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DEVELOPMENT AND VALIDATION OF UV METHOD FOR THE SIMULTANEOUS ESTIMATION OF RACECADOTRIL AND OFLOXACIN IN BULK DRUGS AND MARKETED FORMULATION

Kaustubh V. Gavali*, Ajij A. Shaikh, Ashpak M. Tamboli and Naziya I. Khan

Department of Pharmaceutics, Sahyadri College of Pharmacy, Methwade, Sangola, 413307, Solapur, Maharashtra, India.

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***Corresponding Author**
Kaustubh V. Gavali
Department of Pharmaceutics, Sahyadri College of Pharmacy, Methwade, Sangola, 413307, Solapur, Maharashtra, India.

ABSTRACT

The Spectrophotometric methods Methanol: Water (80:20) used as a solvent. The wavelength used for Racecadotril and Ofloxacin sodium was 231 nm and 297 nm respectively. The simultaneous equation method was developed and validated according to the ICH guidelines. Racecadotril and Ofloxacin individually follow the Beer-Lamberts law over concentration range 5-25 µg/ml and 5–25 µg/ml respectively. Precision studies were carried out using parameter like intra-day and inter-day precision, the study showed that the results were within acceptance limit. i.e. % RSD below 2.0. The LOD was found to be 1.68µg/ml and 1.27µg/ml for Racecadotril & Ofloxacin respectively. The LOQ was found to be 5.11µg/ml and 3.84µg/ml. Accuracy of method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 99-101%.

KEYWORDS: LOD, LOQ, UV Spectrophotometry.

1. INTRODUCTION

Racecadotril (RS)-BenzylN-[(acetylthio)-2benzylpropanoyl] glycinate, also known as acetorphan, is an antidiarrheal drug which acts as a peripherally acting enkephalinase inhibitor. Unlike other opioid medications used to treat diarrhea, which reduce intestinal motility, racecadotril has an antisecretory effect-it decreases the secretion of water and electrolytes into the intestine.

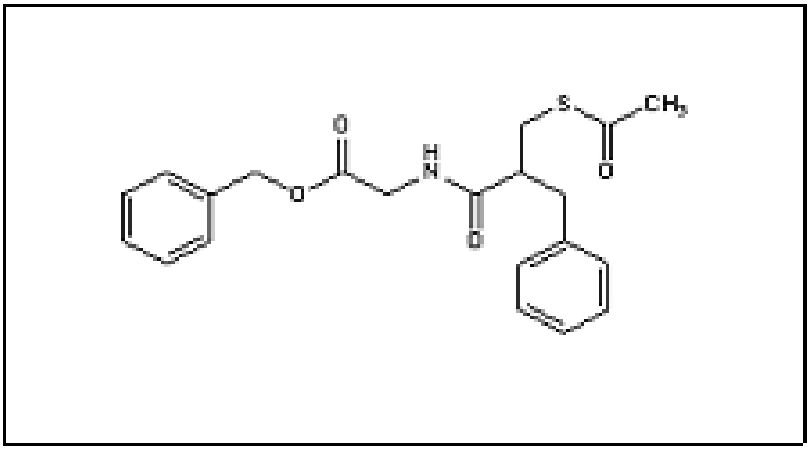

Fig. 1: Structure of Racecadotril.

Molecular Formula: C21H23NO4 S

Molecular Weight: 385.4766 g/mole

OfloxacinOfloxacin±9-Fluoro-2,3-dihydro-3-methyl piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxy-lic acid. is an antibiotic useful for the treatment of a number of bacterial infections. When taken by mouth or injection into a vein this includes pneumonia, cellulitis, urinary tract infections, prostatitis, plague and certain types of infectious diarrhea. Other uses, along with other medications, include multidrug resistant tuberculosis. An eye drop may be used for a superficial bacterial infection of the eye and an ear drop may be used for otitis media when there is a hole in the ear drum.

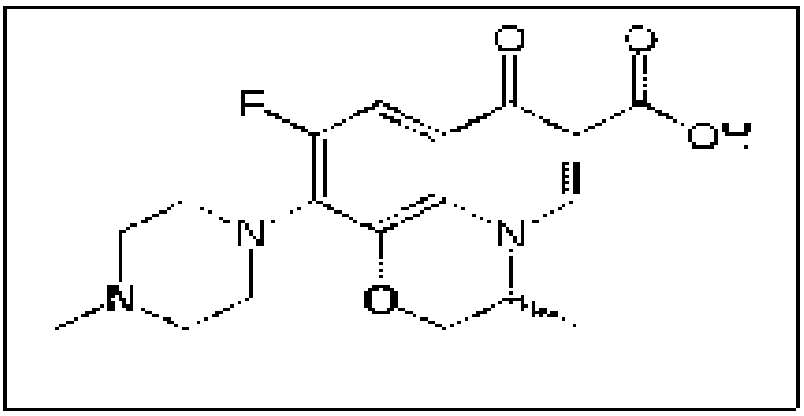

Fig. 2: Structure of ofloxacin.

Molecular Formula: C18H20N3O4

Molecular Weight: 385.47 g/mole

2. MATERIAL AND METHODS

i. Material

ii. Drug sample suppliers

Table No 1: Drug sample suppliers.

Sr. No.	Name of drug	Drug suppliers
1.	Racecadotril	Dr. Macs Biopharma Ltd.
2.	Ofloxacin	Dr. Macs Biopharma Ltd.

iii. Marketed preparation

Table No. 2: Marketed preparation.

Brand Name	Manufactured by	Content
Racigyl-O	Microlabs Ltd. Unit3	1. Racecadotril - 100mg
Tablet	Puducherry.	2. Ofloxacin – 200mg

3. EXPERIMENTAL

i. Standard stock solution of racecadottion

10 mg of Racecadotril was weighed accurately and transferred to a 10 ml volumetric flask, dissolved in sufficient quantity of acetonitrile and water (80:20) then sonicated for 15 min and diluted to 10 ml with the same solvent so as to get the concentration of 1000µg/ml.

ii. Standard stock solution of ofloxacin

10 mg of Ofloxacin was weighed accurately and transferred to a 10 ml volumetric flask, dissolved in sufficient quantity of Acetonitrile and water (80:20) then sonicated for 15 min and diluted to 10 ml with the same solvent so as to get the concentration of 1000 µg/ml.

iii. Determination of λ max of racecadotril

Standard solution of Racecadotril was prepared. The standard drug solution at different concentration was scanned on UV spectrophotometer in the range of 400- 200 nm and the λ max was found to be 231 nm against Acetonitrile: water. The UV spectrum is shown in (fig. No.3).

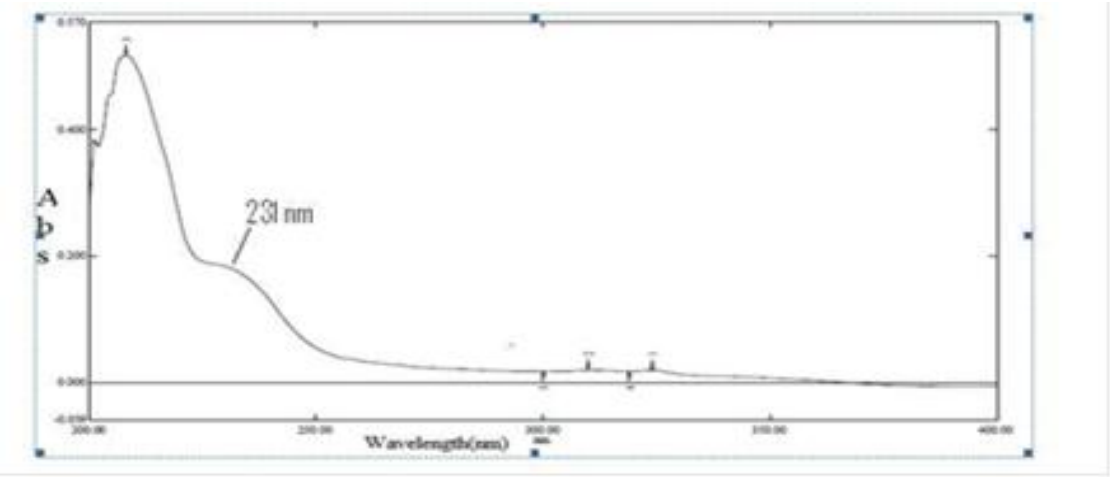

Fig. 3: Individual spectra of Racecadotril.

4. Determination of λ max of Ofloxacin

Standard solution of Ofloxacin was prepared. The standard drug solution at different concentration was scanned on UV spectrophotometer in the range of 400-200 nm & the λ max was found to be 297 nm against Acetonitrile: water. The UV spectrum is shown in (Fig. No.4.).

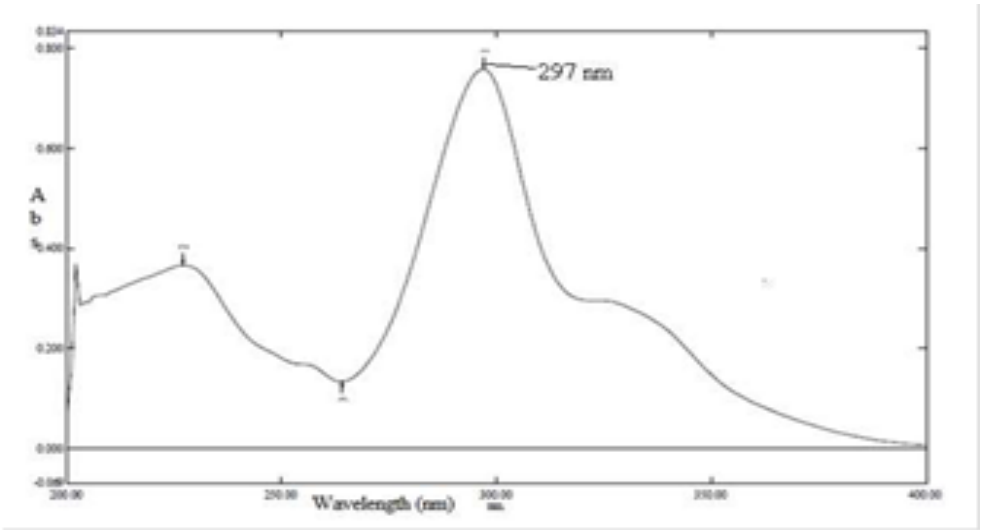

Fig. 4: Individual spectra of ofloxacin.

5. Determination of absorption maxima

Appropriate dilution of two drugs were prepared separately using standard stock solutions containing Racecadotril and Ofloxacin were scanned in the range of 400 nm to 200 nm to determine the wavelength of maximum absorption for both the drugs. Racecadotril and Ofloxacin showed absorbance maxima at 231 nm and 297 nm respectively. The overlain spectra showed λ max of both drugs is found to be 217 nm. (Fig. No.5).

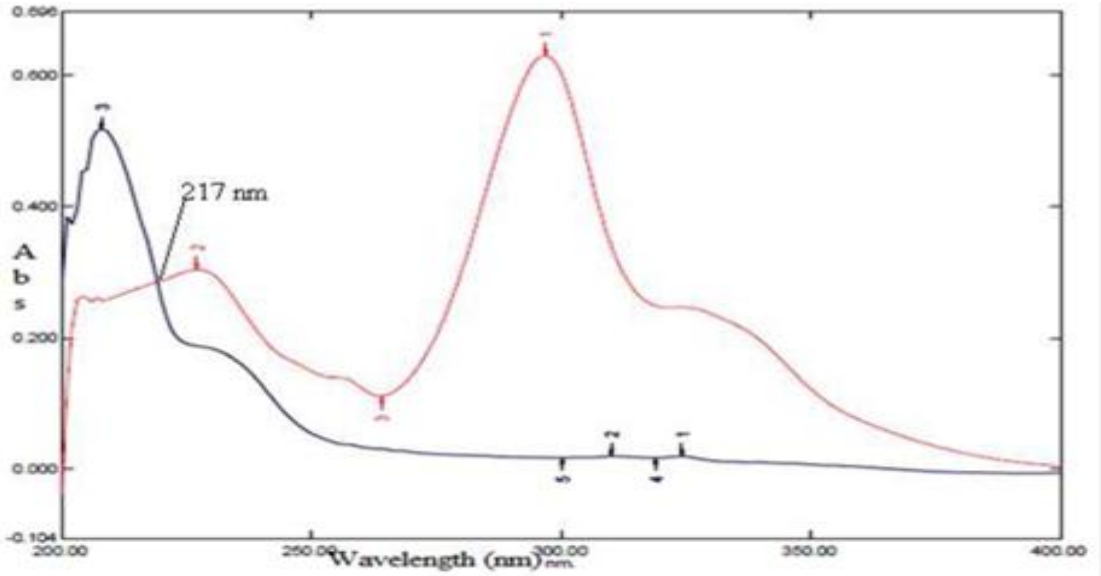

Fig. 5: Overlain spectra of Racecadotril & Ofloxacin.

6. Determination of absorptivity values

The standard drug solutions were prepared to get a concentration of 10 µg/ml of Racecadotril and 10 µg/ml of Ofloxacin and volume was made up to the mark with Acetonitrile: water. The absorbance of each solution was measured at 231 nm and 297 nm. The absorbance was then divided by concentration to get Absorptivity. The detail was shown in (Table No. 3.)

Table No. 3: Absorptivity value of Racecadotril and Ofloxacin.

Components	Absorptivity at 231nm	Absorptivity at 297 nm
Racecadotril (x)	ax1= 530	ax2 = 974
Ofloxacin(y)	ay1= 286	ay2= 91

7. Analysis of marketed formulation by proposed method

Ten tablets of brand name Racigyl-o were accurately weighed and were reduced to fine powder and mixed thoroughly. A quantity of tablet powder equivalent to Racecadotril (10 mg) and Ofloxacin (10 mg) was transferred to 10 ml volumetric flask and dissolved in Acetonitrile: water (80: 20) with vigorous shaking. The volume was made up to the mark using Acetonitrile: water. The solution was filtered through Whatman filter. The aliquot portion of filtrate was further diluted to get Racecadotril (10 µg/ml) and Ofloxacin (10µg/ml) respectively. The absorbance of sample solution was measured at 231.0 nm and 297.0 nm and the results are shown in (Table No. 4).

Table No 4: Result of analysis of Racecadotril and Ofloxacin in table formulation.

Sr. No.	Amount present in mg		Amount found in mg		% Assay	
	Racecadotril	Ofloxacin	Racecadotril	Ofloxacin	Racecadotril	Ofloxacin
1	100	200	99.31	199.14	99.31	99.57
2	100	200	100.12	198.98	100.12	99.49
3	100	200	99.74	200.12	99.74	100.06
4	100	200	99.14	198.21	99.14	99.105
5	100	200	100.24	200.9	100.24	100.45
Mean	-	-	-	-	99.71	99.735
SD	-	-	-	-	0.48342528	0.52464274
%RSD	-	-	-	-	0.48483129	0.52603673

8. Method validation

The method was developed and validated according to analytical procedure as per the ICH guidelines for validation of analytical procedures in order to determine linearity, precision, LOD, LOQ and accuracy for the analyte.

i. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to concentration of an analyte in the sample. The calibration plot was constructed after analysis of five different (from 5 to 25µg/ml for Racecadotril and (10 to 50µg/ml) for Ofloxacin) concentrations from standard stock solution respectively. Absorbances for each concentration were measured at 231 nm (λ max of Racecadotril) and 297 nm (λ max of Ofloxacin) respectively (Table 2). The calibration curves are shown in (Fig. 3). The result of Racecadotril and Ofloxacin optical parameter are as shown in (Table No.5).

Table No. 5: Linearity of racecadotril.

Sr. No.	Concentration	Absorbance
1.	5	0.186
2.	10	0.379
3.	15	0.591
4.	20	0.775
5.	25	0.942

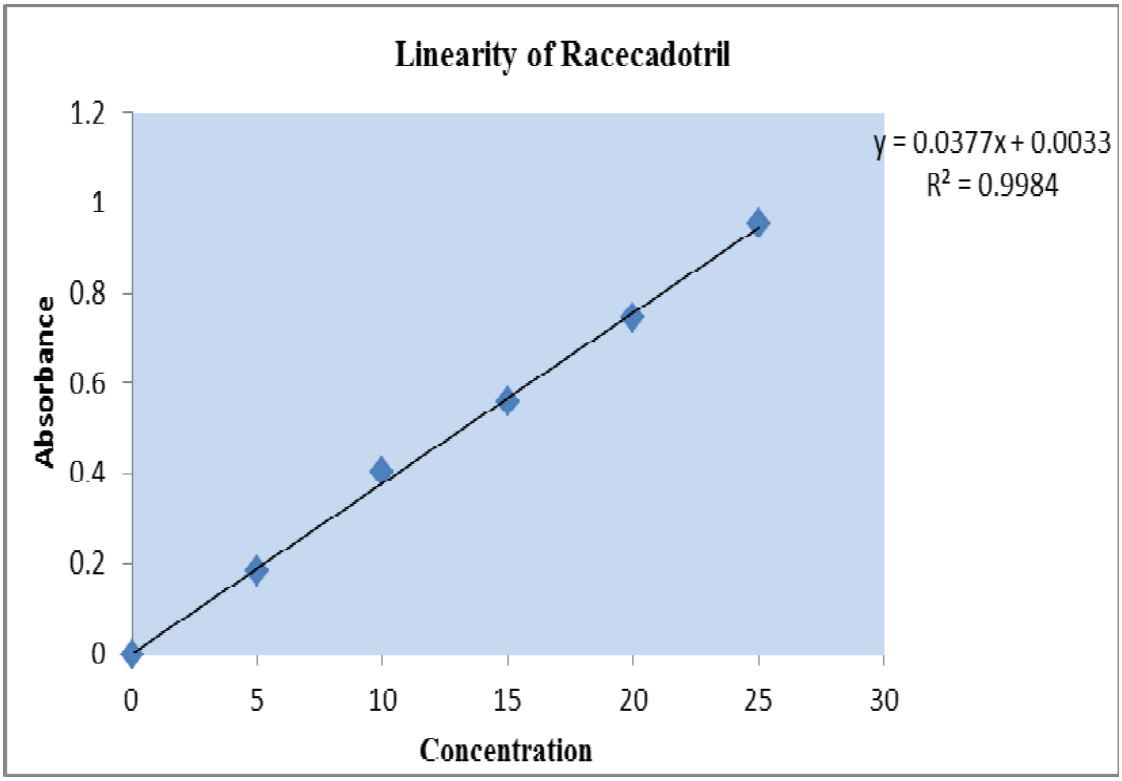

Fig. 6: Standard calibration curve of racecadotril.

Table No 6: Linearity of ofloxacin.

Sr. No.	Concentration	Absorbance
1.	10	0.309
2.	20	0.567
3.	30	0.7868
4.	40	0.987
5.	50	1.234

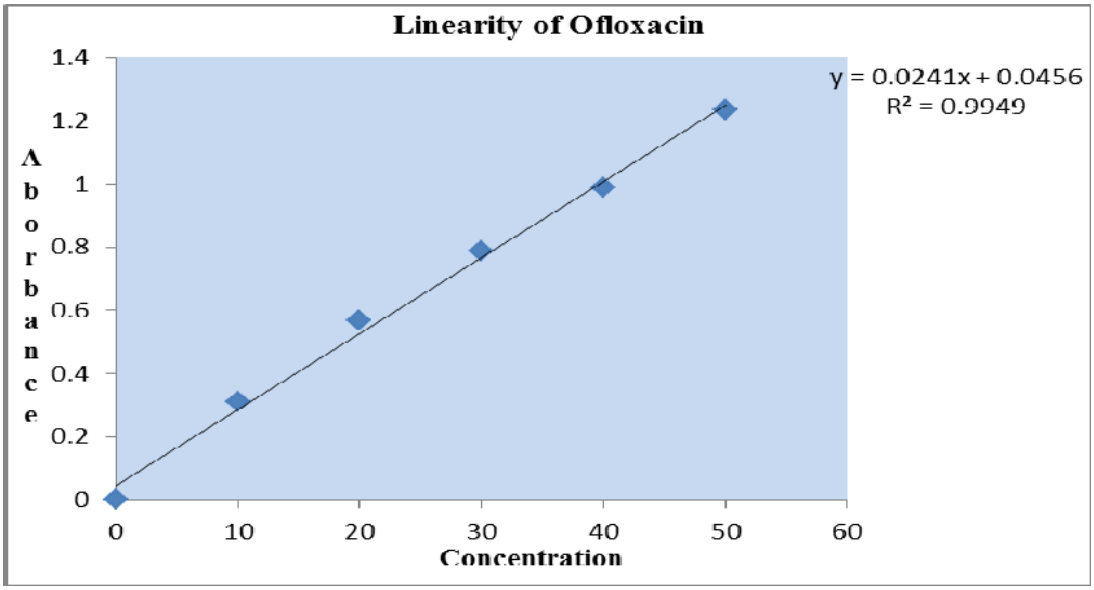

Fig. 7: Standard calibration curve of Ofloxacin.

ii. Precision

The method was established by analyzing various replicates samples of Racecadotril and Ofloxacin. Precision was carried out by performing inter-days variation and intra-day variation. In inter-days variation the sample was analyzed on three consecutive days. In intraday variation the absorbances were measured three times in a day. The results for intra-day precision are shown in (Table No.7,8) and for inter-day precision are shown in (Table No.9,10).

Table No. 7: Intra-day precision study of racecadotril.

Conc µg/ml	Absorbance			Mean Absorbance	SD	% RSD
	Trial 1	Trial 2	Trial3			
10	0.381	0.383	0.380	0.381	0.002	0.525
15	0.547	0.544	0.541	0.544	0.003	0.551
20	0.726	0.725	0.723	0.725	0.002	0.276

Table No. 8: Intra-day precision study of ofloxacin.

Conc. µg/ml	Absorbance			Mean Absorbance	SD	%RSD
	Trial 1	Trial 2	Trial3			
10	0.379	0.376	0.376	0.377	0.002	0.531
15	0.586	0.584	0.588	0.586	0.002	0.341
20	0.771	0.769	0.773	0.771	0.002	0.259

Table No. 9: Inter-day precision study of racecadotril.

Conc. µg/ml	Absorbance			Mean Absorbance	SD	%RSD
	Day 1	Day 2	Day3			
15	0.543	0.536	0.539	0.539	0.003	0.557
20	0.716	0.719	0.721	0.719	0.002	0.278
25	0.946	0.949	0.943	0.946	0.003	0.317

Table No. 10: Inter-day precision study of ofloxacin.

Conc µg/ml	Absorbance			Mean Absorbance	SD	%RSD
	Day 1	Day 2	Day3			
15	0.582	0.579	0.582	0.581	0.002	0.344
20	0.764	0.767	0.771	0.767	0.004	0.522
25	0.931	0.934	0.935	0.933	0.002	0.214

iii. Recovery studies

Recovery studies were performed to validate the accuracy of developed method. To preanalysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added, the absorbances are recorded at 231 nm, 297 nm.(λ max of Racecadotril &

Ofloxacin respectively) and then its recovery was analyzed and result was shown in (Table No. 11) and statistical validation of recovery studies shown in (Table No. 12).

Table No. 11: Recovery studies of Racecadotril and Ofloxacin.

Level of Recovery (%)	80		100		120	
	Race	Oflox	Race	Oflox	Race	Oflox
	10	20	10	20	10	20
Amount present (mg)	10	20	10	20	10	20
	10	20	10	20	10	20
Amount of Std. added (mg)	8 8 8	8 8 8	10 10 10	10 10 10	12 12 12	12 12 12
Level of Amount recovered(mg)	18.2 17.9 18.1	28.11 27.94 28.14	20.12 19.97 20.13	29.99 30.14 29.84	22.01 22.14 21.98	31.87 32.01 31.7
	101.1111	100.392	100.6	99.96667	100.04545	99.59375
% Recovery	99.44444	99.7857	99.85	100.4667	100.63636	100.0313
	100.5555	100.5	100.65	99.46667	99.909091	99.0625
M % R	100.3703	100.226	100.36667	99.96667	100.19697	99.5625
S D	0.848625	0.38520	0.4481443	0.5	0.3865864	0.48513
% RSD	0.845493	0.38433	0.4465071	0.500167	0.3858264	0.487262

Table No. 12: Statistical validation of recovery studies.

Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
80	RACE	99.92	0.716	0.717
	OFLOX	100.13	1.378	1.376
100	RACE	100.91	0.351	0.347
	OFLOX	99.81	0.652	0.653
120	RACE	99.50	0.710	0.713
	OFLOX	99.95	0.446	0.447

***Denotes average of three determinations.**

iv. LOD (Limit of detection) and LOQ (Limit of quantitation)

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in Result. (Table No. 13)

LOD = 3.3 (σ / S)

Where,

S = slope of calibration curve**,**

σ = standard deviation of the response.

The Limit of Quantitation (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Result (Table No. 13)

LOQ = 10 (σ / S)

Where,

S = slope of calibration curve**,**

σ = standard deviation of the response.

Table No 13: Result of LOD and LOQ.

Racecadotril		Ofloxacin	
LOD	**LOQ**	**LOD**	**LOQ**
1.68µg/ml	5.11 µg/ml	1.27 µg/ml	3.84 µg/ml

RESULT AND DISCUSSION

From the individual spectra of Racecadotril and Ofloxacin in methanol: water (Fig.No. 3,4) at concentration of 10 µg/ml of Racecadotril and 10 µg/ml Ofloxacin, two wavelengths 231 nm and 297 nm were selected for simultaneous estimation of drugs respectively. The relation between concentration and absorbance for individual drug was studied. Racecadotril and Ofloxacin individually follows the Beer-Lamberts law over concentration range 5-25 µg/ml and 5–25 µg/ml respectively. The absorptivity values for both the drugs were determined at the selected wavelengths for Racecadotril and Ofloxacin respectively**.** (Table No. 3.)

SUMMARY AND CONCLUSION

For the Spectrophotometric methods Methanol: Water (80:20) used as a solvent. The wavelength used for Racecadotril and Ofloxacin sodium was 231 nm and 297 nm respectively. The simultaneous equation method was developed and validated according to the ICH guidelines. The linearity, precision, LOD, LOQ, recovery by the simultaneous equation method was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, and economical for routine analysis of Racecadotril and Ofloxacin in bulk drug as well as formulations.

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