



Application of High-Performance Thin-layer Chromatographic Method for Simultaneous Determination of Co-formulated Ofloxacin and Racecadotril in their Oral Dosage Form

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ABSTRACT

Objectives: This study aimed to develop and validate a simple, rapid, economical, precise and accurate HPTLC method for simultaneous determination of ofloxacin and racecadotril has been developed. **Methods:** Chromatographic separation was achieved using silica gel aluminum plate 60 F₂₅₄ (10*10) as a stationary phase and dichloromethane: methanol: tri-ethylamine (95:5:0.1 by volume) as a mobile phase. The developed plates scanned densitometrically using UV detector. Detection was carried out at 254 nm over the concentration range of 100-800 ng/spot for ofloxacin and 30-240 ng/spot for racecadotril. The R_f value of ofloxacin and racecadotril was found to be 0.15 and 0.85 respectively. **Results:** The method is validated for different validation parameter such as linearity, accuracy, precision, LOD, LOQ and robustness and the result were found to be within the acceptance limit as per the guideline of international conference on harmonization (ICH). **Conclusion:** The described TLC-densitometric method was successfully applied to simultaneous determination of ofloxacin and racecadotril in their pure forms and pharmaceutical dosage form without previous separation.

Keywords: Ofloxacin; Racecadotril; HPTLC method development; Simultaneous determination; ICH guidelines.

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 intestine¹. 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 fluoroquinolone⁴. Chemically ofloxacin is (±)-9-fluoro-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 BP, IP, USP, and EP.^{3,5-7}

The review of literature revealed that various analytical methods involving spectrophotometry, HPLC have been reported for racecadotril in single form.⁸⁻¹⁶ Several analytical methods including UV, HPLC, electrophoresis, chemiluminescence have been reported for ofloxacin in single form and in combination with other drugs.¹⁷⁻³⁷

The present paper describes a simple, accurate and precise method for simultaneous determination of racecadotril and ofloxacin in co-formulated oral dosage form. 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.³⁸

2. MATERIAL AND METHODS

Materials and Chemicals

Pure racecadotril (99.35%) and ofloxacin (99.55%) were kindly supplied by National Organization for Drug Control and Research, Giza, Egypt. Enuff-O[®] oral suspension was purchased from the Indian pharmaceutical market Batch No. E1705004 (labeled to contain 15 mg racecadotril and 50 mg ofloxacin per 5ml of constituted suspension). All chemicals and reagents used throughout this work were of HPLC grade. Methanol, dichloromethane and tri ethylamine were purchased from (Sigma-Aldrich, Germany). Whatman filter paper No 41.

Instruments

The chromatography was performed by a CAMAG HPTLC System with Linomat V Automatic Sample Applicator, while a 100 μ L syringe (Hamilton, Bonaduz, Switzerland) was used for sample application. Densitometric scanning was performed on a Camag HPTLC scanner III in the reflectance absorbance mode at 254 nm and operated by CATS software (V 3.15, Camag). The source of radiation utilized was deuterium lamp emitting continuous UV spectrum between 190 and 400 nm. Pre coated Silica Gel Aluminum plate 60F254, (10 \times 10cm; E. Merck) were used for separation of mixture components.

Preparation of standard stock solution

Ofloxacin stock solution

A stock solution of Ofloxacin (1000 μ g/ml) was prepared by dissolving 100 mg Ofloxacin in 100ml volumetric flask with Methanol. Withdraw 10ml of stock solution and dilute up to 100ml with methanol to prepare 100 μ g/ml.

Racecadotril stock solution

A stock solution of Racecadotril (1000 μ g/ml)

was prepared by dissolving 100 mg Racecadotril in 100ml volumetric flask with Methanol. Withdraw 3ml of stock solution and dilute up to 100 ml with methanol to prepare 30 μ g/ml.

Preparation of pharmaceutical sample solution

Enuff-O[®] 10 gm powder for 30ml suspension (each 5ml of constituted suspension labeled to contain 15 mg of racecadotril and 50 mg ofloxacin). A portion of powder equivalent to 1.667 gm was accurately weighed, transferred to 100 ml volumetric flask and the volume was made up to 50 ml with methanol. The solution was shaken vigorously for about 10 minutes, then sonicated for 30 minutes and filtered. The volume was completed to 100 ml with methanol to produce a stock solution labeled to contain 500 μ g/ml of Ofloxacin and 150 μ g/ml of Racecadotril, respectively.

Procedures

TLC-Densitometric Conditions

A pre-coated silica gel TLC plates were washed with methanol and dried at 60°C for 5 min in order to be activated. Samples were applied on these plates in the form of bands (6 mm length, 10 mm spacing, and 10 mm from the bottom edge of the plate). The plates were put in a chromatographic tank, previously saturated with the developing system consisted of dichloromethane: methanol: tri-ethylamine (9.5:0.5:0.1 by volume) for 30 min at room temperature. Ascending development of this developing system was preceded and the plates were air dried and finally scanned at 254 nm.

Construction of Calibration Graph

Aliquots equivalent to 100–800 μ g and 30–240 μ g of Ofloxacin and Racecadotril respectively were separately transferred from their standard solutions to a two set of 10 ml volumetric flasks and diluted to volume with methanol. Triplicate applications of 10 μ l from each solution were performed on the TLC plates to obtain the concentration range of 100–800 ng band⁻¹ and 30–240 ng band⁻¹ of Ofloxacin and Racecadotril respectively. The procedure under TLC-densitometric conditions was then followed. The peak area values were calculated and plotted against the corresponding concentrations of Ofloxacin and Racecadotril in ngband⁻¹ to get the calibration graphs. The regression equations were finally derived.

Assay of the Laboratory Prepared Mixtures:

Into a series of 10 ml volumetric flasks, aliquots of standard solutions equivalent to (100-800 μ g) and (30-240 μ g) of Ofloxacin and Racecadotril respectively, were transferred and diluted to volume

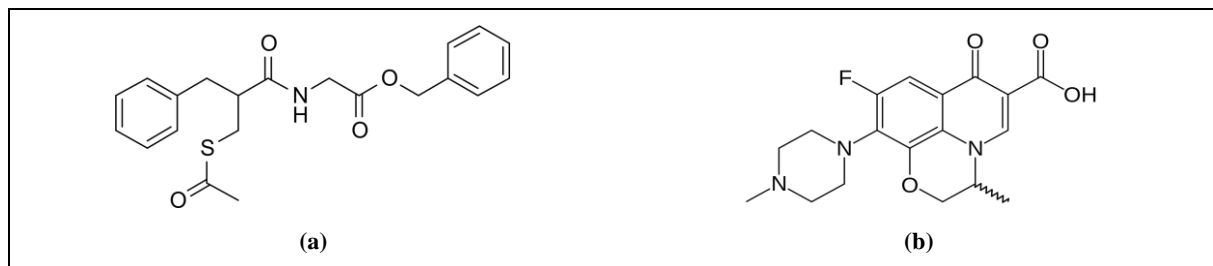


Figure 1. Structure formula of (a) Racecadotril and (b) Ofloxacin.

Table 1. Regression and validation data for determination of Ofloxacin and Racecadotril by the proposed method

Parameter	Ofloxacin	Racecadotril
Slope	13.903	64.22
Intercept	5845.5	5293.6
Coefficient of determination(r^2)	0.9992	0.9994
Range (ng band ⁻¹)	100–800	30–240
Accuracy (mean %R) *	100.02	99.66
Repeatability (%RSD) *	1.178	0.806
Intermediate precision (%RSD) *	1.391	1.123
LOD (ng band ⁻¹)	16.11	3.252
LOQ (ng band ⁻¹)	48.82	9.854
Robustness (%RSD)	Methanol volume \pm 2%	0.835
	Saturation time \pm 2 min	0.689

* Average of three determinations for three concentrations repeated three times.

Table 2. Determination of Ofloxacin and Racecadotril in Enuuff-O[®] powder for oral suspension by the proposed method and application of standard addition technique

Drug	Pharmaceutical taken (ng/spot)	Pharmaceutical found ^a (ng/spot)	Pure added (ng/spot)	Pure found (ng/spot)	% Recovery
Racecadotril	30	30.01	30	29.92	99.89
			60	60.83	100.94
			90	91.60	101.44
		Mean \pm %RSD			100.75 \pm 0.787
Ofloxacin	100	99.98	100	102.01	100.99
			200	202.12	100.70
			300	296.92	98.974
		Mean \pm %RSD			100.22 \pm 1.093

*Average of five determinations

**Average of three determinations.

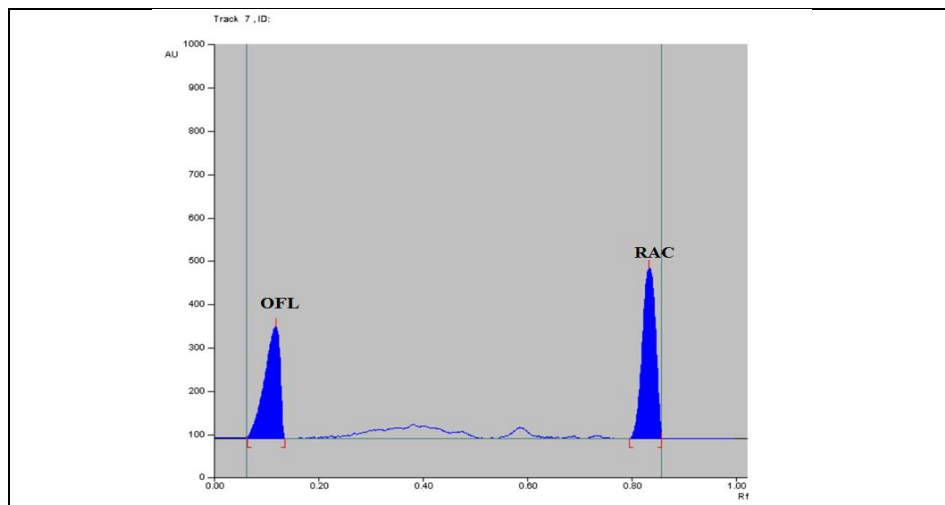


Figure 2. TLC-densitogram of OFL (200ng band⁻¹) and RAC (60 ng band⁻¹) using dichloromethane: methanol: tri-ethylamine (95:5:0.1 by volume) as a mobile phase with UV detection at 254 nm.

Table 3. System suitability testing parameters of the developed TLC–densitometric method

Parameters	Ofloxacin	Racecadotril	Reference value
Capacity factor (K)	1.03	9.76	1-10
Tailing factor (T)	0.676	1.16	< 2
Resolution factor (R)	7.216		> 2

Table 4. Statistical comparison of the results obtained by applying the proposed method and the reported method²³

Parameters	Proposed method		Reported method*	
	Ofloxacin	Racecadotril	Ofloxacin	Racecadotril
n**	5	5	5	5
%R***	99.96	100.44	100.13	100.45
%RSD	1.367	1.159	1.046	0.658
t-test (2.306) ****	0.222	0.017	—	—
F-test (6.388) ****	1.702	3.1	—	—

*UV spectrophotometric absorbance correction method for determination of racecadotril by subtracting absorbance of ofloxacin from total absorbance of sample at 231nm (λ_{max} of racecadotril). Ofloxacin concentration was determined directly from calibration plot by measuring absorbance at 323.40 (λ_{max} of ofloxacin), where racecadotril shows zero absorbance.

** Number of experiments.

*** The mean of percent recovery of pharmaceutical preparation.

**** The values in parenthesis are tabulated values of “t” and “F” at (P = 0.05).

with methanol. 10 µl of each solution were applied to a TLC plate following the above mentioned specific chromatographic conditions and scanned at 254 nm. The concentrations of Ofloxacin and Racecadotril have been calculated from the regression equations.

Application to Pharmaceutical Preparation

Aliquots from pharmaceutical sample solution solutions equivalent to (100-800 µg) and (30-240 µg) of Ofloxacin and Racecadotril respectively, were transferred into a series of 10 ml volumetric flask and the volume was completed with methanol. 10 µl of each solution were applied to a TLC plate following the above mentioned specific chromatographic conditions and scanned at 254 nm. The analysis was repeated in triplicate. The content of each drug in the Formulation was calculated by putting respective response into regression line equation for Ofloxacin and Racecadotril. The % recovery of the drugs was calculated and the results are given in **Table 2**.

RESULTS AND DISCUSSION

To date, The literature survey revealed that there is no separating technique had been developed for the determination and quantification of Ofloxacin and Racecadotril in its combined dosage forms. only spectrophotometric method (absorbance correction) were reported for simultaneous estimation of Ofloxacin and Racecadotril.³⁹. Simultaneous determination of co-formulated drugs is an important part in the field of pharmacy as it reduces the effort and time of extraction. This fact promotes our interest in the development of a simple and sensitive HPTLC method for the simultaneous quantitative determination of both drugs. TLC provides advantages over the previously reported technique in terms of higher sensitivity, fast analysis times, smaller quantities of solvents.

Method Optimization

Selection of suitable mobile phase is carried out by controlled trials and errors and search of the literature. To obtain the most appropriate mobile phase, several trials were tested. Different developing systems of different compositions and different percentages of each component were tried, such as methanol-acetonitrile- tri ethylamine (20: 80: 0.1, by volume), methanol- hexane-acetic acid (40: 60: 0.1, by volume), methanol- ethyl acetate- tri ethylamine (50: 50: 0.1, by volume) which gave poor resolutions, band broadening, tailing and an asymmetric peaks. Upon using a mixture of dichloromethane- methanol- tri ethylamine in different ratios, resolution was obtained with tailed bands to some extent until using the ratio (95: 5: 0.1, by volume) which gave the optimum resolution. Quantitative determination of Ofloxacin and

Racecadotril was performed by scanning the bands at 254 nm. The R_f values were 0.15 and 0.85 for Ofloxacin and Racecadotril, respectively **Figure 2**.

Method validation

Validation of the described methods was performed in a compliance with International Conference of Harmonization (ICH) guidelines [38].

Linearity and range

Under the optimum TLC-densitometric conditions, calibration graphs for Ofloxacin and Racecadotril were constructed by plotting the peak area values of the separated bands versus the drugs concentrations in ngband^{-1} . The regression plot was found to be linear over the range of 100–800 ngband^{-1} and 30–240 ngband^{-1} for Ofloxacin and Racecadotril, respectively. Values of slopes, intercepts and coefficient of determination (r^2) are presented in **Table 1**.

Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines from the following equations:

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where, σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

The standard solutions of Ofloxacin and Racecadotril were analyzed using the developed method and minimum detectable and quantifiable limits were measured, the results are given in **Table 1**.

Accuracy

Accuracy was calculated as a mean percent recovery of three determination for three concentration levels of standard solutions of Ofloxacin and Racecadotril and the results are presented in **Table 1**. Moreover, standard addition technique was applied to assess the accuracy and there was no interference from excipients **Table 2**.

Precision

Three replicate determinations of three different concentrations of Ofloxacin and Racecadotril in pure forms within linearity range were performed in the same day (repeatability) and on three successive days (intermediate precision) for the analysis of the three chosen concentrations using the proposed method. Acceptable % RSD was obtained, confirming the precision of the method as shown in **Table 1**.

Specificity

The specificity of the proposed procedure was assured by applying it to laboratory prepared mixtures

of Ofloxacin and Racecadotril using the standard addition technique. Laboratory prepared mixtures of Ofloxacin and Racecadotril are subjected to analysis by the proposed method in presence of the same concentration of pharmaceutical formulation. The obtained results were satisfactory as shown in **Table 2**. The method was suitable for the determination of the Ofloxacin and Racecadotril in raw materials and pharmaceutical formulation.

Robustness

The method was found to be robust, as it wasn't appreciably influenced by minor deviation in experimental parameters, e.g.: changing methanol volume in the developing system $\pm 2\%$ and changing saturation time ± 2 min. These proved by smaller values of RSD as shown in **Table 1**.

System suitability

System suitability parameters were applied to a representative chromatogram to confirm that, the system is working correctly during the analysis operation. Parameters including capacity factor (K), tailing factor (T) and resolution factor (R) were calculated to determine if the operating system were performed properly. The obtained values were in the acceptable ranges as shown in **Table 3**.

Application to Pharmaceutical Formulations

The described method was applied for determination of ofloxacin and racecadotril in Enuff-O[®] oral suspension. Satisfactory results were obtained in good agreement with the label claim. Standard addition technique was applied, and the results indicate no matrix interference. Statistical analysis of applying t-test and F-test at 95% confidence level of the results obtained by proposed method and those obtained by the reported method³⁹, indicate no significant differences, as shown in **Table 4**.

CONCLUSION

In this study; sensitive and selective TLC-densitometric procedure for the simultaneous determination of ofloxacin and racecadotril in their pure form and in their pharmaceutical preparation has been developed and validated. The developed TLC-densitometric procedure if compared to the reported methods, it has the advantage of being more sensitive and selective. Furthermore this TLC-densitometric procedures can replace the reported HPLC method when HPLC requirements are unavailable. The developed method is time saving where many bands can be run at the same time. This method is also economic since a small quantity of mobile phase as a developing system was used unlike HPLC procedures. Finally we

can conclude that the described TLC-densitometric procedure can be used in routine analysis of ofloxacin and racecadotril in their pure forms and pharmaceutical dosage form without previous separation.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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