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DEVELOPMENT AND VALIDATION OF FT-IR SPECTROMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF SERRATIOPEPTIDASE AND ROXITHROMYCIN IN BULK AND DOSAGE FORM

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ABSTRACT

A simple, economic, specific, accurate and precise validated FT-IR spectrometric method has been developed for the simultaneous estimation of Roxithromycin and Serratiopeptidase in bulk and dosage form. Here in present method spectrometry was carried out by using FT-IR spectrophotometer, Model FT/IR 4600 (JASCO). The linearity range for serratiopeptidase is 0.24-0.4mg and for roxithromycin 3.6-6mg. The selected spectral region is 1720-1740cm-1 and 1620-1680cm-1, related to a C=O stretch and C=C stretch bands of Roxithromycin and Serratiopeptidase respectively. The LOD and LOQ were 1.428mg and 4.328mg for Serratiopeptidase and 1.372mg and 4.159mg for Roxithromycin respectively. The percentage purity of Roxithromycin and Serratiopeptidase was found to be 97.02% and 92.79% respectively. This method was found to be accurate, precise, stable, robust and rugged as indicated by low values of %RSD. The developed method could be successfully applied for the simultaneous determination of Roxithromycin and Serratiopeptidase in combined pharmaceutical dosage form.

INTRODUCTION

Roxithromycin is a semi-synthetic 14-membered ring macrolide antibiotic derived from erythromycin, with a methyl-substituted nitrogen atom incorporated into the lactone ring. It is more stable than erythromycin under acidic conditions and thus exhibits improved pharmacokinetic properties. Roxithromycin is chemically (3R,4S,5S,6R,7R,9R,11S,12R,13S, 14R) -6-[(2S,3R,4S, 6R)-4-dimethylamino-3-hydroxy-6-methyloxan-2-yl] Oxy-14 -ethyl-7,12, 13-trihydroxy-[(2R,4R,5S,6S) -5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl] Oxy - 10- (2 - methoxy methoxyimino) -3,5,7,9,11,13-hexamethyl-1-oxacyclo Tetradecan -2-one. It is used to treat respiratory tract, urinary and soft tissue infections [1-5].

Serratiopeptidaseis a proteolytic (protein digesting) enzyme produced by *Enterobacterium Serratia* sp. It is present in the digestive system of silkworms. It is the enzyme responsible for dissolving a silkworm's cocoon. Used as an anti-inflammatory agent. This also enhances tissue repair and reduces pain. Pain is also reduced by this enzyme's ability to block amines. It also has the unique ability to dissolve the dead and damaged tissue, which is the byproduct of the healing response without harming living tissue [6-11].

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EXPERIMENTAL METHOD

Materials and Instruments

· Shimadzu electronic balance AY 220
· FT-IR spectrophotometer, Model FT/IR 4600 (JASCO). KBr Hydraulic Press

Drug samples

Pure drug

Roxithromycin and Serratiopeptidase were purchased from Yarrow Chem. Products Pvt Ltd, Mumbai.

Formulation

Roxidase tablets were purchased from Thulasi pharmacy Coimbatore. Each tablet contains 150mg of Roxithromycin and 10mg of serratiopeptidase.

Chemicals and reagents

KBr for FT-IR used was of analytical grade and were obtained from S.D.Fine Chemicals, Mumbai.

METHOD DEVELOPMENT

Obtaining of Analytical curve

Equivalent amounts of 0.24, 0.28, 0.32, 0.36, 0.4mg of Serratiopeptidase and 3.6, 4.2, 4.8, 5.4; 6mg of Roxithromycin pure drug (previously diluted in potassium bromide) were taken and diluted with sufficient amount of potassium bromide to obtain 100mg pellets. The powder were mixed and ground until obtaining a homogeneous mixture. Thus, this mixture was compressed in a mechanical die press with 5 ton pressure for 2min to obtain translucent pellets, through which the beam of the spectrometer can pass. After obtaining the FT-IR spectrum and with the assistance of the IR solution software, quantitative analysis was carried out in the spectral region 1720-1740cm-1 and 1620-1680cm-1, related to a C=O stretch and C=C stretch bands of Roxithromycin and Serratiopeptidase respectively, and these bands had its height analyzed in terms of absorbance [12-14].

Determination of Serratiopeptidase and Roxithromycin in combined dosage form

Preparation of standard pellets

Amount of powder equivalent to 0.32mg of Serratiopeptidase and 4.8mg of Roxithromycin were taken and diluted with sufficient amount of potassium bromide to obtain 100mg pellets. Absorbance was measured in the spectral region 1620-1680cm-1 and 1720-1740cm-1, for Serratiopeptidase and Roxithromycin respectively [15].

Preparation of sample pellets

20 tablets were weighed accurately and the average weight was calculated. The tablets were ground to a fine powder. An accurately weighed tablet powder equivalent to 10mg of Serratiopeptidase and 150mg of roxithromycin mixed with potassium bromide to obtain 500mg mixture. Amount of powder equivalent to 0.32mg of Serratiopeptidase and 4.8mg of Roxithromycin were taken and make the pellet. Absorbance was measured in the spectral region 1620-1680cm-1 and 1720-1740cm-1 for Serratiopeptidase and Roxithromycin respectively. The determinations were performed in triplicate [16].

Calculation of the amount of active pharmaceutical ingredient in the sample

C sample = (A sample / A standard) x C standard

Amount of API = (C sample x equivalent weight)/ C standard

Percentage purity = (Amount of API/ Label claim) x 100

METHOD VALIDATION

The method was validated by determining the following parameters: linearity, accuracy, precision, robustness, ruggedness, detection limit and quantification limit.

Linearity

With the intension of validate the method, five concentration of standard Serratiopeptidase (0.24, 0.28, 0.32, 0.36,0.4mg) and Roxithromycin (3.6, 4.2, 4.8, 5.4, 6mg) were used. Linearity was evaluated by linear regression analysis.

Accuracy

Accuracy was attained via the recovery assay, in which known quantity of pure drugs was added to known quantity of the sample. The recovery was performed in the three levels, 80%, 100% and 120%, and the pellets were prepared in three replicate.

Precision

The precision of the method was evaluated in two requisites: repeatability and intermediate precision. Repeatability (intra-day) was studied by the performance of three determinations of the sample in a concentration 0.28mg of Serratiopeptidase and 4.2mg of Roxithromycin per pellet, all in the same day and identical working conditions. Intermediate precision (inter-assay) was assessed by performing the assay in three different days under the same experimental conditions. At the end of test, the percentage relative standard deviation (%RSD) values of the determinations were analyzed.

Robustness

By introducing changes in the compressed pressure for making pellet and the effects on the results were examined.

Ruggedness

Ruggedness was evaluated by performing the analysis following the recommended procedures by three different analysts. From the % RSD values presented, one can conclude that the proposed method is rugged.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD & LOQ Values were calculated to check the deduction limit and the quantification limit of the method by using the following equations.

$$LOD = \frac{3.3\sigma}{S}, LOQ = \frac{10\sigma}{S}$$

Where, σ is the standard deviation and S is the slope of the curve.

RESULTS AND DISCUSSION

Assay results

The percentage purity limit for serratiopeptidase is not less than 90% and for roxithromycin 96% to 102%.

Linearity and rang

The linearity range for serratiopeptidase is from 0.24mg to 0.4mg and for roxithromycin is from 3.6mg to 6mg.Calibration curves of serratiopeptidase and roxithromycin at1620-1680cm-1 and 1720-1740cm-1 respectively shown high linearity. The correlation between sample concentrations and their absorbencies complied with Beer's law.

Accuracy

Accuracy results were obtained with very less % RSD (relative standard deviation) values and those are in within the limit.

Robustness

Limit of detection (LOD) and Limit of quantification (LOQ).

Table 1. Assay results for marketed formulation

Marketed formulation	Drug	Label claim(mg)	Estimated amount(mg)	% purity	Mean ± SD(standard deviation) for % purity	% RSD
Roxidase (160mg)	Serratiopeptidase (SERR)	10	9.27	92.79	92.79±0.01	0.015
			9.05	92.78		
			9.28	92.81		
	Roxithromycin (ROX)	150	145.7	97.13	97.02±0.073	0.101
			145.5	97		
			145.4	96.93		

Table 2. Accuracy results are within the limit

Drug	Theoretical percentage target level	Amount added (mg)	Amount recovered(mg)	% Recovery	Mean ± SD for % recovery	% RSD
SERR	80	0.224	9.09	90.98	90.83± 0.21	0.277
			9.05	90.51		
			9.10	91		
	100	0.28	9.35	93.57	93.48± 0.14	0.188
			9.32	93.26		
			9.36	93.60		
	120	0.336	9.97	99.70	100.62± 0.61	0.826
			10.13	101.3		
			10.08	100.86		
ROX	80	3.36	145.89	97.26	97.08± 0.14	0.190
			145.33	96.89		
			145.65	97		
	100	4.2	144.78	96.52	96.50± 0.131	0.186
			144.45	96.30		
			145.01	96.67		
	120	5.04	152.30	101.53	101.51± 0.155	0.225
			152.59	101.73		
			151.92	101.28		

Table 3. Precision results are within the limit

Drug	Amount (mg)	Intra-day			Inter-day		
		% Content	Mean ± SD for % content	% RSD	% Content	Mean ± SD for % content	% RSD
SERR	0.28	101.01	100.85± 0.24	0.31	98.57	98.25± 0.21	0.32
		100.97			98.26		
		100.50			97.93		
ROX	4.2	97.74	97.28± 0.30	0.43	96.26	96.08± 0.12	0.17
		97.22			95.91		
		96.89			96.08		

Table 4. Robustness results are beyond the limit

Drug	Amount Taken (mg)	Parameters Altered Pressure in tons	Amount Found (mg)	% Content	Mean ± SD For % content	% RSD
SERR	0.28	4	15.89	158.9	156.85± 1.36	1.95
			15.50	155		
			15.67	156.7		
		6	15.88	158.8	159.62± 0.55	0.71
			16	160		
			16.07	160.07		
ROX	4.2	4	155.73	103.82	103.67±0.11	0.16
			155.26	103.50		
			155.54	103.69		
		6	157.98	105.32	105.15±0.18	0.24
			157.89	105.26		
			157.32	104.88		

Table 5. Ruggedness results are within the limit.

Drug	Analyst	Amount taken(mg)	Amount found(mg)	% Content	Mean ± SD For % content	% RSD
SERR	I	0.28	10.09	100.9	100.7±0.13	0.173
	II		10.06	100.6		
	III		10.06	100.6		
ROX	I	4.2	152.59	101.73	101.73±0.013	0.02
	II		152.63	101.75		
	III		152.56	101.71		

Table 6. LOD & LOQ results

SERR		ROX	
LOD (mg)	LOQ (mg)	LOD (mg)	LOQ (mg)
1.428	4.328	1.372	4.159

Fig 1. Roxithromycin

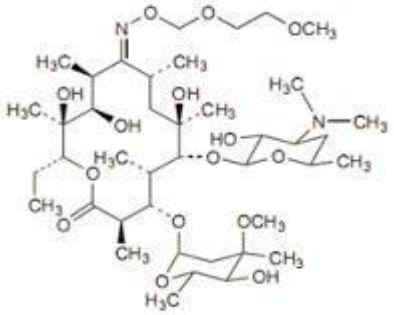


Fig 2. Serratiopeptidase

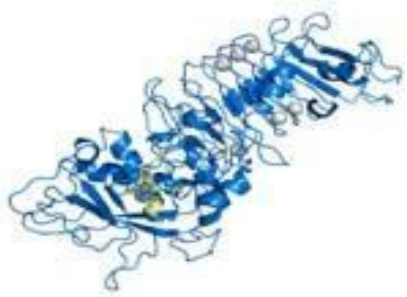
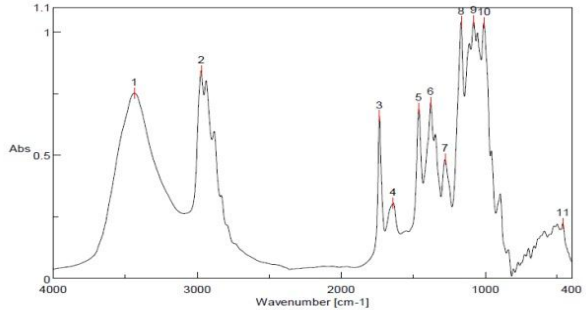
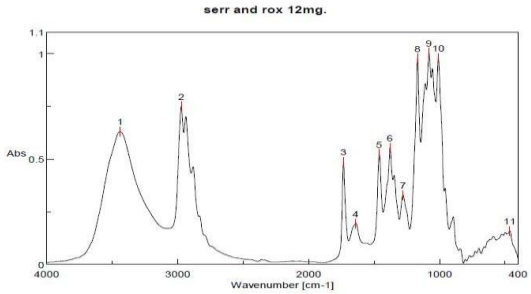


Fig 3. FT-IR spectrum for marketed formulation



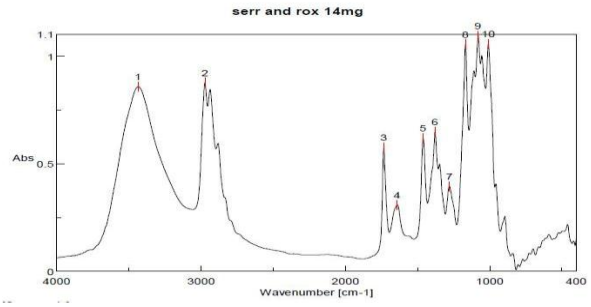
In the above spectrum 3&4 peaks are at 1720-1740 cm-1 and 1620-1680cm-1 respectively

Fig 4. FT-IR spectrum for serratiopeptidase 0.24mg and roxithromycin 3.6mg in 12mg KBr mixture



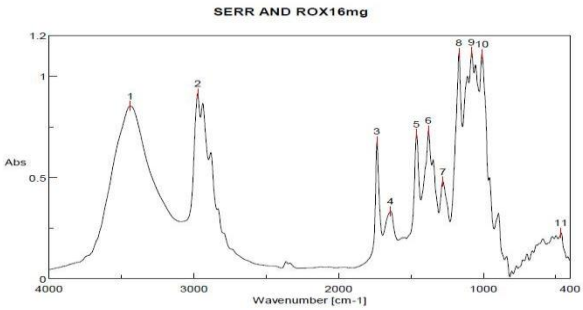
In the above spectrum 3&4 peaks are at 1720-1740 cm-1 and 1620-1680cm-1 respectively.

Fig 5. FT-IR spectrum for serratiopeptidase 0.28mg and roxithromycin 4.2mg in 14mg KBr mixture



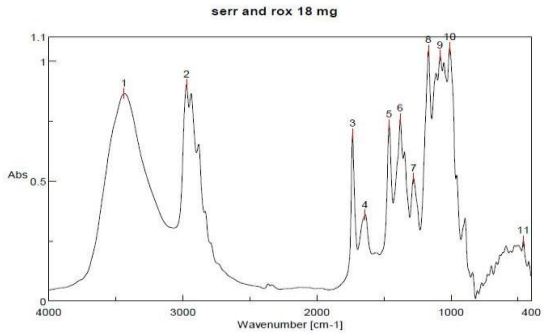
In the above spectrum 3&4 peaks are at 1720-1740 cm-1 and 1620-1680cm-1 respectively

Fig 6. FT-IR spectrum for serratiopeptidase 0.32mg and roxithromycin 4.8mg in 16mg KBr mixture



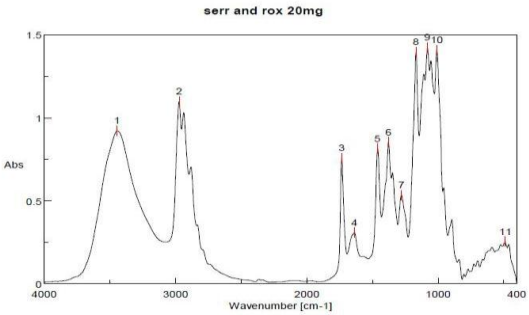
In the above spectrum 3&4 peaks are at 1720-1740 cm-1 and 1620-1680cm-1 respectively.

Fig 7. FT-IR spectrum for serratiopeptidase 0.36mg and roxithromycin 5.4mg in KBr mixture



In the above spectrum 3&4 peaks are at 1720-1740 cm-1 and 1620-1680cm-1 respectively

Fig 8. FT-IR spectrum for serratiopeptidase 0.4mg and roxithromycin 6mg in 20mg KBr mixture



In the above spectrum 3&4 peaks are at 1720-1740 cm-1 and 1620-1680cm-1 respectively

Fig 9. Calibration curve for serratiopeptidase

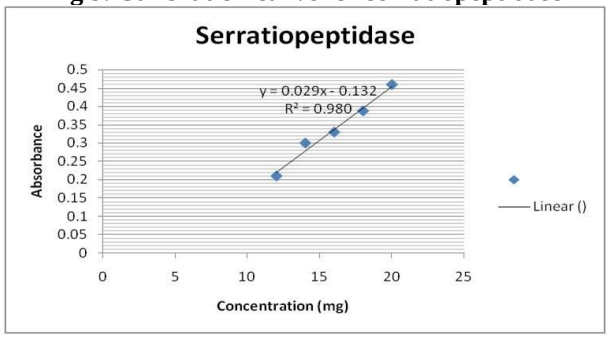
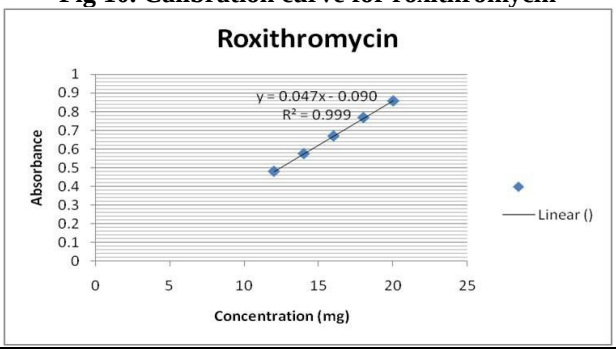


Fig 10. Calibration curve for roxithromycin



CONCLUSION

The developed method is specific, linear, accurate, precise, robust and rugged. Acceptable regression values, % RSD and standard deviations which make it a versatile and valuable for simultaneous estimation of two drugs in tablet formulation. The developed FT-IR method could be conveniently adopted for quality control analysis of serratiopeptidase and roxithromycin simultaneously from tablet dosage forms.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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