WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 5, 1798-1807. Research Article ISSN 2277– 7105

SIMULTANEOUS ESTIMATION OF RACECADOTRIL AND OFLOXACIN IN COMBINED TABLET DOSAGE FORM

Ashish kumar S. Makadiya[1]*, Laxman M. Prajapati[1], Amit K. Joshi[1], Mohammed Ali L. Kharodiya[1]

[1]Department of Quality Assurance, Shri B. M. Shah College of Pharmaceutical Education & Research, Modasa-383315, Gujarat, India.

Article Received on
28 Feb 2015,

Revised on 19 March 2015,
Accepted on 12 April 2015

***Correspondence for Author**
Ashish kumar S. Makadiya
Department of Quality Assurance, Shri B. M. Shah College of Pharmaceutical Education & Research, Modasa-383315, Gujarat, India.

ABSTRACT

A simple, accurate, precise and specific spectrophotometric method has been developed for simultaneous determination of racecadotril and ofloxacin in its combined tablet dosage form by using methanol as a solvent. The method involves absorbance correction based on measurement of absorbance at two wavelengths at 231 nm and 323.40 nm. Method follows Beer's linearity in the range of 10-24 µg/ml for racecadotril and 20-48 µg/ml Ofloxacin both. The mean % recoveries were found to be in the range of 99.22 – 100.66 % and 99.62 – 100.19 % for racecadotril and ofloxacin respectively. LOD and LOQ were found to be 0.1922µg/ml and 0.5826 µg/ml for racecadotril and 0.1063 µg/ml and 0.3223 µg/ml for ofloxacin respectively. Assay results of market formulation were found to be 99.24 % and 99.25 % for racecadotril and ofloxacin respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of racecadotril and ofloxacin in their combined Tablet dosage form.

KEYWORDS: Racecadotril, Ofloxacin, absorbance correction method, simultaneous, tablet.

INTRODUCTION

Racecadotril, 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 reduces the secretion of water and electrolytes into the intestine.[1] Chemically it is Benzyl N-[3-(acetylsulfanyl)-2-benzylpropanoyl]glycinate (Figure 1).[2] It is official in BP.[3]

Ofloxacin is a synthetic antibiotic of the fluoroquinolone drug class considered to be a second-generation fluoroquinolone.[4] Chemically ofloxacin is (±)-9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6 carboxylic acid (Figure 1).[2] It is official in IP, BP, USP, and EP.[3,5-8]

The review of literature revealed that various analytical methods involving spectrophotometry, HPLC, NMR have been reported for racecadotril in single form.[9-12] Several analytical methods including UV, HPLC, electrophoresis, chemilumiscence have been reported for ofloxacin in single form.[13-18] Several methods have also been reported for oflaxacin in combination with other drugs.[19-22]

To the best of our knowledge, there is no published spectrophotometric method for this combination. So, the present paper describes a simple, accurate and precise method for simultaneous estimation of racecadotril and ofloxacin in combined tablet dosage form by Simultaneous equation method. The developed method was validated in accordance with ICH guidelines and successfully employed for the assay of racecadotril and ofloxacin in their combined dosage form.[23]

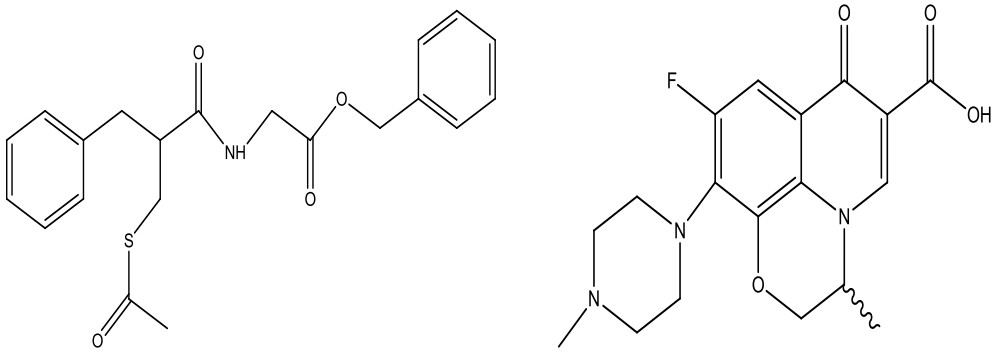

Figure 1: Chemical structure of (a) Racecadotril and (b) Ofloxacin

MATERIALS AND METHODS

Reagents and chemicals

Analytically pure racecadotril and ofloxacin were kindly provided Precious Pharma Ltd. Bombay, India and Megh Pharma Ltd. Modasa, India respectively as gratis samples. Analytical grade methanol was purchased from RFCL limited, New Delhi, India. Tablet of racecadotril and ofloxacin in combined dosage form, RACIGYL-O, was procured from local market.

Instruments

Two spectrophotometers were used for study, A Shimadzu UV/Vis 1800 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 2.32 software was used for all the spectral measurements and Shimadzu UV/Vis 1601 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm) and 1 cm matched quartz cells was used for reproducibility study.

Preparation standard stock solutions

Accurately weighed 100 mg of racecadotril and ofloxacin standard were transferred to a separate 100 ml volumetric flask and dissolved in 50 ml methanol. The flasks were shaken and volume was made up to the mark with methanol to give solutions containing 1000 µg/ml racecadotril and 1000 µg/ml ofloxacin. From this solution 10 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 100µg/ml of racecadotril and 100 µg/ml ofloxacin.

Absorbance correction method

This method involves the absorbance correction for racecadotril determination by substrating absorbance of ofloxacin from total absorbance of sample at 231nm (λmax of racecadotril). Ofloxacin concentration was determined directly from calibration plot by measuring absorbance at 323.40 (λmax of ofloxacin), where racecadotril shows zero absorbance. The equations obtained for the determination of concentration are

$$Cx = \frac{A1}{0.041 * b}$$

Where,

1) A1 is absorbance of sample at 323.40nm.

2) Cx is concentration of Ofloxacin in µg/ml.

$$Cy = \frac{A2 - (cx * 0.047)}{0.013}$$

Where,

1)A2 is absorbance of sample at 231 nm.

2)Cy is concentration of Racecadotril in µg/ml.

3) Cx is concentration of Ofloxacin in µg/ml.

Method validation

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (% RSD) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations. After validation, the developed method has been applied to pharmaceutical dosage form.

Linearity

Appropriate volume of aliquot from racecadotril and ofloxacin standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol to give solutions containing different concentration of racecadotril and ofloxacin. All absorbance were measured at 231 nm and 323.40 nm for racecadotril and ofloxacin respectively (n=8). Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 80, 100 and 120 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured.

Precision

The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. The intraday and interday precision study of racecadotril and ofloxacin was carried out by estimating different concentrations of racecadotril and ofloxacin, 3 times on the same day and on 3 different days (first, second, fifth) and the results are reported in terms of % RSD.

Detection limit and Quantitation limit

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ

were based on the third approach and were calculated according to the 3.3σ/S and 10σ/S criterions, respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

Determination of racecadotril and Ofloxacin in their Combined Dosage

Twenty tablets were weighed and powdered. A powder quantity equivalent to 100 mg racecadotril and 200 mg ofloxacin was accurately weighed and transferred to volumetric flask of 100 ml capacity. 60 ml of methanol was transferred to this volumetric flask and sonicated for 15 min. The above solution was filtered through whatman filter paper (0.45µ).The flask was shaken and volume was made up to the mark with methanol. From this solution 1 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 10µg/ml of racecadotril and 20µg/ml of ofloxacin. The resulting solution was analyzed by proposed methods.

RESULTS AND DISCUSSION

Owing to the solubility of Racecadotril and ofloxacin in the methanol it was selected as solvent. From overlain spectra of racecadotril and ofloxacin it is clear that racecadotril exhibits λmax at 231 nm and ofloxacin exhibits λmax at 323.4 nm (Figure 2). The overlain spectra of racecadotril and ofloxacin reveal that the racecdotril shows zero absorbance at 323.4nm (λmax of ofloxacin). So concentration of ofloxacin was directly measured at 323.4nm (λmax of ofloxacin). In this method two wavelengths are required. One wavelength is selected at which racecadotril shows maximum absorbance (231 nm), while second wavelength is selected at which racecadotril shows zero absorbance & ofloxacin shows absorbance (323.40 nm).

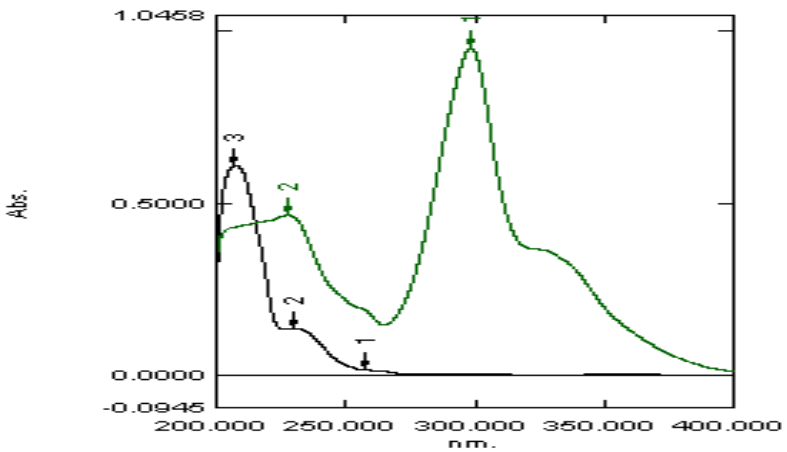

Figure 2: Overlain spectrum of Racecadotril (10µg/ml) and Ofloxacin (10µg/ml) in methanol

For racecadotril the Beer- Lambert's law is obeyed in concentration range of 10 to 24 µg/ml 231 nm (Table 1, Figure 3). For oflaxacin the Beer- Lambert's law is obeyed in concentration range of 20 to 48 µg/ml at 231 nm and 323.40 nm both (Table 2, Figure 4-5). The % recoveries were found to be in the range of 99.22 – 101.66% for racecadotril and 99.62-100.19 % for ofloxacin (Table 3). The precision of method was determined by repeatability, intraday and interday precision and was expressed as the % RSD, which indicates good method precision (Table 4). The Limit of detection for racecadotril and ofloxacin was found to be 0.192µg/ml and 0.1063 µg/ml respectively. Limit of quantification for racecadotril and ofloxacin was found to be 0.5826 µg/ml and 0.323 µg/ml at 323.40 nm (Table 3). There was no significant change in absorbance up to 24 hours of preparation of solution in methanol. The proposed spectrophotometric method was successfully applied to racecadotril and ofloxacin combined dosage form. Racecadotril and ofloxacin content in marketed tablet was found to be 99.24 % and 99.25 % respectively (Table 5).

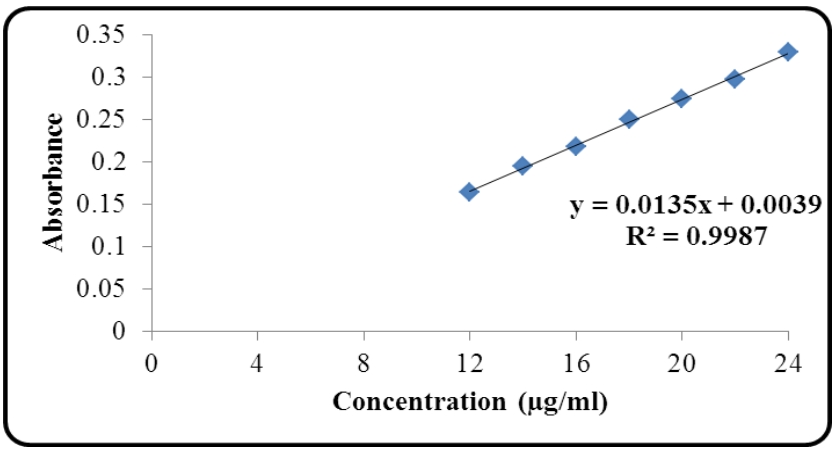

Figure 3: Calibration curve of racecadotril in methanol at 231nm.

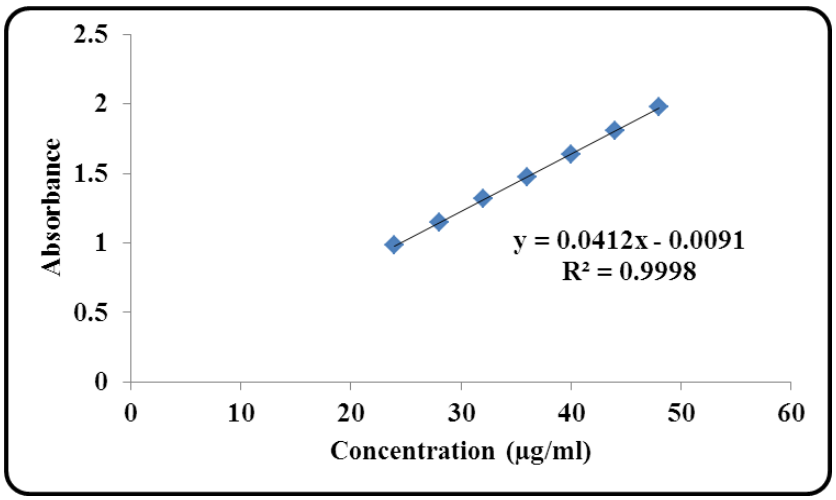

Figure 4: Calibration curve of ofloxacin in methanol at 323.4 nm

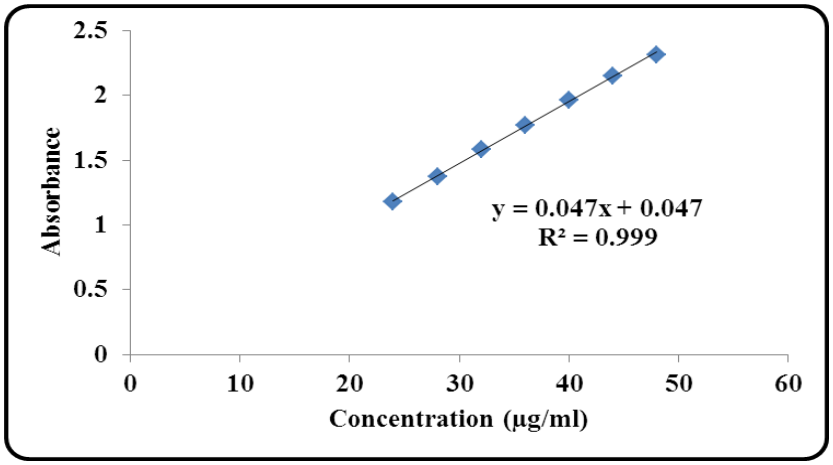

Figure 5: Calibration curve of ofloxacin in methanol at 231 nm

Table 1: Statistical data racecadotril by absorbance correction method

Parameters (n=3)	Racecadotril (231 nm)
Range	10-24 (µg/ml)
Slope	0.013
Intercept	0.003
Regression Coefficient (r2)	0.998
Standard deviation of Slope	0.000147
Standard deviation of Intercept	0.00124

Table 2: Statistical data ofloxacin by absorbance correction method

Parameters (n=3)	Ofloxacin (323.40 nm)	Ofloxacin (231 nm)
Range	20-48 (µg/ml)	20-48 (µg/ml)
Slope	0.041	0.047
Intercept	0.009	0.047
Regression coefficient (r2)	0.999	0.999
Standard deviation of Slope	0.000142	0.000184
Standard deviation of Intercept	0.00174	0.00165

Table 3: Accuracy data for racecadotril and ofloxacin by absorbance correction method

% Level (n=3)	Amount of drug added (µg/ml)		Amount recovered (µg/ml)		% Recovery	
	Racecadotril (µg/ml)	Ofloxacin (µg/ml)	Racecadotril (µg/ml)	Ofloxacin (µg/ml)	% Racecadotril	% Ofloxacin
80%	18	36	18.29	35.88	100.66	99.68
100%	20	40	20.19	39.85	100.99	99.62
120%	22	44	21.82	44.08	99.22	100.19

Table 4: Summary of validation parameters of absorbance correction method

Parameters (n=3)		Racecadotril	Ofloxacin
% Recovery		99.22-101.68	99.62-100.19
Repetability (n=6)		0.324	0.115
Precision	Inter day (n=3)	0.37-0.90	0.41-0.75
	Intraday (n=3)	0.24-0.54	0.14-0.65
Specificity		Specific	Specific
Solvent Suitability		Suitable for 24hrs	Suitable for 24hrs
Limit of Detection (µg/ml)		0.192	0.107 (323.4 nm) 0.113 (231 nm)
Limit of Quantitation (µg/ml)		0.586	0.323 (323.4 nm) 0.331 (231 nm)

Table 5: Assay results of marketed formulation

Formulation	Drug	Ammount taken (µg/ml)	Amount found (µg/ml) (n=3)	Labeled claim (mg)	Amount found per Tablet(mg)	% Label claim ±SD
RACIGYL-O TABLET	Racecadotril	10	9.92	100	99.24	99.24±0.02714
	Ofloxacin	20	19.85	200	198.5	99.25±.02714

CONCLUSION

The proposed absorbance correction method provides simple, precise, accurate and reproducible quantitative analysis for simultaneous determination of racecadotril and ofloxacin in combined dosage form. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of racecadotril and ofloxacin in combined dosage form.

Conflict of Interest

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

Authors are thankful to Precious Pharma Ltd. (Bombay, India) and Megh Pharma Ltd. (Modasa, India) for providing gift samples of racecadotril and ofloxacin.

REFERENCES

1. Matheson AJ, Noble S. Racecadotril. Drugs, 2000; 59(4): 829–35.
2. Budavari S. The Merck Index. 13th ed. Whitehouse Station, NJ: Merck and co., Inc; 2001.
3. British Pharmacopoeia, 6th Edn; Vol-II, British Pharmacopoeia Commission, London, 2010.

4. Nelson, JM, Chiller, TM, Powers, JH, Angulo, FJ. Fluoroquinolone-resistant Campylobacter species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. Clin Infect Dis, 2007; 44(7): 977–80.
5. Indian Pharmacopoeia, 13th Edn; Vol-II, The Indian Pharmacopoeia Commission, Ghaziabad, Govt. of India, Ministry of Health and Family Welfare, 2010.
6. United State Pharmacopoeia, 28th Edn; The United State Pharmacopeial Convention, Washington DC, Board of Trustees, 2005.
7. European Pharmacopoeia, 4th Edn, Vol-II, Published in accordance with convention on the elaboration of European Pharmacopoeia, 2004.
8. Tank P, Zanwar A, Seth AK and Kumar S, "Development of new analytical methods for quantitative estimation of racecadotril as an active pharmaceutical ingredient by UV spectrophotometer. Int J.Pharm Sci Res, 2012; 3(5): 1495-97.
9. Srinivasa RP, Nappinnai.M, UV and RP-HPLC estimation of Racecadotril. , Asian J Chem, 2007; 19(5): 3697-702.
10. Rao Seshagiri. Bhanu Prakash.P, Muralikrishna.M, Ravikumar.P. RP-HPLC method for the estimation of Racecadotril in bulk and in tablets. Asian J Chem, 2007; 19(4): 2623-26.
11. Prabu.SL, Singh.T, Joseph.A, Dinesh.C. Determination of racecadotril by HPLC in capsules, Indian J Pharm Sci, 2007; 69(6): 819-21.
12. Reddy KM, Babu JM, Sudhakar P, Sharma MS, Reddy GS, Vyas K. Structural studies of racecadotril and its process impurities by NMR and mass spectroscopy. Pharmazie, 2006; 61(12); 994-08.
13. Ev LS. and Schapoval EES. Microbiological assay for determination of ofloxacin injection. J Pharm Biomed Anal, 2002; 27: 91-96.
14. Fierens C, Hillaert S, Van den BW. The qualitative and quantitative determination of quinolones of first and second generation by capillary electrophoresis. J Pharm Biomed Anal, 2000; 22: 763- 72.
15. Liang YD, Song JF, Yang XF. Flow-injection chemiluminescence determination of fluoroquinolones by enhancement of weak chemiluminescence from peroxynitrous acid. Anal Chim Acta, 2004; 510: 21-28.
16. Francis PS, Adcock JL. Chemiluminescence methods for the determination of ofloxacin. Anal Chim Acta, 2005; 541: 3-12.
17. Du LM, Yang YQ, Wang QM. Spectrofluorometric determination of certain quinolone through charge transfer complex formation. Anal Chim Acta, 2004; 516: 237-243.

18. Burana-Osot J, Saowakul K, Charoensilpchai C, Surapeepong N. Stability-indicating HPLC method for determination of ofloxacin in bulk drug and tablets using trifluoroacetate as counter anions. Journal of Liquid Chromatography & Related Technologies, 2012; 35(13): 1909-19.
19. Dube A, Pillai S, Sahu S, Keskar N, Spectrophotometric estimation of cefixime and ofloxacin from tablet dosage form. Int J of Pharm & Life Sci, 2011; 2(3): 629-632
20. Premanand DC, Senthilkumar KL, Senthilkumar B, "A Validated RP-HPLC method for simultaneous estimation of nitazoxanide and ofloxacin in pharmaceutical formulation. Der Chemica Sinica,. 2010; 1(2): 1-5.
21. Gandhi VM, Nair SB, Menezes C, Narayan R, Development of UV-spectrophotometric method for the quantitative estimation of ofloxacin and ornidazole in combined liquid oral dosage form by simultaneous equation method. Int J Res.Pharm Chem, 2013; 3(1): 6-11.
22. Bhusari KP, Chaple DR, Simultanious spectrophotometric estimation of ofloxacin and ornidazole in tablet dosage form. Asian J Res Chem., 2009; 2(1): 60-62.
23. Validation of Analytical Procedure: Text and Methodology, ICH Harmonized Tripartite Guideline, Q2 (R1), 2005; 1-13.