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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF ROXITHROMYCIN FROM HUMAN PLASMA

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ABSTRACT

A stable, simple, rapid, precise, accurate HPLC method for analysis of Roxithromycin was developed and validated as per ICH guidelines without need of any internal standard. Separation was carried out using X'terra RP18 (250*4.6) mm, 5µ column with potassium dihydrogen orthophosphate buffer (pH 3): acetonitrile (30:70 v/v) as mobile phase with flow rate 1 mL min^{-1}. The parameters studied were retention time, linearity and range, accuracy, precision. The proposed method can be used for determination of Roxithromycin from Human plasma.

Keywords: Roxithromycin, HPLC, Validation.

INTRODUCTION

Roxithromycinis an azalide, a subclass of macrolideantibiotics. Roxithromycin is one of the world's best-selling antibiotics. It is derived from erythromycin, with a methyl-substituted nitrogenatom incorporated into the lactone ring, thus making the lactone ring 15-membered.

Roxithromycin is used to treat or prevent certain bacterial infections, most often those causing middle ear infections, strep throat, pneumonia, typhoid, bronchitis and sinusitis. In recent years, it has been used primarily to prevent bacterial infections in infants and those with weaker immune systems [1-5]. It is also effective against certain sexually transmitted infections, such as nongonococcal urethritis, chlamydia, and cervicitis. Recent studies have indicated it also to be effective against late-onset asthma, but these findings are controversial and not widely accepted.

HPLC METHOD DEVELOPMENT FOR PURE ROXITHROMYCIN

Today the 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 some way relate or compare to existing, literature-based approaches. The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final needs or requirements of the method. Method development usually requires selecting the method requirements and deciding on what type of instrumentation to utilize and why. The extraction reported to detect Roxithromycin was liquid-liquid extraction [6-10].

They were reported for the determination of Roxithromycin and its related substances in biological fluids like plasma, blood, and urine only but, very few methods have been reported for its determination in bulk and solid (tablet) dosage forms by reversed phase high-performance liquid chromatographic (RP-HPLC) method. However, these methods presented some disadvantages such as being of low sensitivity, time consuming, and costly. This study was designed to develop a simple and reliable method to quantitate Roxithromycin in a relatively short time with high linearity.

Therefore, this study involves the development of simple and rapid isocratic RP-HPLC method which can be employed for the routine analysis of ROXITHROMYCIN. The established method was validated with respect to specificity, linearity, precision, accuracy, and ruggedness [11-13].

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Reagents

Water : Milli-Q / HPLC Grade
Ortho phosphoric acid (88%): GR Grade
Trimethyl amine : GR Grade
Acetonitrile : HPLC Grade
Methanol : HPLC Grade

The linearity of the response of drug was verified from 1 g/ml to 10 g/ml concentrations. The calibration graphs were obtained by plotting the response versus the concentration.

Preparation of Mobile Phase

The separation was carried out under isocratic elution with mobile phase was a mixture (75 volumes) of 1.4 mL of *ortho*-phosphoric acid in 1000 mL of water and adjust the pH 3.0 by using triethyl amine and acetonitrile (25 volumes), was filtered through 0.4 µm nylon membrane filter before use.

Chromatographic Conditions

Column: C8 column (250 mm × 4.6 mm), 5-µm particle size SS column
Flow: 1.0 ml/min
Wavelength: 220 nm
Injection volume: 20µl

Standard Preparation

A standard stock solution of 50 mg of Roxithromycin in mobile phase was prepared in a volumetric flask. From this stock solution, about 10 mL was diluted to 100 mL with mobile phase.

Hplc Method Development

Preparation of sample solution for Roxithromycin in tablet dosage form

Twenty tablets were weighed and crushed to a fine powder. The powder equivalent of 50 mg of Roxithromycin was taken in a 100-mL volumetric flask containing mobile phase and kept sonication for 10 min and made up to mark with mobile phase. The resultant mixture was filtered through 0.45 µm nylon filter. The desired concentration for the drug was obtained by accurate dilution, and the analysis was followed up as in the general analytical procedure.

Evaluation of system suitability

1. The column efficiency determined for the Roxithromycin peak from the standard preparation should not be less than 5000 theoretical plates and tailing factor for the same peak should not be more than 2.0.
2. The percentage relative standard deviation for five replicate injections of standard preparations should not be more than 2.0.

Assay calculation for Roxithromycin Tablet formulations

% Assay

$$\frac{AT1}{AS} X \frac{W}{100} X \frac{5}{50} X \frac{5}{50} X \frac{100}{W1} X \frac{50}{5} X \frac{P}{PC}$$

Where,

AT1 : Average area counts of Roxithromycin peak in sample preparation.
AS : Average area counts of Roxithromycin peak in standard preparation.
W : Weight of Roxithromycin working standard, in mg.
P : Potency of Roxithromycin working standard, on as is basis.
LC : Label claim of Roxithromycin in mg / gm
W1 : Weight of sample in gm

Method precision was evaluated by carrying out the independent assays of Roxithromycin. The sample of known concentration was injected thrice for every formulation. The relative standard deviation was then calculated.Accuracy or recovery test was studied by adding known amount of drug in the blood samples. The recovery was performed at about 50%, 100% and 150% of Roxithromycin. The method used in determining the accuracy of the samples was adopted to prepare the samples for the recovery studies. The solutions were analyzed and the percentage recoveries were calculated [14-18].

Validation of HPLC method for Roxithromycin tablet formulation

Preparation of sample solution for ROXITHROMYCIN in tablet dosage form

Twenty tablets were weighed and crushed to a fine powder. The powder equivalent of 50 mg of Roxithromycin was taken in a 100-mL volumetric flask containing mobile phase and kept sonication for 10 min and made up to mark with mobile phase. The resultant mixture was filtered through 0.45 µm nylon filter. The desired concentration for the drug was obtained by accurate dilution, and the analysis was followed up as in the general analytical procedure.

Sample Injection Procedure

Six injections of each of the Roxithromycin sample were injected into the chromatographic system. The chromatograms were recorded and the peak area counts were measured for the Roxithromycin peak.

Specificity / Purity plots

The Roxithromycin samples prepared as per the above mentioned methodology were foremost analyzed for the purity of the samples and the purity peaks were obtained.

System Precision:

Six replicates of the standard solution were

injected into the HPLC system and the area of the peak and RSD was calculated.

Method Precision:

Assay of method precision (intraday precision) was evaluated by carrying out six independent assays for both formulations of Roxithromycin. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, systems and different days in the same laboratory.

Accuracy (Recovery test)

Accuracy of the developed method was studied by recovery experiments. The same solutions were analyzed for percentage recovery studies at three levels (50%, 100% and 150%) for each formulation. The assay results were expressed as percentage of label claim of amount of Roxithromycin found in the tablet formulations.

These solutions were analyzed for its percentage drug contents with respect to label claim, by a single analyst six times a single day and by another analyst once a day for six days, to calculate the percentage precision of the method [19-26].

RESULTS

This study has demonstrated that all the pharmacokinetic parameters of both the treatments were statistically different from each other. In the fed condition the values of Cmax and AUC were decreased while T max increases than that of fasting which demonstrated that the extent of systemic exposure to Roxithromycinwas affected by the delay in absorption of Roxithromycinin the presence of food. None of the study volunteers reported any serious adverse effects throughout the study. The only two AEs reported were mild and not related to the study medication. The AEs reported were, according to the study medical expert, related to the sampling procedure and were self limiting and did not require any treatment. There was no change in the vital signs of the volunteers throughout the study period. The presented data are of major importance in identifying the optimal dosing regimen for future clinical trials with oral Roxithromycin. In our study, only one type of food (a standardized continental breakfast) was evaluated; further studies are needed to assess the effects of foods with different compositions and contents on the bioavailability of Roxithromycin.

Table 1. Peak Results for Roxithromycin WS

Sr. No.	Name	RT	Area	% Area	USPPlate count	USPTailing Factor
1	Roxithromycin	4.217	4437618	100	12144	1.44

Table 2. Intraday precision characteristics of Roxithromycin

Weight of samples (g)	Injection Volume (µL)	Mean Area	RSD (%)
304.4	20	4429594	0.03
305.6	20	4462525	0.59
308.2	20	4568540	0.23
299.1	20	4319730	0.11
305.6	20	4395803	0.04
300.1	20	4322305	0.01

Table 3.Interday precision characteristics of Roxithromycin

Weight of samples (g)	Injection Volume (µL)	Mean Area	RSD (%)
304.1	20	4446587	0.40
303.7	20	4453466	0.19
307.9	20	4548451	0.00
300.3	20	4333103	0.14
302.7	20	4397236	0.14
304.1	20	4332490	0.40

Table 4. Recovery studies of Roxithromycin

Labeled amount (mg)	Amount added (mg)	Amount recovered (mg)	% Recovery
150.0	40.40	40.38	99.95
150.0	50.90	51.30	100.79
150.0	60.10	59.68	99.29

Table 5. Recovery studies of Roxithromycin

Specificity	Weight of sample (g)	Time (h)	RT of Roxithromycin	RT of degraded Product
Acid stress (0.5 N)	0.305	0	4.300	4.308
		8	4.301	4.310
Base stress (5 N NaOH)	0.305	0	4.325	4.317
		8	4.322	4.314
Peroxide stress (3 % H2O2)	0.305	0	4.233	4.217
		8	4.244	4.221

Table 6. Robustness characteristics of Roxithromycin

Factor	Level	Retention Time
Flow Rate (mL/min)		
0.9	-1	4.675
1.0	0	3.833
1.1	+1	3.825
pH of mobile phase		
2.9	-1	3.667
3.0	0	3.675
3.1	+1	4.808
Percentage acetonitrile in the mobile phase		
22.5	-1	3.800
25.0	0	3.792
27.5	+1	5.233

Table 7. Determination of Precision for HPLC system validation

Sr. No.	Percentage assay value for Precision
1	99.43
2	99.64
3	99.60
4	99.08
5	99.20
6	100.12
Mean	99.50
RSD	0.36

Table 8. Determination of Precision for HPLC method validation

Sample number	Assay of Roxithromycinas % of labeled amount	
	Analyst-I (Intra-day precision)	Analyst-II (Inter-day precision)
1	99.43	99.73
2	99.62	99.20
3	99.50	99.88
4	99.18	99.57
5	99.22	100.00
6	100.10	99.23
Mean	99.50	99.60
RSD	0.38	0.27

Table 9. Recovery Studies for HPLC method validation

Formulation	Level	%Recovery	%RSD*
Roxithromycin Tablet formulation	50%	99.20	0.2834
	100%	99.90	0.3050
	150%	99.60	0.3491

* RSD of six observations

Table 10. Analysis of Formulation for HPLC method validation

Formulation	Amount		% label claim	%RSD*
	Labeled	Found		
Roxithromycin Tablet formulation	150 mg	497.9 mg	98.60	0.2223

* RSD of six observations

Figure 1. Spectrum Index Plot of Roxithromycin by HPLC

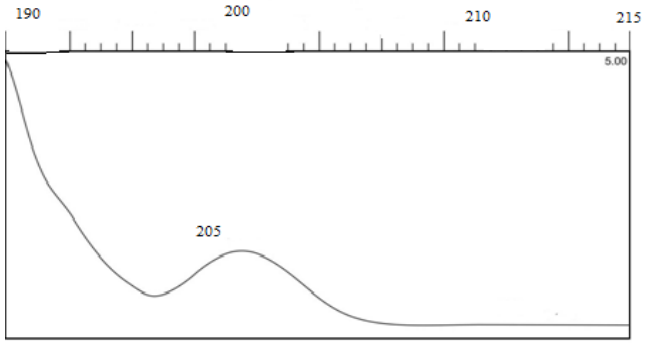


Figure 2. Chromatogram of Roxithromycin

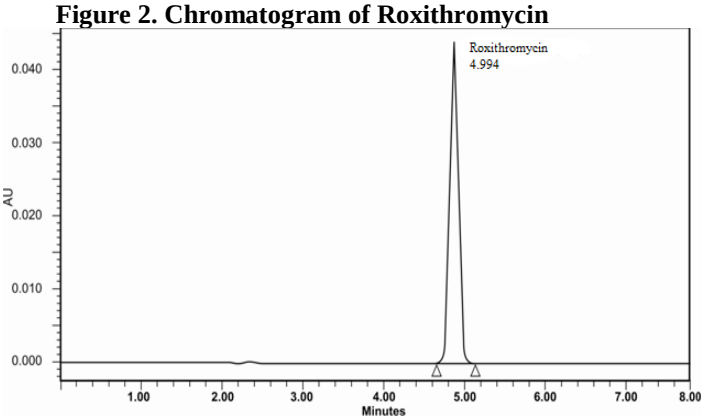
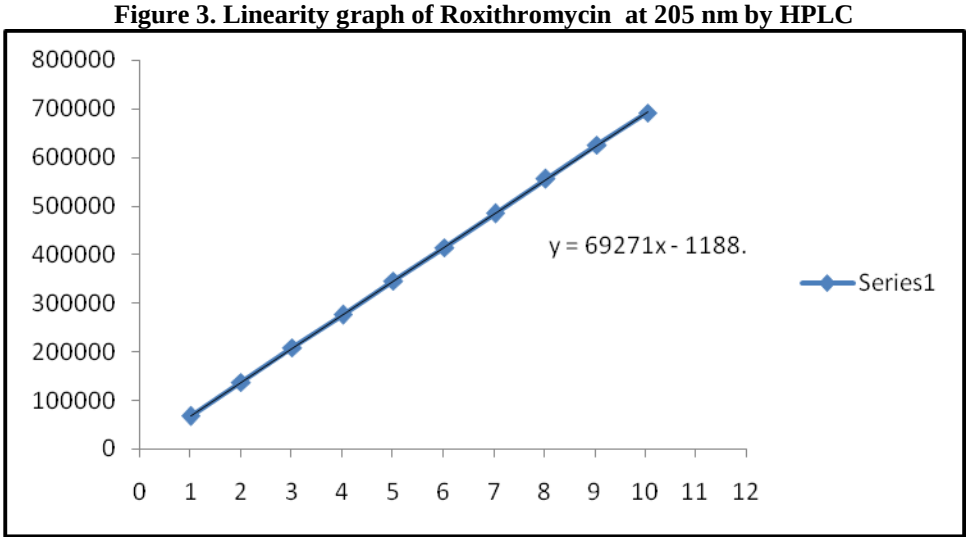


Figure 3. Linearity graph of Roxithromycin at 205 nm by HPLC



CONCLUSION

The HPLC method developed for analysis of Roxithromycin in Human Plasma is found to be accurate, sensitive and robust.

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