

APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF RACECADOTRIL AND OFLOXACIN IN THEIR PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Racecadotril and ofloxacin are co-formulated for treatment of acute symptomatic diarrhea. In the present work RP-HPLC method was developed for simultaneous determination of racecadotril and ofloxacin in the combined dosage form. An isocratic separation was carried out on BDS Equisil C18 (150 X 4.6 mm, 5 μ m particle size) reversed phase column with a mobile phase consists of water and acetonitrile in the ratio of (20:80 % v/v) and pH 3 maintained by 0.1% ortho phosphoric acid. The flow rate was 1 ml/min and UV detection at 230 nm. The linear regression analysis of the calibration graphs showed a good linear relationship over a concentration range of 5-25 μ g/ml with main recovery percent 100.06 ± 0.952 and 99.93 ± 0.798 for racecadotril and ofloxacin, respectively. The proposed method was validated according to ICH guidelines, and has been successfully applied for simultaneous determination of both drugs in both bulk and commercial dosage form.

Keywords: Racecadotril, Ofloxacin, Simultaneous determination, International conference on harmonization.

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 (Eberlin et al., 2012). Chemically racecadotril is benzyl N-[3-(acetylsulfanyl)-2-benzylpropanoyl]glycinate, **Figure 1** (O'Neil et al., 2001). It is official in BP (Pharmacopoeia, 2010).

Ofloxacin is a synthetic antibacterial agent of the fluoroquinolone class (Nelson et al., 2007). Chemically ofloxacin is (\pm)-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** (O'Neil et al., 2001). It is official in BP, IP, USP, and EP (Pharmacopoeia, 2010; Pharmacopoeia, 2010 ; Pharmacopoeia, 2008; Pharmacopoeia, 2004).

The review of literature revealed that various analytical methods involving spectrophotometry, HPLC have been reported for determination of racecadotril in single dosage form (Akifulhaque et al., 2012; Annapurna et al., 2014; Bh et al., 2016; Kharodiya., 2015; Patel et al., 2012; Raju et al., 2011; Sankar and Arulantony, 2017; Tank et al., 2012; Vetrichelvan and Prabakaran, 2007; Yuanyuan et al., 2007). Several analytical methods including spectrophotometry, HPLC, electrophoresis, chemilumiscence have been reported for ofloxacin in single form and in combination with other drugs (Alnajjar, 2013; Bhusari and Chaple, 2009; Bindaiya et al., 2010; Chen et al., 2014; Hancu et al., 2012; John et al., 2015; Liu et al., 2015; Nebsen et al., 2013; Patel Sanket and Patel Satish, 2011; Patel Satish and Patel Natavarlal, 2011; Prabhu et al., 2010; Premanand et al., 2010; Puranik et al., 2010; Rane and Shinde, 2009; RP-HPLC, 2010; Singh et al., 2011; Sivakumar et al., 2012; Sun et al., 2010; Vinay et al., 2009; Wang et al., 2009; Zhu et al., 2017).

The present paper describes a simple, accurate and precise method for simultaneous determination of racecadotril and ofloxacin. 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 (ICH guidelines, 2005).

Experimental:

Materials and Chemicals:

Pure racecadotril (99.35%) and ofloxacin (99.55%), the purity was checked by applying the official method described in the British Pharmacopeia (Pharmacopoeia, 2010), were kindly supplied by National Organization for Drug Control and Research, Giza, Egypt. E努ff-O[®] powder for 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). Acetonitrile, HPLC grade (Sigma Aldrich, Germany), orth-phosphoric acid (El-Nasr company, Egypt). Whatman filter paper N^o 41.

Instruments:

HPLC Analytical (Agelint, USA), equipped with Diode-array UV-Visible detector and auto sampler injector. The chromatographic analysis was carried out using (Thermo ChromQuest 4.2.34, version 3.1.6) data analysis program.

Standard solutions:

A Standard stock solutions of racecadotril and ofloxacin were prepared separately by dissolving the appropriate amount of pure powder of the drugs in acetonitrile to yield a final concentration of 1mg/ml. Further, working solutions were obtained by serial dilutions of stock solutions with acetonitrile.

Chromatographic conditions:

At ambient temperature, isocratic separation was carried out on a BDS Equisil C18 column (150 mm X 4.6 mm ; 5 μ m particle size) using a mobile phase consists of water and acetonitrile in the ratio of (20:80 % v/v), pH 3 maintained by 0.1% ortho phosphoric acid. The mobile phase was degassed before pumping at the flow rate of 1 ml/min. Before injecting the solution, the column was equilibrated for at least 30 minutes with the mobile phase flowing through the system. The injected volume of the standard solution was 20 μ l and UV detection at 230 nm. The Solutions and the mobile phase were freshly prepared and filtered using membrane filter at the time of use.

Procedures:**Construction of calibration graphs:**

Accurately measured volumes (0.5-2.5 ml) of racecadotril and ofloxacin standard solutions (100 μ g/ml) were transferred into two separate series of 10 ml volumetric flasks and diluted to volume with the acetonitrile to obtain final concentrations of (5 – 25 μ g/ml) for both drugs. Twenty μ l aliquots were injected (in triplicate) and eluted with the mobile phase under the optimum chromatographic conditions. The average peak areas of racecadotril and ofloxacin were plotted versus the corresponding drug concentrations in μ g/ml to get the calibration graphs. Alternatively, the corresponding regression equations were derived.

Assay of the laboratory-prepared mixtures:

Aliquots of standard solutions equivalent to (100 μ g/ml) of racecadotril and ofloxacin were transferred into a series of 10 ml volumetric flasks keeping the ratio between them as in the pharmaceutical preparation (1:3.3 v/v respectively). The solutions were diluted to the volume with the acetonitrile and mixed well. Twenty μ l aliquots were injected (in triplicate) and eluted with the mobile phase under the optimum chromatographic conditions. The mean percent recoveries of each drug were calculated using the corresponding regression equation.

Application to pharmaceutical preparation:

Enuff-O[®] 10 gm powder for 30 ml suspension (each 5 ml 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 acetonitrile. The solution was shaken vigorously for about 10 minutes, then sonicated for 30 minutes and filtered. The volume was completed to 100 ml with acetonitrile to produce a stock solution labeled to contain 0.15 mg/ml of racecadotril and 0.5 mg/ml of ofloxacin, respectively. Different concentrations of racecadotril and ofloxacin have to be obtained by dilutions of the stock solution of each one

with the acetonitrile to covering the concentration range. Twenty μl aliquots were injected and eluted with the mobile phase under the optimum chromatographic conditions. Contents of both drugs were calculated using the corresponding regression equation.

RESULT AND DISCUSSION :

To date, The literature survey revealed that there is no separating technique had been developed for the determination and quantification of racecadotril and ofloxacin in its combined dosage forms. Only one spectrophotometric method (absorbance correction) were reported for simultaneous estimation of racecadotril and ofloxacin **Figure 2** (Kharodiya, 2015).

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 reversed phase HPLC method for the simultaneous quantitative determination of both drugs.

The HPLC technique has been chosen due to its ability to determine quantitatively many individual components present in mixture by single analytical procedure without preliminary separation. . The developed method if compared to the reported method, it has the advantage of being more sensitive, Furthermore it doesn't need extra mathematical processing steps as in spectroscopic methods.

Method development and optimization:

The chromatographic conditions were carefully optimized to determine the most optimum chromatographic parameters required for good separation and quantitative determination for both racecadotril and ofloxacin.

Firstly, the selection of the optimum wavelength was carried out to provide maximum sensitivity for both racecadotril and ofloxacin. By trial, a wavelength of 230 nm had been selected since it provided a maximum sensitivity for both racecadotril and ofloxacin. Then, different developing isocratic systems with different composition and ratios were tried for separation using C18 packing as a stationary phase. Isocratic elution exhibits some advantages over the gradient one, such as greater simplicity, lower cost, simpler instrumentation and no need of column re equilibration between consecutive injections.

The flow rate of the mobile phase is also very important parameter in the separation of the studied drugs. So, the effect of the flow rate and pH were studied consequently, the flow rate of 1 ml/min is the optimum and pH 3 is the best of choice since it keeps the drugs in its unionized form to increase hydrophobicity. Hence, the separation and resolution were improved.

A mixture of [acetonitrile and 1% ortho-phosphoric acid in water (pH 3) in the ratio of (80: 20, v/v)] was used as the mobile phase at a flow rate of 1 ml/min to exhibit good separation (reasonable resolution).

By optimization of the previous significant chromatographic condition, a chromatogram of racecadotril and ofloxacin was obtained with well-defined symmetrical peaks. In this chromatogram, both racecadotril and ofloxacin were separated with good resolution and their corresponding peaks were developed in retention times of 1.643 ± 0.017 and 2.094 ± 0.021 minutes for racecadotril and ofloxacin respectively, as shown in **Figure 3**.

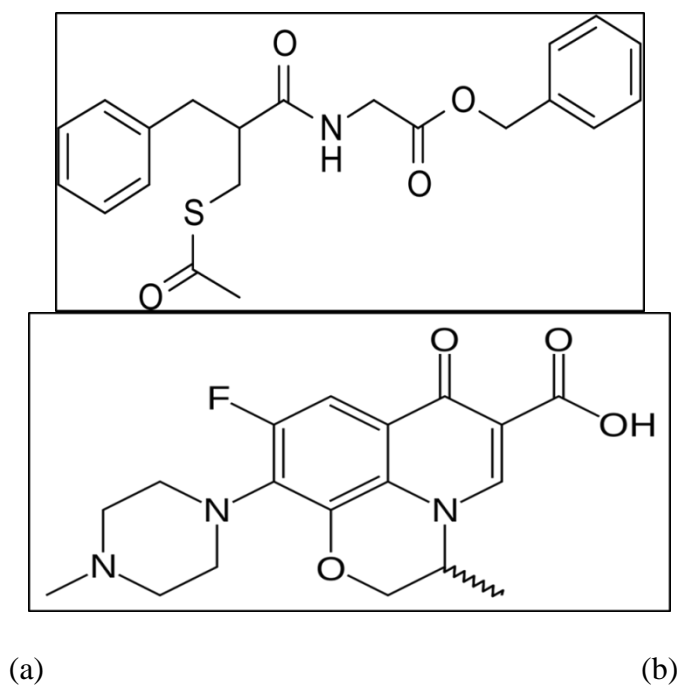


Figure 1: Structure formula of (a) Racecadotril and (b) Ofloxacin.

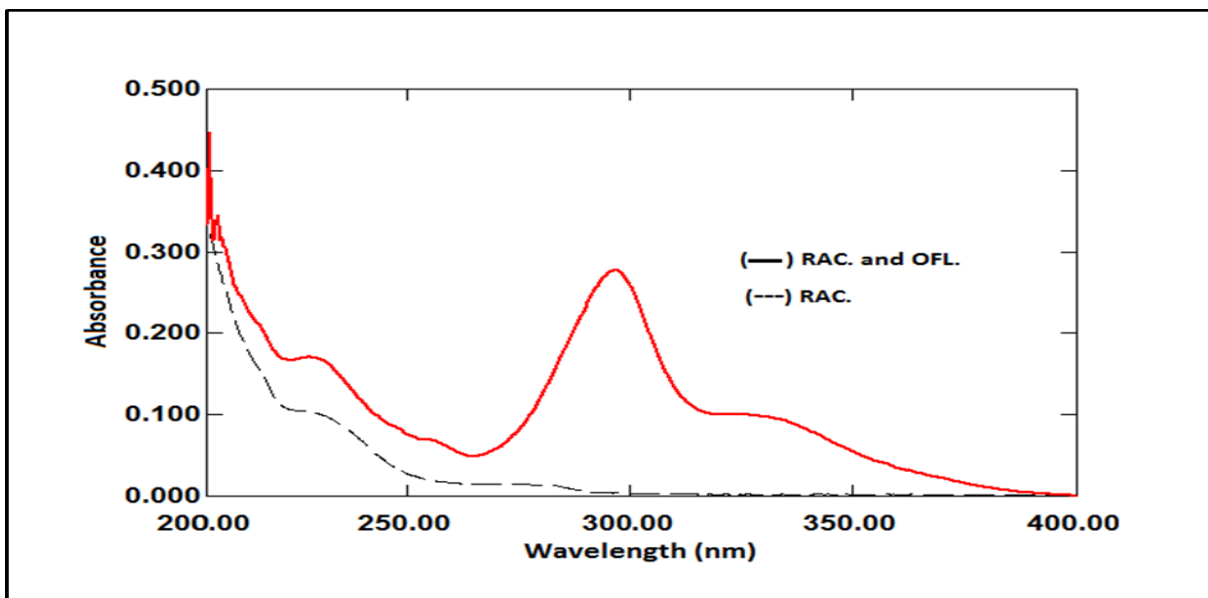


Figure 2: UV Absorption spectra of mixture of racecadotril (24 $\mu\text{g/ml}$), ofloxacin (24 $\mu\text{g/ml}$) and racecadotril (24 $\mu\text{g/ml}$) in methanol.

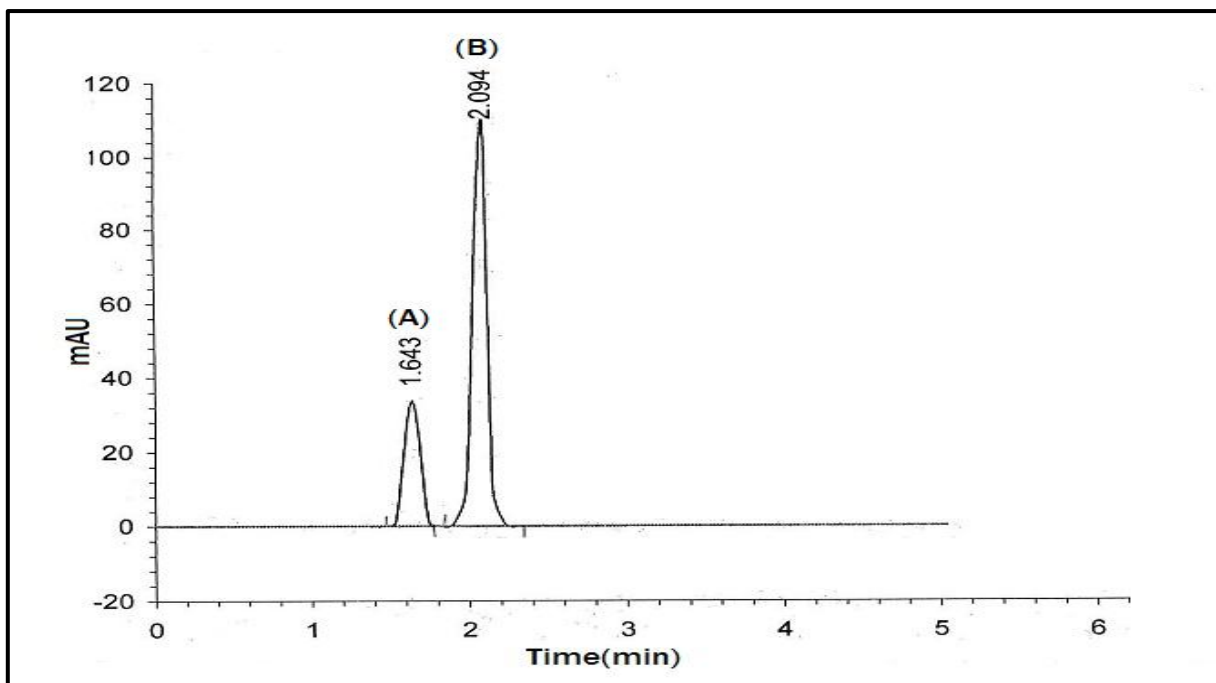


Figure 3: HPLC Chromatogram of racecadotril (A), (20 $\mu\text{g/ml}$) and ofloxacin (B), (20 $\mu\text{g/ml}$) at 230 nm.

Method validation:

Validation of the described methods was performed in a compliance with International Conference of Harmonization ICH guidelines (ICH guidelines,2005).

Linearity and range:

Under the described experimental conditions, the calibration graphs were constructed in the ranges of 5 – 25 µg/ml for both drugs by plotting the average areas of the chromatographic peaks versus drug concentrations in µg/ml for both racecadotril and ofloxacin. Linearity ranges, regression equations, intercepts, slopes and coefficient of determination of the calibration data were 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 racecadotril and ofloxacin were analyzed using the developed method and minimum detectable and quantifiable limits were measured, the results are given in **Table 1**.

Accuracy:

Accuracy of the proposed method was assessed by applying the proposed procedure for triplicate determination of three concentration levels (5, 10, 15 µg/ml for each drug). The mean percent recovery revealed excellent accuracy as shown in **Table 1**.

Precision:**Repeatability:**

Triplicate determinations of standard drug solutions at three different concentrations of racecadotril and ofloxacin within one day using the proposed method. The resultant of standard deviations was measured, as shown in **Table 1**.

Intermediate precision:

Triplicate determinations of standard drug solution at three different concentration levels of racecadotril and ofloxacin on three successive days using the proposed method. The % RSD was calculated and was found very satisfactory, as shown in **Table 1**.

Table (1): Regression and validation parameters of the proposed HPLC method.

Parameters		Racecadotril	Ofloxacin
Wavelength (nm)		230	230
Linearity range (µg/ml)		5 – 25	5 – 25
- Regression Equation		$y^* = b x^{**} + a$	$y^* = b x^{**} + a$
- Slope (b)		8.1403	26.2401
- Intercept (a)		-0.4948	2.2585
Coefficient of determination(r^2)		0.9999	0.9999
LOD (µg/ml)		0.825	0.782
LOQ (µg/ml)		2.500	2.371
Accuracy(%R)***		100.06±0.952	99.93±0.798
Precision (%RSD)****	Repeatability	0.914	0.890
	Intermediate precision	1.299	1.008

* Peak area of RAC or OFL.

** Concentration of RAC or OFL in µg/ml.

***Average of nine determinations (triplicate determination of three concentration level).

****%RSD of %Recovery of nine determinations (triplicate determination of three concentration level).

Specificity:

By visual inspection of the standard chromatogram, assay sample chromatogram, demonstrated that the separation of two drugs has a good resolution within suitable analysis time. To check the interference from the excipients present in the dosage form, the specificity of the proposed method was checked by applying the standard addition

technique by addition of known quantities of the studied drugs in their pure form to already analyzed commercial tablet and the percent recovery of the pure added of three experiments was calculated. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives, as shown in **Table 2**.

Table 2: Quantitative determination of both Racecadotril and Ofloxacin in Enuff-O[®] oral suspension by HPLC method using standard addition technique:

Drug	Pharmaceutical taken ($\mu\text{g/ml}$)	Pharmaceutical found* ($\mu\text{g/ml}$)	Pure added ($\mu\text{g/ml}$)	Pure found** ($\mu\text{g/ml}$)	%Recovery
Racecadotril	10	9.95	10	10.09	100.99
			20	19.85	99.26
			30	29.90	99.67
			Mean \pm %RSD		
Ofloxacin	10	10.06	10	10.00	100.06
			20	19.92	99.64
			30	30.20	100.68
			Mean \pm %RSD		

*Average of five experiments.

**Average of three experiments.

System suitability:

System suitability parameters were applied to a representative chromatogram to confirm that, the system is working correctly during the analysis operation. The final system suitability test parameters for the method were presented in **Table 3**.

Table 3: System suitability results for the determination of Racecadotril and Ofloxacin by the proposed HPLC method.

Parameters	Racecadotril	Ofloxacin	Reference value
Retention time (t_R)	1.630±0.017	2.097±0.021	---
Retention factor (K')	1.143	1.594	1-10
Theoretical Plates (N)	4526	5244	>2000
Tailing factor (T)	1.01	1.09	<2
Resolution factor (R_s)	2.678		>2

Robustness:

It was evaluated by slight changes in the chromatographic conditions such as flow rate (± 0.1 ml/min.) and mobile phase concentration. In each case, only one parameter was changed while other conditions were kept constant. These minor changes did not affect the separation and resolution of racecadotril and ofloxacin, confirming the reliability of the proposed method. The results are given in **Table 4**.

Table 4: Robustness results for the determination of Racecadotril and Ofloxacin by the proposed HPLC procedure:

Parameters		Retention time (t_R)		Tailing factor (T)		Resolution (R)
		RAC	OFL	RAC	OFL	
Flow rate (ml/min.)	0.9	1.644	2.091	1.057	1.125	2.452
	1	1.630	2.081	1.012	1.094	2.522
	1.1	1.614	2.067	1.003	1.055	2.631
Mobile phase ratio (water: acetonitrile)	22:78	1.646	2.072	1.026	1.055	2.749
	20:80	1.639	2.086	1.015	1.078	2.396
	18:82	1.627	2.094	1.009	1.117	2.225

4.Application:

The proposed method was successfully applied for the simultaneous determination of racecadotril and ofloxacin in Enuff-O[®] oral suspension without interference of the excipients and additives and without prior separation. The obtained results were statistically compared to those obtained by the reported method (Kharodiya, 2015). No significant differences were found by applying t-test and F-test at 95% confidence level, as shown in Table 5.

Table 5: Determination of Racecadotril and Ofloxacin in Enuff-O[®] oral suspension by the proposed HPLC method and the reported methods.

Parameters	Proposed HPLC method		Reported method* [39]	
	Racecadotril	Ofloxacin	Racecadotril	Ofloxacin
Mean	99.63	100.23	100.45	100.13
%RSD	0.709	0.493	0.658	1.046
Number of measurements	5	5	5	5
Student's <i>t</i> -test	1.441 (2.306)**	0.537 (2.306)**	—	—
<i>F</i> -value	1.933 (6.388)**	1.242 (6.388)**	—	—

*UV Spectrophotometric method (absorbance correction) for determination of racecadotril by substrating 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 (Kharodiya, 2015).

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

Conclusion:

From the above discussion, we can conclude that the proposed method was found to be accurate, precise and sensitive for simultaneous determination of racecadotril and

ofloxacin in both bulk and commercial dosage form. The proposed method suitable for routine analysis and quality control laboratories.

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تطبيق طريقة الكروماتوغرافيا السائلة عالية الأداء لتحديد المتزامنة لسباق السكروتريل والأوفلوكساسين في شكل جرعة الدواء

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الخلاصة:

راسيكادوترل و اوفلكساسين يتم صياغتهما بشكل مشترك لعلاج الإسهال الحاد الأعراض.

في العمل الحالي ، تم تطوير طريقة RP-HPLC من أجل التحديد المتزامن لراسيكادريل وأوفلوكساسين في شكل جرعة مشتركة.

تم إجراء فصل انفرادي على عمود المرحلة العكسي BDS Equisil C18 (بحجم 150×4.6 مم ، 5 ميكرون) مع مرحلة متقلة تتكون من الماء وأسيتونيتريل بنسبة (20 : 80 % ت / ت) والحفاظ على درجة الحموضة 3 بنسبة 0.1 % حمض الفوسفوريك أورثو. كان معدل التدفق 1 مل / دقيقة واكتشاف الأشعة فوق البنفسجية عند 230 نانومتر. أظهر تحليل الانحدار الخطي لرسومات المعايرة وجود علاقة خطية جيدة على مدى تركيز يتراوح بين 5-25 ميكروغرام / مل مع انتعاش رئيسي بنسبة 100.06 ± 0.952 و 99.93 ± 0.798 لسباق كادوتريل وأوفلوكساسين ، على التوالي. تم التحقق من صحة الطريقة المقترحة وفقاً لإرشادات ICH، وتم تطبيقها بنجاح من أجل التحديد المتزامن لكل من العقاقير في شكل جرعة سائبة أو تجارية.