Original Article

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Analysis of Racecadotril in Pharmaceutical Formulations Using Ultra Performance Liquid Chromatography (UPLC) Method

Ultra Performanslı Sıvı Kromatografisi (UPSK) Yöntemi Kullanılarak Rasekadotrilin Farmasötik Formülasyonlarda Analizi

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ABSTRACT

Objective: In this study, a simple and selective ultra-performance liquid chromatography method for the determination of racecadotril in pharmaceutical dosage forms has been developed.

Methods: The method is in reverse phase C18 column; based on the chromatographic analysis of racecadotril with acetonitrile-water (70: 30, v/v) mobile phase at a flow rate of 0.7 mL/minimum.

Results: The most suitable wavelength for racecadotril has been determined 220 nm. The linearity range for racecadotril was found 5-100 µg/mL.

Conclusion: This method was applied to the analysis of racecadotril drug substance in tablets. The results were evaluated statistically. The developed method is easy, precise and repeatable and can be used safely in racecadotril pharmaceutical dosage forms analysis.

Keywords: Racecadotril, ultra-fast liquid chromatography, pharmaceutical dosage form analysis, validation, degredation product

ÖZ

Amaç: Bu çalışmada, rasekadotril'in farmasötik preparatlarda tayini için basit ve seçici yeni bir yüksek performanslı sıvı kromatografisi yöntemi geliştirilmiştir.

Yöntemler: Yöntem ters fazlı C18 kolonda; asetonitril-su (70: 30, h/h) hareketli fazı ile 0,7 mL/minimum akış hızında rasekadotril'in kromatografik olarak analiz edilmesi esasına dayanır.

Bulgular: Rasekadotril için en uygun dalga boyu 220 nm olarak belirlenmiştir. Rasekadotril için doğrusallık aralığı 5-100 µg/mL olarak bulunmuştur.

Sonuç: Elde edilen sonuçlar istatistiksel olarak değerlendirildi. Geliştirilen yöntem kolay, kesin ve tekrarlanabilir olup rasekadotril'in farmasötik preparatlarının analizlerinde güvenle kullanılabilir.

Anahtar Sözcükler: Rasekadotril, ultra fast sıvı kromotografisi, farmasötik preparat analizi, validasyon, bozunma ürünleri

Introduction

Diarrhea is defined as three or more soft or watery stools per day (or more than a normal defecation habit). Acute gastroenteritis is defined as softening of stool consistency and/or increased frequency (3 or more per day). Cholera is a pathogen in the group of bacterial gastroenteritis and causes acute diarrhea without causing intestinal damage (1). Thiorphan is an inhibitor of cell membrane peptidase enzyme enkephalinase, which is found in various tissues, especially in the epithelium of the small intestine. This enzyme ensures the digestion of exogenous peptidase and degradation of endogenous peptidase such as enkephalin. Racecadotril (acetorphan) is a prodrug that hydrolyzes to its active metabolite, thiorphan (Figure 1) (2). Racecadotril protects enkephalins from enzymatic degradation. Thus, it prolongs

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its effect on enkephalinergic synapses in the small intestine and reduces hypersecretion. Racecadotril is a pure intestinal antisecretory drug. It reduces water and electrolyte loss caused by cholera toxin or inflammation and does not affect basal secretory activity. Racecadotril rapidly performs its antidiarrheal effect without changing the duration of intestinal transit (3).

When the analyzes of racecadotril in pharmaceutical preparations were examined, it was found to be analyzed by spectrophotometric (4,5), HPTLC (6), and HPLC (7-14). In this study, it was planned to develop a new method for the analysis of racecadotril in tablets, using ultra-performance liquid chromatography (UFLC) method, which was more sensitive, reproducible and had a shorter analysis time than high-performance liquid chromatography (HPLC). This proposed method was also validated and its applicability to analyzes in pharmaceutical preparations was tested.

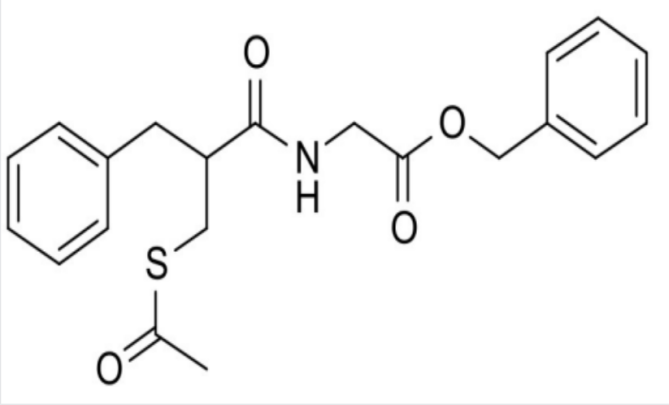

Figure 1. Chemical structure of racecadotril

Methods

Chemicals and Solvents

The racecadotril substance was obtained from Ilko Pharmaceuticals. The pharmaceutical preparation was purchased from the pharmacy (raxerin 10 mg & 20 mg Tablet).

Acetonitrile: Merck (Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur. Acetonitrile CAS 75-05-08, molar mass 41.05 g/mol, and chemical formula CH_3CN.)

Racecadotril Standard Solution

Stock solution: Twenty-five mg of racecadotril was weighed exactly and transferred to a 25 mL flask, 10 mL of dilution solution (as mobile phase) was added and dissolved with the help of vortex, and its volume was completed with the mobile phase (equivalent to 1 mg/mL racecadotril). Racecadotril stock solution is stable for approximately 29 days at +40 °C.

Tablet Sample Solutions

80.0 mg of raxerin 10 mg dispersible tablet and raxerin 30 mg dispersible tablet powders were weighed separately, transferred to a 100 mL flask, 50 mL of mobile phase was added, dissolved in an ultrasonic bath for about 30 minutes and allowed to cool, the volume was completed with mobile phase (0.1 mg/mL). Stock solution is stable for approximately 29 days at +40 °C, 7 days at room conditions.

Chromatographic System

UFLC Device (Shimadzu Nexera X2, Binary Pump)

LC 20AB pump system, SPD M20A Detector (Photo Diode Array), LC Solution System software, SIL 20AC Autosampler,

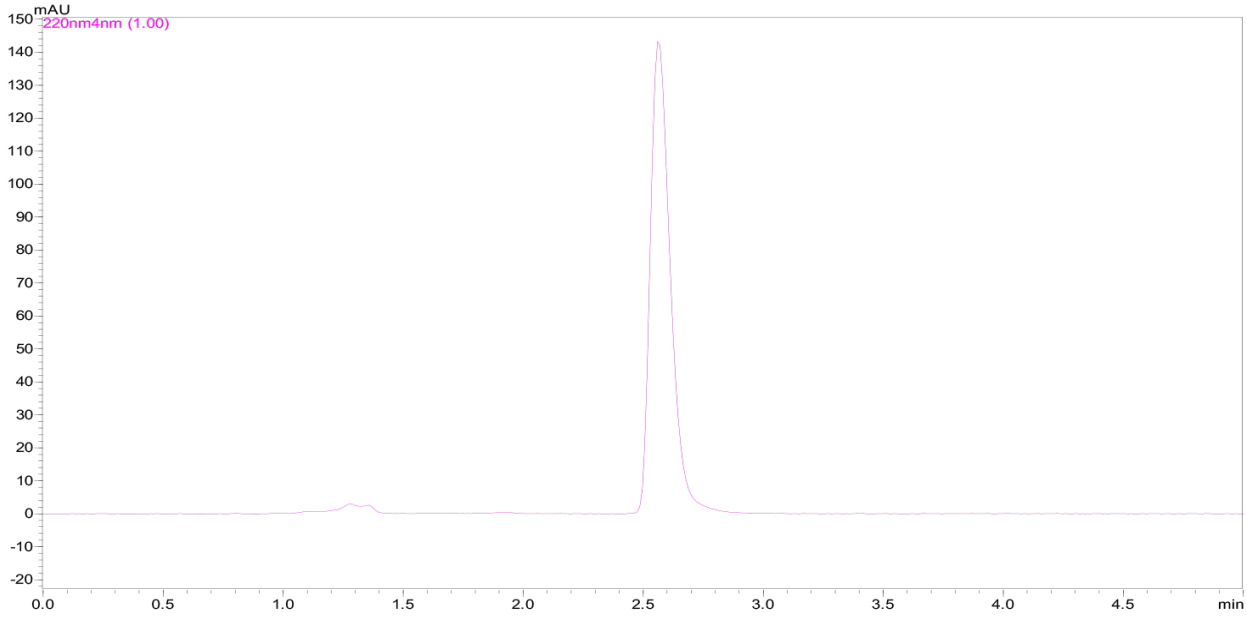

Figure 2. Standard chromatogram of racecadotril at 220 nm wavelength

CTO-10As Column furnace, Analytical column: Inertsustain C18 (4.0x100 mm, 3 µm; GL Sciences, Tokyo- JAPAN). Mobile phase is consisted of 70% ultra pure water & 30% acetonitrile

Method Validation

After method development was completed, analytical method validation was performed according to ICH guideline Q2(R1) (15).

Acuracy

The standard addition method was used to determine the accuracy of the developed method. For this purpose, 40 mg of tablet powder in two different forms (10 mg-30 mg) was weighed separately and transferred to a 10 mL balloon. 2, 5, 10 µg/mL concentrations of racecadotril standard solutions were added onto the sample solutions at a concentration of 5 µg/mL and completed to 10 mL with mobile phase (dilution solution).

Inter-day and Intra-day Reproducibility

In order to determine the precision of the method, a reproducibility study was carried out for 6 repetitions for 3 different days for solutions with active substance content at a concentration of 20 µg/mL.

Lineer Range

In order to show this linearity range, solutions containing racecodotril at 5 different concentrations were prepared. Peak areas and concentration versus linearity plots obtained from the chromatograms were plotted.

Robustness study

Three parameters were determined for the robustness test of the method. (Column temperature change (±2 ºC), Filter effect (PTFE, PVDF, Nylon), Flow rate change (±0.1 mL/min). The applicability of the analysis method in these different conditions was tested.

Solution Stability

In order to determine the racecadotril solution stability, standard solutions and sample solutions were kept at room temperature for 7 days and at +4 ºC for 29 days.

Degredation process

In this study, standard solutions with a concentration of 20 µg/mL were prepared. These solutions were subjected to temperature, oxidation, acidic and basic stress conditions and analyzed.

Analysis of Racecadotril in Pharmaceutical Preparations

Analysis with the Developed Method

In order to see the applicability of this developed method in tablets, 80 mg of the powder of the tablets in two different forms (10 mg and 30 mg) was weighed and transferred into a 10 mL flask. Adding 50 mL of mobile phase, it was dissolved in an ultrasonic bath for about 30 minutes, allowed to cool, and completed to its volume with mobile phase (0.1 mg/mL). Two mL of this prepared stock solution was taken and transferred to a 10 mL balloon flask, and its volume was completed with mobile phase (20 µg/mL).

Analysis by Comparison Method

The racecadotril method in the Pharmacopeia was determined as the comparison method (16). By following the sample preparation conditions in the Pharmacopoeia method, separate solutions were prepared for 10 mg and 30 mg tablets with a concentration of 20 µg/mL.

Results

Determination of Chromatographic Conditions

As a result of the experiments, the best results were obtained in a C18 column with a length of 10 cm, an inner diameter of 4.0 mm, a particle diameter of 3 µm, acetonitrile-water (70:30) mobile phase system, at 30 ºC, at a flow rate of 0.7 mL/min. The PDA detector was studied in the range of 190-300 nm, and the most sensitive results were obtained at a wavelength of 220 nm. The retention time in this chromatographic system is 2.56 minutes for racecadotril. In Figure 2, the standard chromatogram of racecadotril at 220 nm wavelength is indicated.

Accuracy

The standard addition method was used to determine the accuracy of the developed method.

The data obtained as a result of the analysis and the recovery percentages were calculated from the formula $(Ct-C_1) \times 100/C_2$.

Ct: Total concentration of racecadotril found,

C_1: Analyte concentration from the pharmaceutical preparation,

C_2 : The concentration of the added standard solution.

Recovery results were found in the range of 98.28-101.17% (Table 1). The results obtained proved that the additives in the tablets did not interfere.

Inter-day and Intra-day Reprocubility

Relative standard deviation (RSD) values of the analyzes performed on the same day, with 6 repetitions on three different days, were calculated between 0.25-0.40. The intraday standard and RSD between tablets were calculated as 0.57% (Table 2). The RSD values obtained as a result of the analyzes performed on different days were calculated between 0.47 and 0.49. The RSD between the standard and tablets for the different day was calculated as 0.35% (Table 2). The results obtained were the analysis values for three consecutive days.

Lineer Range

For racecadotril, 5 different concentrations of standard solutions were studied in the concentration range of 5-100 µg/mL. The line equation was found to be y=10362x+9666.4 r^2=0.9989.

Robustness study

Different conditions were applied to determine the robustness of the developed method. As a result of the analysis, the retention time at 0.60 mL/min flow for the flow test was 2.98 min; it was observed as 2.25 min for 0.80 mL/min and 2.55 min for normal conditions. The data obtained are shown in Table 3.

Solution Stability

To determine the solution stability, standard and sample solutions were kept in the dark for 7 days at room temperature, 24 hours and 48 hours in the automatic sample sampler, and 29 days at +4.00 °C. As a result of the evaluations, it was determined that the solutions remained stable. There was no deviation of more than 1.00% within 48 hours.

Degradation Procedure

Stress studies were carried out to show the effect of the related stress conditions in the developed method. The results are shown in Table 4. Chromatograms are shown in Figure 3.

Analysis of Racecadotril with the Method Developed in Pharmaceutical Preparations

It was studied to see the applicability of the developed method on tablets. The amount of racecadotril obtained in tablet samples was calculated using the measurement curve equation. In addition, the comparison method was also studied (16). The results obtained and the t- and F-test values are shown in Table 5.

Discussion

In this study, a new method was developed for the analysis of racecadotril in tablets, using UFLC method, which was more sensitive, reproducible and had a shorter analysis time than HPLC. The UFLC method offers benefits as an alternative to conventional HPLC. It does not only reduce the period required to complete the analyses and conserves the solvent used for the process, but it also allows for separating and determining the drug substance reliably in pharmaceutical formulation. These advantages of the UFLC method provides increased efficiency

Table 1. The results of accuracy study

Taken concentrations (µg/mL)	Added concentration (µg/mL)	Teorical concentration (µg/mL)	Found concentration (µg/mL)	Recovery (%)
	2.0000	7.000	7.000	100.02
5	2.0000	7.000	6.993	99.63
	2.0000	7.000	6.988	99.41
	5.0000	10.000	9.974	99.47
5	5.0000	10.000	10.059	101.17
	5.0000	10.000	10.016	100.32
	10.0000	15.000	14.970	99.70
5	10.0000	15.000	14.997	99.97
	10.0000	15.000	14.828	98.28
Mean				99.8
SD				0.78
RSD				0.78

RSD: Avarage standart deviation, SD: Standart deviation

Table 2. The results of Inter-day and Intra-day reprocudubility

Inter-day				
	Added concentration (µg/mL)	Found concentration(µg/mL)	RSD (%)	Mean average (RSD)
Standard	20	20.06	0.254	
10 mg tablet	20	20.29	0.335	0.570
30 mg tablet	20	20.18	0.400	
Intra-day				
	Added concentration (µg/mL)	Found concentration (µg/mL)	RSD (%)	Mean average (RSD)
Standart	20	20.10	0.469	
10 mg tablet	20	20.24	0.398	0.352
30 mg tablet	20	20.19	0.486	

RSD: Relative standard deviation

of the analysis (17). In addition to the developed methods, a degradation study was also carried out. In this study; acid, base, peroxide decomposition and heat decomposition processes were applied. When the results obtained were examined, it was observed that racecadotril was slightly affected by acid, peroxide and heat, but completely decomposed in the base environment. This developed and validated method is reliable, fast, and can be used routinely in pharmaceutical analysis and quantification.

Ethics

Ethics Committee Approval: Since we do not work with biological materials in our study, ethical committee approval is not necessary.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: E.B., Concept: A.Ö., E.B., Design: A.Ö., E.B., Data Collection or Processing: A.Ö., E.B., Analysis or Interpretation: A.Ö., E.B., Literature Search: E.B., Writing: A.Ö., E.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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Table 3. Analysis results of different conditions for method robustness and -statistical evaluation of the results

		10 mg tablet		30 mg tablet	
%		RSD %	%	RSD %	
Column temperature (±2 °C)	28 °C	99.51	0.15	100.16	0.407
	Normal (30 °C)	100.06	0.291	100.20	0.349
	32 °C	99.93	0.376	100.13	0.406
	Mean	99.83	0.272	100.16	0.387
Filter effect (0.45 µ)	PTFE	100.44	0.238	100.58	0.285
	PVDF	100.49	0.134	100.52	0.589
	NYLON	100.66	0.450	100.43	0.293
	Mean	100.53	0.274	100.51	0.389
Flow rate (±0.1 mL/min)	0.6	100.48	0.190	99.76	0.209
	Normal (0.7)	99.40	0.134	99.43	0.589
	0.8	99.52	0.069	98.64	0.202
	Mean	99.80	0.131	99.28	0.333

Table 4. Degradation study results

Stress conditions	Area	Concentration (%)	Degredation (%)	Purity index	Purity threshold
Heat	214236	98.50	1.50	0.999999	0.999225
Asidic	179065	82.33	17.67	0.999999	0.998217
Basic	0	0	100.00	0	0
Oxidation	208128	95.69	4.31	0.999999	0.999580

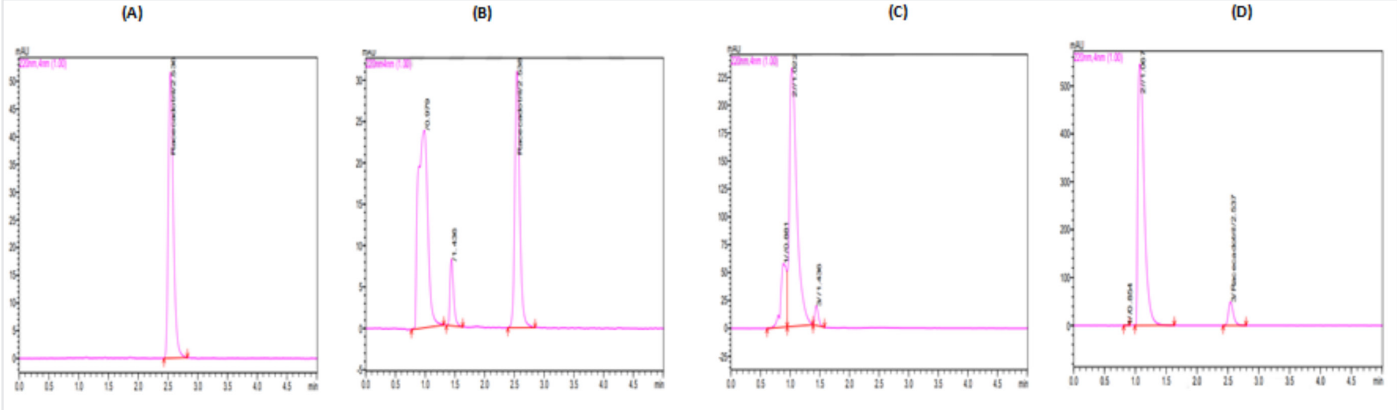

Figure 3. Chromatograms of the racecadotril; **A)** Temperature degradation study, **B)** Acid degradation study, **C)** Base degradation study, **D)** Oxidation degradation study

Table 5. Concentration values obtained from the methods according to the comparison method of 30 mg tablet sample and t- and F- test data

	Proposed method		Pharmacopoeia method	
Sample	Concentration (µg/mL)	Concentration (%)	Concentration (µg/mL)	Concentration (%)
1	20.13	100.64	20.09	100.47
2	20.23	101.16	20.15	100.77
3	20.24	101.20	20.17	100.84
4	20.18	100.91	20.13	100.64
5	20.18	100.91	20.11	100.55
6	20.11	100.55	20.33	101.64
Meanaa	20.179	100.89	20.164	100.82
SDb	0.053		0.085	
% RSDc	0.26		0.42	
Confidence interval	0.055		0.089	
Confidence range	20.124-20.234		20.075-20.253	
t-testd	t= 0.715			
F-testd	F= 2.593			

[a]$n_1=n_2=6$, [b]Standard deviation, [c]Relative standard deviation, [d]p=0.05, t_{table}: 2.57, F_{table}: 5.05

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