solution).

Determination of wavelength of maximum absorption: Stock-B solution scanned under UV Visible spectrophotometer against reagent blank at 200-400 nm.

Procedure of Calibration curve for Roxithromycin: Appropriate aliquots were pipette out from the standard stock B solution in to a series of 10 ml volumetric flasks. The volume was made up to the mark with water to obtaina set of solutions having the concentration ranging from 0.1, 0.2, 0.3, 0.4, 0.5, 0.6µg/ml and 0.7µg/ml of Roxithromycin. Then take the absorbance of the above solutions were measured at 205nm, and a calibration curve of absorbance against concentration was plotted.

Procedure for the assay of Roxithromycin: Take 10 tablets of roxithromycin, powdered and weigh the powder equivalent to 10mg and dissolved in acetonitrile and make up to 10ml with water (1000 µg/ml). Working Standard solution of 10µg/ml was prepared. From this solution take 0.5ml sample to a 10 ml volumetric flask. Then measure the absorbance at 238nm against Reagent blank. The content of Roxithromycin in the marketed formulations was determined by above method.

Method B: RP-HPLC Method

Chromatographic conditions

Analytical C_{18} column (250mm x 4.6mm i.d, 5µm) was used for separation and the mobile phase, acetonitrile and water (80:20), was pumped at a flow rate of 1 mL/min. It was filtered through 0.20/0.45µm filter and degassed before use. The elution was monitored at 238 nm and the injection volume was 20µL. The oven temperature was 40°C.

Preparation of mobile phase: A mixture of acetonitrile and water at a ratio of 80:20 was prepared with vigorous shaking. The solution was sonicated and degas before use.
Preparation of Standard Solution: 10mg of Roxithromycin was weighed and transferred in to 10 ml volumetric flask. The drug was made up to the mark with mobile phase (acetonitrile and water - 80:20) to obtain final concentration of 1000 µg/ml (Stock -A solution).
From the above stock -A solution, 1ml of aliquot was pipette out in 100 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain the final concentration of 10 µg/ml (stock –B solution).

Determination of wavelength of maximum absorption: Stock-B solution scanned under UV Visible spectrophotometer against reagent blank at 200-400 nm.
Procedure of Calibration curve for Roxithromycin: From Stock solution –B, different volume of standard solutions was taken and prepares 0.1, 0.2, 0.3, 0.4, 0.5, 0.6µg/ml concentration of Roxithromycin solutions. Then inject into the chromatographic system at a flow rate of 1ml /minute and lamda max of 205nm. Peak areas were recorded for all the peaks and a standard calibration curve of area under curve against concentration was plotted.

Procedure for the assay of Roxithromycin: Ten tablets were weighed and finely powdered. From the powdered tablet, a quantity of powder equivalent to 10 mg was weighed and extracted with mobile phase having acetonitrile and water (80:20) and finally made up with mobile phase to get a concentration of 1000µg/ml. From this, prepare 10µg/ml of working solution. 20µl of each concentration of the drug were injected into the HPLC system and their chromatograms were recorded.

2.4. Method Validation[10]

The developed methods were validated for the estimation of Roxithromycin in accordance with ICH guidelines.

Accuracy / Recovery study: In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. Accuracy of the method was carried out by the analysis of standard additions at the three levels (50%, 100% and 150%) that is multi-level recovery studies. HPLC method and absorbance and peak area were recorded. The amount of drug present, percentage recovery, percentage relative standard deviation (% RSD) was calculated.

Precision: Precision of method was demonstrated by

Intra-day precision: Intraday precision was carrying out by analysis of sample solution (Roxithromycin) at different concentrations were prepared and analyzed. The variation of the results on different days was analyzed and statistically validated.
Inter-day precision:: Inter day precision was carrying out the analysis of sample solution (Roxithromycin) at different concentrations were prepared and analyzed at same time on different days. The variation of the results on different days was analyzed and statistically validated.
Linearity: For the establishment of linearity, a minimum of six different concentrations is recommended. Linearityshould be evaluated by visual inspection of a plot of signals as a function of analyte concentration. If there is a linear relationship, test results should be evaluated by appropriate statistical methods.
Range: The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy, and precision when applied to samples containing amount of analyte within or at the extremes of the specified range of the analytical procedure.
Limit of detection (LOD) and Limit of quantification (LOQ): The LOD and LOQ values were separately determined based on the standard calibration curve. The residual standard deviation of y-intercept of regression lines may be used to calculate LOD and LOQ. Ruggedness: The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, analysts, instruments.

Robustness: The robustness of an analytical procedure is done by hree replicates were made for the same conc. (5µg/ml,) in 10 mL, 50 mL and 100mL volumetric flasks and the recording of absorbance and peak area were done on both the UV-Vis spectrophotometer and RP-HPLC method. The result is expressed in Percentage RSD.

3. RESULTS AND DISCUSSION

3.1. Method A: UV- Spectrophotometric Method

Development of Spectrophotometric Method: The main step in the development of an analytical method is to improve the conditions and parameters which should be followed in the development and validation. Different solvents were studied (methanol, ethanol, acetone, water and acetonitrile), the criteria employed were the sensitivity of the method and availability of the solvent. From a solvent effect studies and spectral behaviors of Roxithromycin in acetonitrile and water were selected as solvents for the suggested spectrophotometric method. Proper wavelength selection of the methods dependsupon the nature of the sample and its solubility. The method sensitivity such as LOD and LOQ and analytical parameters such as correlation coefficient, intercept and slopeof the calibration equations was tested. Optimize the reaction conditions such as selection of solvent, pH, effect of sample concentration and volume etc.

Determination of Maximum Absorbance

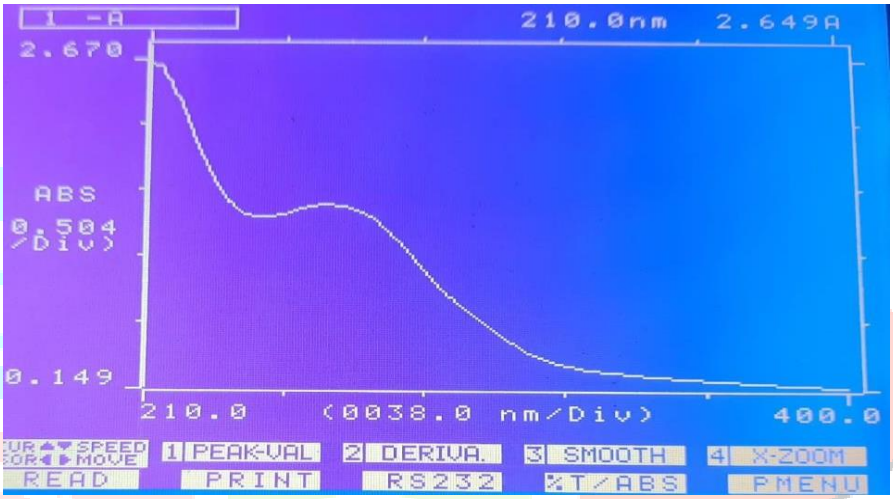

Figure 2: UV Spectrum of Roxithromycin

3.1.1. Method Validation

a)Accuracy: The recovery studies [Table 1 &2] were carried out 3 times at 50%, 100% and 150% levels and the percentage recovery and percentage relative standard deviation of the percentage recovery were calculated. Recoveries studies revealed that results within the specified limits and the method were found to be accurate.

Table 1: Recovery Studies for Roxithromycin

Level of addition (%pure drug)	Con.of drug in formulations (µg/ml)	Con.of pure drug (µg/ml)	Total conc. ofdrug found (µg/ml)
50%	0.5	0.25	0.747 0.748 0.747
100%	0.5	0.5	1.007 1.005 0.998
150%	0.5	0.75	1.244 1.245 1.244

Table 2: Statistical validation data for accuracy determination of Roxithromycin

Level of % Recovery	Mean	Standard Deviation	%RSD	% Analytical recovery
50%	0.7473	0.00057	0.076	99.64
100%	0.1017	0.412	0.578	101.7
150%	1.244	0.0016	0.012	99.52

b).Precision: The intra-day and inter-day precision and repeatability studies [Table 3] of the developed method confirmed adequate sample stability and method reliability, where all the RSDs were less than 2%.

Table 3: Precision studies for Roxithromycin:

INTRADAY [n=6]				INTERDAY [n=6]			
Conc. (µg/ml)	Ab.(1)	Ab.(2)	Ab.(3)	Conc. (µg/ml)	Ab. (D-1)	Ab. (D-2)	Ab. (D-3)
0.5	0.278	0.279	0.277	0.5	.278	0.276	0.279
0.5	0.278	0.278	0.278	0.5	0.278	0.278	0.278
0.5	0.279	0.278	0.277	0.5	0.277	0.277	0.278
0.5	0.278	0.279	0.277	0.5	0.278	0.276	0.277
0.5	0.279	0.278	0.278	0.5	0.277	0.278	0.278
0.5	0.278	0.278	0.278	0.5	0.278	0.278	0.278
MEAN	0.278	0.278	0.277	MEAN	0.277	0.277	0.278
S.D	0.000516	0.000516	0.00058	S.D	0.000516	0.00098	0.00063
%RSD	0.183	0.183	0.194	%RSD	0.186	0.354	0.227

c) Linearity and Range: For linearity seven points calibration curve were obtained in a concentration range 0.1- 0.6µg/ml. The response of the drug was found to be linear in the investigationconcentration range and the linear regression equation for Roxithromycin was y = 0.7321x+0.0031 with correlation coefficient 0.9996 (Fig. 2). Where x is the concentration in mg/ml and y is the peak area in absorbance unit.The absorbance and concentration value and calibration data are shown in [Table: 4 &5]

Figure 3: Calibration curve of Roxithromycin

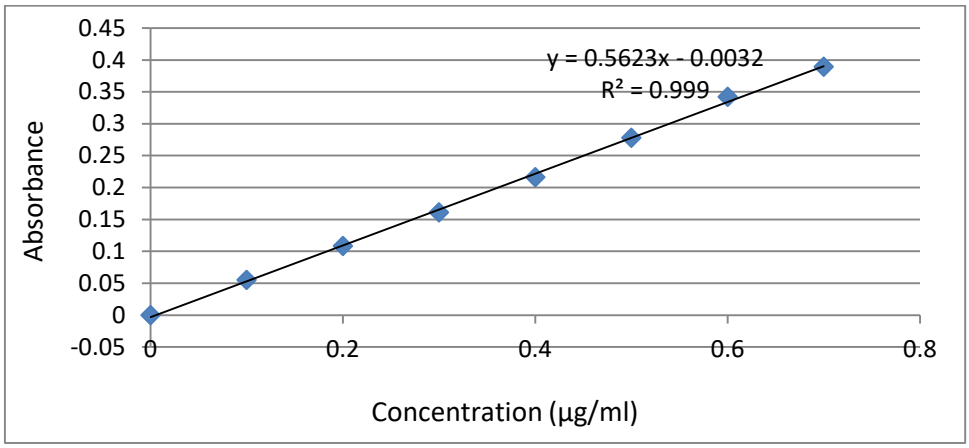

Table 4: Absorbance Vs Conc. Table for Roxithromycin.

Conc. (µg /ml)	Absorbance
0	0
0.1	0.055
0.2	0.108
0.3	0.161
0.4	0.216
0.5	0.278
0.6	0.342
0.7	0.389

Table 5: Calibration Data for Roxithromycin.

Parameters	Method
λmax	238 nm
Linearity(µg/ml)	0.1-0.7
Correlation coefficient(r^2)	0.999
Slope(m)	0.0562
Intercept(c)	0.0032

d).Limit of Detection (LOD) & Limit of Quantification (LOQ): The limit of detection and limit of quantification [Table 6] were evaluated by serial dilutions of Roxithromycin std. solution. The LOD value for Roxithromycin was found to be 0.02µg/ml. The LOQ value was found to be 0.06 µg/mL

Table 6: Limit of Detection (LOD) & Limit of Quantification (LOQ) for Roxithromycin.

Detection Wavelength(nm)	LOD µg/mL	LOQ µg/mL
238 nm	0.02	0.06

e).Ruggedness: The ruggedness of the methods was demonstrated (Table 7) by conducting the experiment on two different analyst and %RSD was calculated. Variation in percentage content was found to be within the limit so the method is rugged.

Table 7: Ruggedness of Roxithromycin.

SL NO:	Conc.(µg/ml)	Method	
		Analyst-1	Analyst-2
		Absorbance	Absorbance
1	0.5	0.278	0.277
2		0.278	0.278
3		0.279	0.277
4		0.278	0.277
5		0.279	0.278
6		0.278	0.278
Ab. Mean		0.278	0.277
S.D		0.000516	0.000548
%RSD		0.183	0.194

f).Robustness: The result of robustness study of the developed assay method was established in [Table 8 &9]. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. Hence the analytical method would be concluded as robust.

Table 8: Robustness for Roxithromycin

Vol. of volumetricflask	Conc. (µg/ml)	Absorbance Mean, n=3	Label claim (g/tab)	Estimated amount(g/tab)mean
10ml	5	0.36	0.05	0.0504
25ml	5	0.359	0.05	0.0502
50ml	5	0.357	0.05	0.0498

Table 9: Statistical Validation of Robustness of Roxithromycin

Vol. of volumetric flask	%Label Claim	Standard Deviation	%RSD
10ml	100.8	0.0007	0.19
25ml	100.4	0.0011	0.306
50ml	99.6	0.0020	0.560

***g)* Estimation**: The assay procedure was repeated for 6 times, mean weight of standard drugs, of sample were taken and calculated. Prepare 0.5 µg/ml of standard and sample solution were also prepared and assayed for content of Roxithromycin against the reference standard. The content of Roxithromycin in the marketed brands was determined. The percentages of individual drugs found in formulations, amount and relative standard deviation in formulations were calculated. The result of analysis shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation [Table 10].

Table 10: Estimation of Roxithromycin

Tablet Brand name	Labelled amount (g)	Estimated amount (mean ± SD,g)	%label claim	%RSD
Brand A	0.15	0.151±0.00021	100.06%	0.417
Brand B	0.15	0.1496 ±0.00048	99.2%	0.967

3.2. Method B: RP-HPLC Method

3.2.1. Method development

In this proposed method, RP-HPLC method based on UV detection was developed and validated for the estimation of RXM in pure and dosage forms. Analytical make C_{18} column (250mm × 4.6 mm i.d., 5µm particle size) column and mobile phase composition consisting of a mixture of Acetonitrile: Water (80:20v/v) found to be giving satisfactory results. For the selection of organic constituent of mobile phase, acetonitrile was chosen to reduce the longer retention time and to attain good peak shape and [Fig 4] represent the chromatograms of standard.

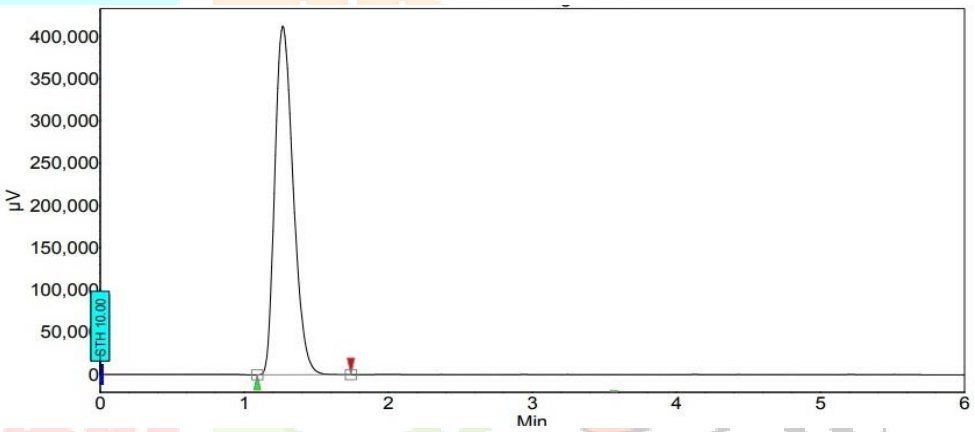

Figure 4: Chromatogram for Roxitromycin Standard

3.2.2. Method validation parameters

a) System suitability

All the efficiency parameters like theoretical plates were observed to be more than 7000 for RXM. The peak tailing was not more than 2.0. The %RSD for the five replicate injections was not more than 2.0 and ensured that the entire testing system and chemicals used could generate an accurate and precise result by showing all the efficiency parameters within the specified limits. Reported the results in Table 11.

Table 11: System suitability parameters of Roxithromycin

Parameter	Roxithromycin
Theoretical plate	7524
Tailing factor	0.8
Retention time	1.301
Peak symmetry	1.15
Area of peak	319747

b) Linearity: For linearity seven points calibration curve [Table 11] were obtained in a concentration range from 0.1-0.6 µg/ml for RXM. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for RXM was y = 3E+06x-27559 with correlation coefficient 0.9991[Fig 5]. Where x is the concentration in µg/ml and y is the peak area in absorbance unit. Concentration and peak area data and linearity data expressed in [table 12 &13]

Fig. 5: Calibration curve of Roxithromycin

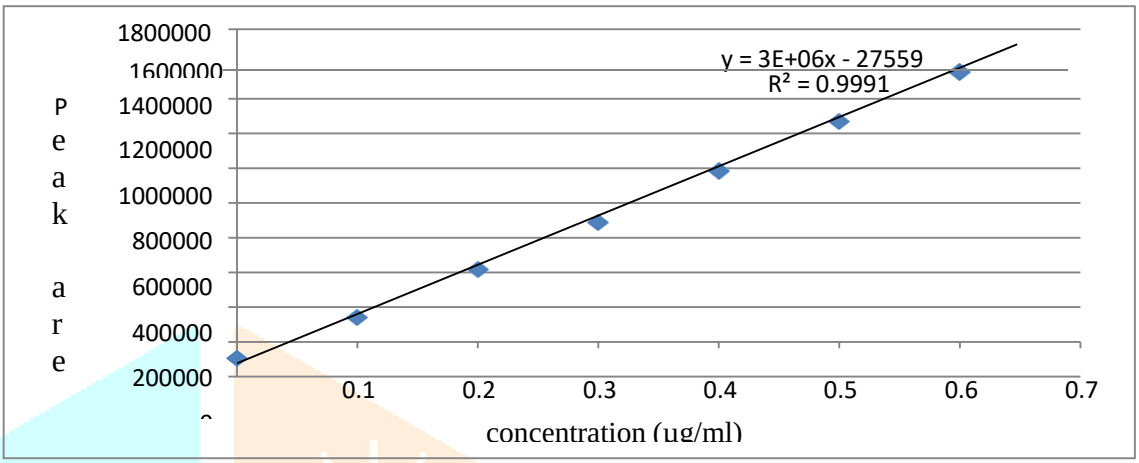

Table 12: Concentration Vs Peak area data for Roxithromycin.

Concentration (µg/ml)	Peak area
0	0
0.1	225487
0.2	485692
0.3	742365
0.4	1156248
0.5	1295815
0.6	1565981

Table 13: Linearity data for Roxithromycin

Parameters	Data's
λmax	238 nm
Linearity(µg/ml)	0.1-0.6
Correlation coefficient(r^2)	0.9991
Slope(m)	3E+06x
Intercept(c)	27559

λmax=Maximum absorbance, µg/ml =microgram per milliliter

c) LOD and LOQ: The limit of detection and limit of quantification were evaluated by serial dilutions of Roxithromycin stock solution. The LOD value for Roxithromycin was 0.02µg/ml and LOQ was found to be 0.07µg/ml. LOD and LOQ study values were shown in table [Table 14]

Table 14: LOD and LOQ for Roxithromycin

Method	Detection Wavelength(nm)	LOD (µg/ml)	LOQ (µg/ml)
ProposedMethod	238 nm	0.02	0.07

LOD=Limit of Detection, LOQ=Limit of Quantification, µg/ml =microgram per milliliter

d) Accuracy: The HPLC area responses for accuracy determination are depicted in [Table 15]. The result shown that best recoveries (98-102 %) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Table 15: Accuracy data for Roxithromycin

Level of addition (%pure drug) n=3	Con. of drug in formulations (µg/ml)	Conc. drug (µg/ml) of Pure	Total conc. of drug found (µg/ml)	%Analytical recovery	Data
50%	0.5	0.25	0.747	99.6	Mean=0.747 SD=0.00047 %RSD=0.0629
50%	0.5	0.25	0.748		
50%	0.5	0.25	0.747		
100%	0.5	0.5	0.998	99.7	Mean=0.997 SD=0.0047 %RSD=0.4714
100%	0.5	0.5	0.997		
100%	0.5	0.5	0.997		
150%	0.5	0.75	1.248	1.248	Mean=1.248 SD=0.00081 %RSD=0.0649
150%	0.5	0.75	1.249		
150%	0.5	0.75	1.247		

SD =Standard deviation, RSD= Relative standard deviation, n=number, µg/ml =microgram per milliliter

e) Precision: The result of repeatability of standard and inter-day and intra-day precision study are shown in [Table 16]. The developed method was found to be precise as the % RSD values for the repeatability and inter and intra-day precision studies were < 0.096 % and < 0.268 %, respectively, which confirm that method was precise.

Table 16: Precision for Roxithromycin

INTRADAY [n=6]					INTERDAY [n=6]		
Conc. (µg/ml)	**Peakarea (1)**	**Peakarea (2)**	**Peak area(3)**		**Peak area (D-1)**	**Peak area (D-2)**	**Peakarea (D-3)**
0.5	319747	319711	321721		319747	320612	321742
0.5	319632	319521	322562		319741	320532	321611
0.5	319569	319499	322511		319640	320412	321591
0.5	319677	319601	322532		319521	320612	321481
0.5	319721	319699	322612		319631	320112	321362
0.5	319512	319493	322461		319721	320711	321355
MEAN	319643	319587.3	322399.83		319666.8	320498.5	321523.6
S.D	76.358	90.416	307.05		79.72	195.32	139.18
%RSD	0.0238	0.0282	0.0952		0.0249	0.0609	0.0432

SD =Standard deviation, RSD= Relative standard deviation, n=number, µg/ml =microgram per milliliter

f) Ruggedness: The robustness method was studied and the results are given in [Table 17]. The ruggedness of the methods was conducted by two different analyst and %RSD was calculated. Variation in percentage content was found to be within the limit so the method is rugged.

Table 17: Ruggedness of Roxithromycin

Sl no:	**Conc. (µg/ml) (n=6)**	**Analyst-1**	**Analyst-2**
1	0.5	319747	319746
2		319746	319747
3		319747	319747
4		319747	319747
5		319746	319746
6		319746	319747
Mean		319746.5	319476.6
S. D		0.5	0.47
%RSD		0.00015	0.00014

SD =Standard deviation, RSD= Relative standard deviation, n=number, µg/ml =microgram per milliliter

g) Robustness: The result of robustness study of the developed assay method was established in [Table 18 and 19]. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. Hence the analytical method would be concluded as robust.

Table 18: Robustness studies of Roxithromycin (Change flow rate)

Sl no:	Flow rate ml/min. (n=3)	Peak area	StatisticalData	Retention time
1	1.0ml/min.	319747 319745 319748	Mean=319,746.6 SD=1.2472 %RSD=0.00039	1.301 1.302 1.301
2	0.5ml/min.	317321 317325 317322	Mean=317322.6 SD=1.6996 %RSD=0.000535	1.301 1.295 1.325

SD =Standard deviation, RSD= Relative standard deviation, n=number, µg/ml =microgram per milliliter

Table 19: Robustness of Roxithromycin at change in wavelength

Sl no:	wavelength [n=3]	Peak area	Statistical data	Retention time
1	205nm	318747 318744 318742	Mean=318744.3 SD=2.0548 %RSD=0.00064	1.296 1.305 1.302
2	238nm	319747 319745 319748	Mean=319746.6 SD=1.2472 %RSD=0.00039	1.301 1.302 1.302

SD =Standard deviation, RSD= Relative standard deviation, n=number, µg/ml =microgram per millilitre

h) Estimation: The content of roxithromycin in the marketed brands was determined. The percentages of individual drugs found in formulations, amount and relative standard deviation in formulations were calculated**.** The result of analysis [Table: 20] showsthat the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation. The chromatogram of roxithromycin tablet are shown in Fig.6.

Table 20: Estimation of Roxithromycin in tablet

Tablet Brand name	Labelled amount (g)	Estimated amount (mean ± SD)	%Label claim	%RSD
Brand-A	0.15	0.151±0.00021	100.06%	0.417
Brand-B	0.15	0.1496±0.00048	99.2%	0.967

Amount of Roxithromycin is expressed as mean ± SD (n = 3)

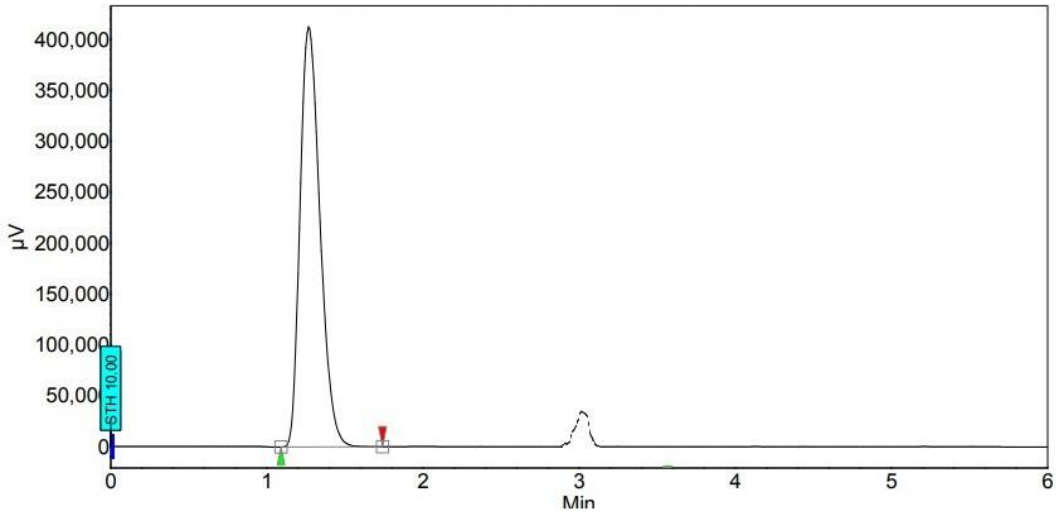

Figure. 6: The chromatogram of Roxithromycin [Sample]

4. CONCLUSION

In the present investigation, simple, precise and accurate UV Spectrophotometric R P - H P L C method was developed for the quantitative estimation ofroxithromycin in its pure and dosage forms. All developed method was statistically validated as per ICH Guidelines. In order to ensure that the data generated by above methods for drug was accurate and precise. High percentage of recovery studies suggests that the developed method as free from interference of excipients used dosage forms. The assay results confirmed to the label claim of the formulation. In addition to positive requirements for analytical methods of all the presently developed methods were economical. Hence the proposed methods were found to be satisfactory and could be used for the routine analysis of roxithromycin in their marketed formulation.

5. ACKNOWLEDGEMENT:

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6. REFERENCES

1. http://en.wikipedia.org/wiki/Roxithromycin
2. The Merck Index, 12th Edn., Merk Research Lab. Publication, Rahway, N.J.1996,(12);8430.
3. Shilpa Jagtap, Smrutidevi Sonavane, Shrikrishna Baokar. In Vitro New Dissolution Method for the Evaluation of Roxithromycin using pH 6.0 Phosphate Buffer and Determination of its Content by Validated UV Spectrophotometric Method. Research J. Pharma. Dosage Forms and Tech. 2013; 5(5): 278-282.
4. G. Sukea, Chahandea A D, Kasliwalb R H, Asnani A J. Roxithromycin potency quantification in pharmaceutical preparation by applying a validated bioassay method and comparison with HPLC analysis. J. Pharma. 2015; 73(5):340-350.
5. K.Ravi Sankar RP-HPLC method for the analysis of Roxithromycin in bulk and pharmaceutical dosage forms Analytical Chemistry An Indian Journal, 2009; 8(1): 1-3
6. Esther Roset Shobana Spectrophotometric and HPLC Methods for Simultaneous Estimation of Roxithromycin and Ambroxol from Tablets Asian Journal of Chemistry ,2008;20(6):4159-4162.
7. Valeria de Oliveira, Ana Maria Bergold, Elfrides Eva Scherman Schpoval. High- performance loquid chromatographic is determination of Roxithromycin in tablets. Analytical letters. 2006;29 (13):2377-2382.
8. Umadevi K Quantitative Estimation of roxithromycin and Ambroxol In bulk and tablet dosage forms by RP-HPLC METHOD.Research journal ofscience and technology 2023;15(1):1-7.
9. Holstein JJ, Luger P, Kalinovsk R, Mebs S, Paulman C, Dittrich B. Validation of experimental charges densities: refinement of the macrolide antibiotic Roxithromycin. Acta Cryst. 2010; 66:568–577.
10. ICH, Q2 (R1), Validation of Analytical Procedures: Text and Methodology. 2005.

11. Osama H Abdelmageed. Development and validation of a spectrophotometric method for the determination of macrolide antibiotics by using 2,4- dinitrophenylhydrazine. J AOAC Int. 2007;90(2):364-71.
12. Olajire A. Adegoke, Monsurat O. Quadri. Novel spectrophotometric determinations of some cephalosporins following azo dye formation with *p*- dimethylaminobenzaldehyde. Arabian. j. of Chemistry. 2016;9:1272-1282.