ORIGINAL ARTICLE

A C G publications *J. Chem. Metrol.* XX:X (202X) X-XX

journal of chemical metrology

In-vitro release study of Racecadotril from granule sachets: influence of Brij-35

Onur Çakır [1], Halil İbrahim Uğraş [1], Yavuz Selim Toksöz [2], Müge Güleli [2] and Cem Çalışkan [2*]

[1] *Istanbul Gedik University, Faculty of Engineering, Department of Metallurgical and Materials Engineering, Cumhuriyet Mahallesi İlkbahar Sokak No:1-3-5 Yakacık 34876 Kartal / İstanbul, Türkiye*

[2] *World Medicine İlaç San. ve Tic. A.Ş. R&D Center, 15 Temmuz Mah. Camiyolu Cad. No:50 K:4 Güneşli/İstanbul, Türkiye*

(July 02, 2023; Revised July 24, 2023; Accepted July 26, 2023)

Abstract: For sachet drug forms containing Racecadotril, which are freely soluble in methanol and methylene chloride but practically insoluble in water, used as an anti-diarrhea, suitable dissolution media meeting the parameters requested by the authorities are not available in the literature and monographs. For this reason, a suitable dissolution medium was determined in accordance with the guidelines and the dissolution profiles of reference product and samples used in the study. The profiles were compared, and the method was validated. In order to determine the most suitable medium for the release of drug product, in vitro dissolution tests were performed in media prepared by different buffers similar to the pH of the gastrointestinal tract (GIT) and media containing Brij 35. In this context, the effects of buffers, pH, and different surfactants were evaluated, and it was found that Polyoxyethylene 23 lauryl ether (Brij-35), a nonionic surfactant, increased the solubility. The results were obtained with RP-HPLC method using Kromasil C18 150 mm x 4.6 mm, 5 µm column with a flow rate of 1.5 ml/min at 210 nm wavelength in 7 minutes for Racecadotril, which dissolves at least 60% of the label value after 45 minutes after the dissolution studies performed with a type II apparatus at 37°C. A suitable dissolution medium was found for sachet drug forms containing Racecadotril, and the analytical method was validated in accordance with the ICH Q2 (R1) guideline. In addition, the difference factor (f_1) and similarity factor (f_2) were calculated to compare the dissolution profiles of the reference product and samples in this determined dissolution medium.

Keywords: Racecadotril; dissolution medium, Brij-35; dissolution rate validation, sink condition.

1. Introduction

Water solubility and dissolution are two of the important factors affecting drug absorption from the gastrointestinal tract (GIT). The solubility behaviour of a drug is often specified as the main determinant of oral bioavailability. Potentially bioavailability problems in the gastrointestinal tract are common due to the unregulated or problematic absorption of highly hydrophobic drugs. Formulation of slightly soluble compounds for the oral route is one of the most frequent and significant problems faced by formulation scientists in the pharmaceutical industry. However, solubility improvement option is concentratedly studied to increase the rate of release in formulation approaches [1].

* Corresponding author: E-Mail: cem.caliskan@worldmedicine.com.tr

The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology Month-Month 202X EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.91.2307.2837
Available online: September 04, 2023

In-vitro release study of Racecadotril

Racecadotril (Figure 1), the prodrug of thiorphan and an enkephalinase inhibitor, is used for the treatment of diarrhea. Compared to other drugs used in the treatment of diarrhea, its antisecretory effect has been reported originating from its ability to reduce the rate of secretion of water and electrolytes into the intestine. In addition, the drug is not formally reported in any pharmacopeia. Racecadotril contains white crystalline powders and it can be dissolved in solvents such as methanol, ethanol, and acetonitrile. Considering that one of the biggest disadvantages of Racecadotril is that it is slight soluble in water and its bioavailability should be improved by increasing with the dissolution medium to be used [2-4].

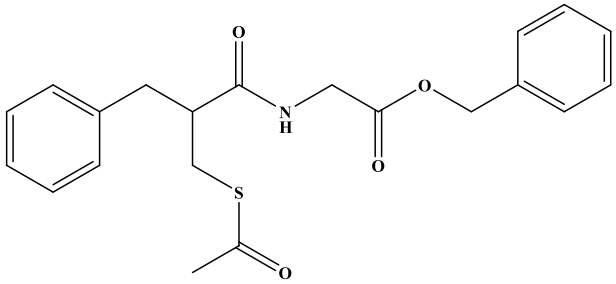

Figure 1. Molecular structure of Racecadotril

Contribution to the literature for Racecadotril at various conditions (0.1N HCl, acetate and phosphate buffers at pH: 4.5 and 6.8) are performed by Singhyi G. et al. However, the solubility could not exceed the specific values in the trial studies carried out in various dissolution media. Phosphate buffer at pH: 6.8 and surfactants are used in another study that showed the favorable effect of the SLS surfactant on dissolution performed by Deng J. et al. Similar results were reported by Prabu S. et al. in various solvent media showing a satisfactory increased solubility even in the presence of excess surfactants (in 0.1N HCl), and the best solubility was measured in the presence of organic solvents [2-5].

As part of the process of choosing the right dissolution medium, the physical and chemical features of the drug ingredient must be determined.When choosing the right dissolution medium, the effect of buffers, pH and, if necessary, different surfactants on the solubility and stability of the active substance should be considered. Taking acount the fact that all typical surfactants including anionic, non-ionic, and cationic types may be added to aqueous solutions (acidic or buffer solutions) in order to improve the solubility of the medicinal ingredient. A list of some of the surfactants used in the dissolution medium was shown in USP ⟨1092⟩ The Dissolution Procedure: Development and Validation monograph [6].

In the pharmaceutical industry, cost-effectiveness, toxic features, and low background absorption are to be considered in the surfactant selection. Non-ionic surfactants without aromatic moieties and branched aliphatic chains, e.g., a known non-ionic and water-soluble surfactant polyoxyethylene 23 lauryl ether - Brij-35-, are recognized as edible by the US FDA. Brij-35 was also found as a "green" modifier in micellar liquid chromatography due to the ability to simulate the bio-partitioning process. In contrast, a significant disadvantage of Brij 35 is that a high cloud point poses a major obstacle to its use in phase separation in aqueous solution at room temperature [7-9].

Although Racecadotril is known to be soluble in methanol, pharmaceutical production requires that organic solvents not be used when preparing dissolution media as they are not suitable for the human body. In this study, Brij-35 is used as dissolution media ingredient. Besides, we completed the method development and validation studies in accordance with the ICH Q2 (RI) guideline. This study contributes to the Racecadotril literature and guides analytical scientists in the pharmaceutical industry [10].

2. Experimental

2.1. Materials

A pharmaceutical-grade sample of Racecadotril CRS (European Pharmacopoeia Reference Standard, the “as is” content is 99.9%) was purchased from EP (European Pharmacopoeia). Potassium dihydrogen phosphate, ortho-phosphoric acid (85%), and triethylamine were purchased from Merck. Acetonitrile for mobile phase preparation and methanol as solvent were obtained from J.T. Baker and Brij-35 used as a dissolution medium component was obtained from Sigma-Aldrich. HPLC-grade water (0.05 µc) was produced by the Sartorius Stedim Biotech system. Racecadotril 30 mg, Granules for oral

suspension products as samples were produced by World Medicine İlaç San. ve Tic. A.Ş (İstanbul, Türkiye).

2.2. Instrumentation

Dissolution studies were carried out with Distek (USA) brand dissolution device. Waters E2695 HPLC (Singapore, Asia) system was used for liquid chromatography method development and validation studies, and Empower 3 Software was used for data processing and evaluations.

2.3. Sink Condition Study and Selection of Suitable Medium for Dissolution

Sink condition studies were carried out to determine the maximum saturation level in different dissolution media for Racecadotril active substance and select the appropriate dissolution medium for the agent. Within this scope, at first, buffers (pH 1.2, 4.5 and 6.8) representing the gastrointestinal tract and additionally purified water media were studied. Due to the low solubility of Racecadotril in these four media, surfactants specified in the USP ⟨1092⟩ The Dissolution Procedure: Development and Validation guideline were used to increase the solubility [6]. Among the anionic, cationic, and non-ionic surfactants given in Table 1, Sodium Lauryl Sulphate (SLS), Cetyltrimethyl Ammonium Bromide (CTAB), and Polyoxyethylene Lauryl Ether (Brij-35) were selected and used in the studies.

Table 1. Anionic, cationic and non-ionic surfactants commonly used in dissolution media

	Surfactant
Anionic	SDS; Sodium Lauryl Sulfate (SLS)
	Taurocholic Acid Sodium Salt
	Cholic Acid Sodium Salt
	Desoxycholic Acid Sodium Salt
Cationic	Cetyltrimethyl Ammonium Bromide (CTAB)
	Benzethonium Chloride (Hyamine 1622)
Nonionic	Polysorbate 20 (Polyoxyethylene (20) sorbitan monolaurate, Tween 20)
	Polysorbate 80 (Polyoxyethylene (20) sorbitan monooleate, Tween 80)
	Caprylocaproyl polyoxyl-8 Glycerides (Labrasol)
	Polyoxyl 35 Castor Oil (Cremophor EL)
	Polyoxyethylene Lauryl Ether (Brij-35)
	Octoxinol (Triton X-100)
Zwitterion	Lauryldimethylamine N-oxide (LDAO)

The sink condition media used for dissolution of the active substance are as follows: Purified water, 0.1 N HCl, pH 4.5 Phosphate, pH 6.8 Phosphate, 0.5% Sodium Lauryl Sulphate (SLS) aqueous solution, %0.5 Cetyltrimethyl Ammonium Bromide (CTAB) aqueous solution and Polyoxyethylene 23 lauryl ether (Brij-35, 5 g/L), (Brij-35, 10 g/L), Brij-35 (37.5 g/L), Brij-35 (50 g/L) aqueous solutions.

In addition, in the bioequivalence studies conducted in line with the EMEA guideline, comparative in-vitro dissolution rate studies were carried out by applying the Brij-35 medium (37.5 g/L) determined by sink condition and three different buffer media (normally pH 1.2, 4.5 and 6.8 which are similar to the pH of the gastrointestinal tract) to the sachet samples [11]. Sampling time points were evaluated as 5, 10, 15, 20, 30, 45, and 60 minutes to follow the dissolution profile.

2.4. Development of the Chromatographic Method

To obtain a sharp and symmetrical peak in the HPLC system, experiments were carried out with columns of different lengths and particle sizes, such as Luna-RP 18, ACE C18, Symmetry C18, and Kromasil C18, which were available from various suppliers. The Kromasil C18 150 mm × 4.6 mm, 5 µm column was determined as the most suitable analytical column by providing both peak symmetry and retention time advantages.

Since precipitation occurs in the mobile phase, studies at different salt ratios and pH tests were performed. The most appropriate buffer solution was determined by adding 0.50 mL of triethylamine to

1.0 g of potassium dihydrogen phosphate dissolved in 1000 mL of purified water and adjusting the pH of the solution to 3.95 ± 0.05 with ortho-phosphoric acid (85%).

In order to prevent interference with blank and placebo peaks, the most proper separation was achieved with 40% buffer solution and 60% acetonitrile as a result of the studies performed at different salt ratios and pH experiments. For the determination of filter to be used in the study, the test sample was filtered through 0.45 µm PTFE (Polytetrafluoroethylene), 0.45 µm Nylon and 0.45 µm RC (Regenerated Cellulose) membrane filters, and the areas of the filtered solutions were compared. The maximum area was obtained from the 0.45 µm PTFE filter and it was chosen as the appropriate filter.

2.5. Dissolution and Chromatographic Conditions

The dissolution experiments were performed with the USP Pedal apparatus (Type II) at a stirring rate of 100 rpm for 45 minutes. 60 mM (37.5 g/L) Brij-35 solution was used as the dissolution medium (900 mL) and the temperature was maintained at 37±0.5°C.

To prepare 60 mM Brij 35 solution, 37.56 g of Brij 35 was weighed and carefully transferred into a 1000 ml volumetric flask. Some purified water was added and it was dissolved completely. After the solution reaches room temperature, it was completed to the total volume with purified water.

The dissolution medium was placed into each of the dissolution vessels and each vessel was heated to 37±0.5°C. A sachet product was placed into each dissolution vessel and the dissolution device was operated as stated in the dissolution parameters. At the end of the test period, some samples were taken from each vessel. In the study, the samples and standard solution were diluted with the dissolution medium to obtain the concentration as 0.0056 mg/mL. Racecadotril standard stock solution was prepared by dissolving with methanol at a concentration of 0.112 mg/mL.

The buffer solution used for chromatographic separation was prepared by adding 0.50 mL of triethylamine to 1.0 g of potassium dihydrogen phosphate dissolved in 1000 ml of purified water and adjusting the pH of the solution to 3.95 ± 0.05 with ortho-phosphoric acid (85%). Buffer solution: acetonitrile are mixed at a ratio of 40:60 (v/v) and the solution was used as mobile phase with the isocratic flow. The analyses were conducted on a Kromasil C18 150 mm × 4.6 mm, 5 µm analytical column. The column and sample temperatures were set to 25°C and 5°C, respectively. The wavelength was 210 nm and the injection volume was set to 100 µL. Analyzes were performed with a total injection time of 7 minutes at a flow rate of 2.0 mL/min.

3. Results and Discussion

3.1. Sink Condition Study

The saturation concentrations obtained for Racecadortil as a result of sink condition studies are given in Table-2. According to the table, dissolution medium containing 37.5 g/L and 50 g/L Brij-35 yielded similar results, therefore 37.5 g/L dissolution medium was preferred as the optimum medium.

Table 2. Saturation concentrations for Racecadotril based on sink condition studies

Dissolution Medium	Saturation Concentration (mg/mL)
Purified Water	0.0778
0.1N HCl	0.0778
pH 4.5 Phosphate	0.0778
pH 6.8 Phosphate	0.0778
Purified Water + %0.5 SLS	0.2500
Purified Water + %0.5 CTAB	0.2500
Brij-35 (5 g/L)	0.2500
Brij-35 (10 g/L)	0.2500
Brij-35 (37.5 g/L)	0.3333
Brij-35 (50 g/L)	0.3333

The concentrations and the corresponding areas obtained based on the amount of each Racecadotril added in the sink conditioning study are shown in Table-3. The total added amount obtained for Brij-35 (37.5 g/L) medium is given in Figure S-1 by drawing an area graph.

Table 3. Total active substance amount, concentration and area results obtained from 37.5 g/L Brij-35 sink condition medium for Racecadotril

Solution	Amount of active substance added(mg)	Total amount of active substance (equivalent mg)	Theoretical concentration of active substance (mg/mL)	DF*	AreaxDF
C_1	10	10	0.0111	1	143657
C_2	20	30	0.0333	1	439993
C_3	30	60	0.0667	1	923212
C_4	30	90	0.1000	1	1463695
C_5	60	150	0.1667	1	2476107
C_6	150	300	0.3333	1	4659678

*DF: Dilution Factor

The concentration determined for Racecadotril during the studies was calculated by dividing the label value of the product (30 mg) by 900 mL (dissolution medium volume) (Ct: 0.0333 mg/mL). In accordance with the EP guideline, the point at which maximum of 10 times dissolution occurs (Cs: max saturation point) was calculated as 0.3333 mg/mL and is shown in Figure S1 in supporting information. [12].

According to the EP guideline, the point at which maximum 10-fold dissolution occurs (Cs: maximum saturation point) was calculated as 0.3333 mg/mL and is shown in Figure S1 [12].

In the dissolution test according to the sink condition, the dissolved active substance concentration (Ct) found based on the label value of the active substance in the product should not exceed 20% of the solubility (Cs) of this active substance in the media (Ct < 0.2xCs). Therefore; 0.0333 < 0.0667 condition was provided and Brij-35 (37.5 g/L) dissolution medium was suitable for Racecadotril. The Cs values for all media are shown in Table 4.

Table 4. Cs values for all sink condition mediums

Dissolution Medium	Cs (mg/mL)
Purified Water	0.0156
0.1N HCl	0.0156
pH 4.5 Phosphate	0.0156
pH 6.8 Phosphate	0.0156
Purified Water + %0.5 SLS	0.0500
Purified Water + %0.5 CTAB	0.0500
Brij-35 (5 g/L)	0.0500
Brij-35 (10 g/L)	0.0500
Brij-35 (37.5 g/L)	0.0667
Brij-35 (50 g/L)	0.0667

3.2. Method Development and Validation

Figure 2 shows the results of dissolution media (2a: pH 4.5, 2b: pH 6.8, 2c: pH 1.2, and 2d: purified water) containing 60 mM Brij 35. Fig. 2a, 2b, and 2c clearly showed that the solubility of both the original product and the developed product at the end of 60 minutes remained below 50% in pH 1.2, 4.5, and 6.8 media. In Brij 35-containing medium (Figure 2d), Racecadotril in the reference product showed more than 50% dissolution after 5 minutes while in the developed product after 10 minutes, however, both reached approximately 90% at the end of 60 minutes.

In-vitro release study of Racecadotril

The specificity, system suitability, precision, robustness, accuracy, and linearity parameters of the RP-HPLC method applied to control the study results of the new dissolution medium, which was determined to increase the solubility of Racecadotril were validated in accordance with the ICH Q2 (R1) guideline.

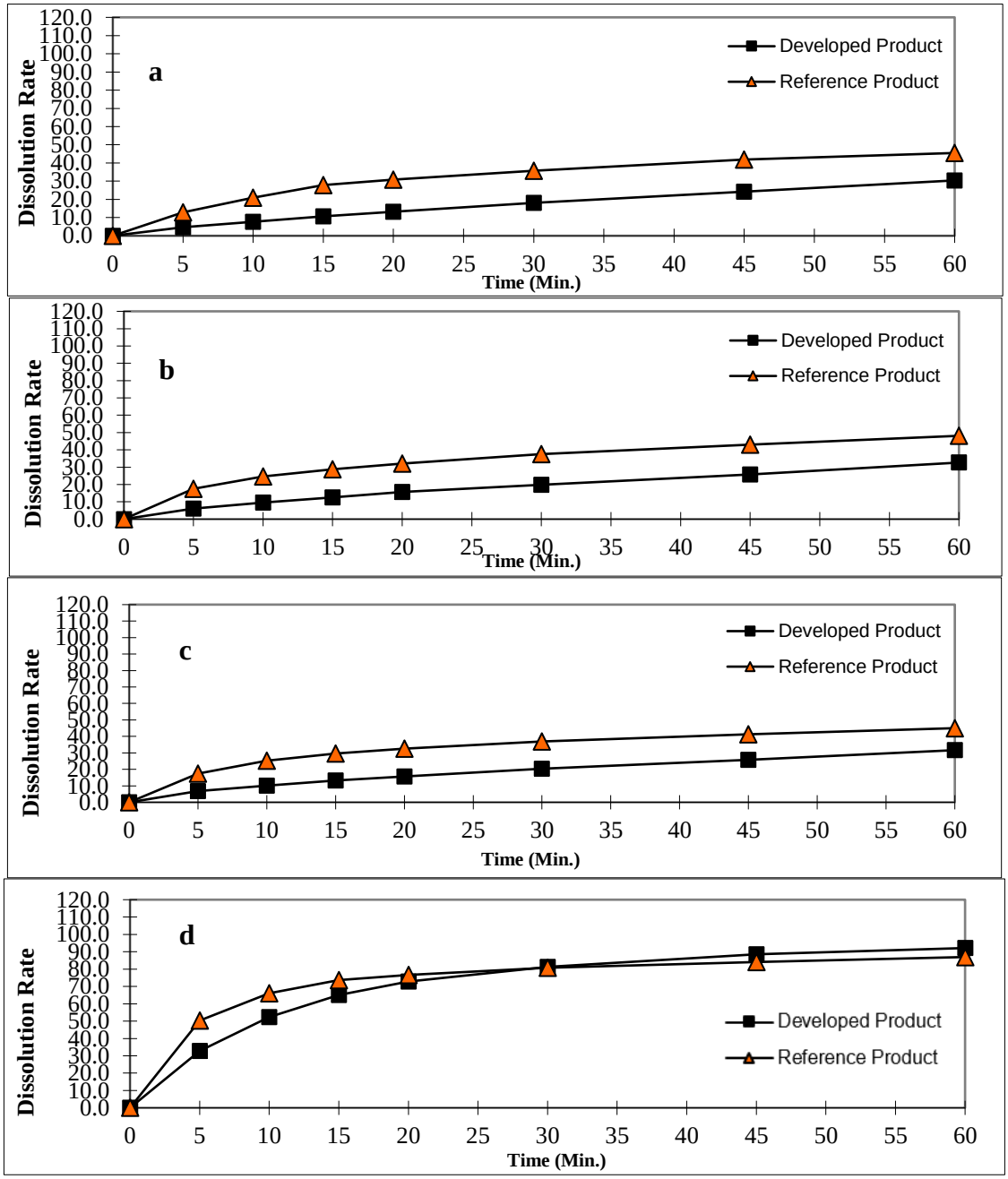

Figure 2. Dissolution rate-time graphs of the in vitro study: a) pH: 4.5 (acetate medium), b) pH: 6.8 (phosphate medium), c) pH: 1.2 (0.1 N Hydrochloric acid medium), d) 60 mM Brij-35 solution

3.3. System Suitability

For the suitability of the chromatographic system, the %RSD value (<2.0%) of the Racecadotril peak areas obtained from 6 consecutive injections of the standard solution, the theoretical plate number of the peak (>2000), and the symmetry factor (<2.0) were checked. 0.05% RSD value, 6646 theoretical plate number, and 1.1 symmetry factor values have been proven to meet the acceptance criteria.

3.4. Specificity

The specificity of the developed method was evaluated by controlling Racecadotril in the sample that did not interfere with other excipients. Blank, placebo, standard solution, sample solution, and 100% concentration recovery solution were prepared and checked with a diode array detector. Appropriate peak purity of all components was demonstrated by the criteria of peak purity < peak purity threshold. Representative chromatograms for specificity were showed in Figure 3.

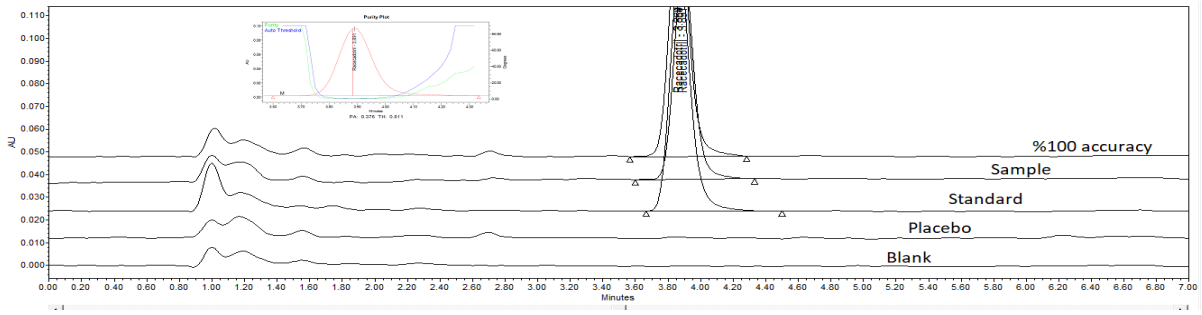

Figure 3. Specificity chromatograms and representative peak purity chromatograms

3.5. Linearity

To obtain standard calibration curves 14 calibrators within a concentration range of 0.007 µg/ml – 7.825 µg/ml were prepared for Racecadotril. 100% standard solution (5.589 µg/ml) was injected 6 times, and other solutions were injected 3 times. The linearity graph was drawn, and slope, interception, and correlation coefficient were obtained. The correlation coefficient (r), determination coefficient (r^2), and linear equation were calculated using the calibration graph and determined as 1.000, 1.000, and y=181648217.883804x-22.028113, respectively. These results showed that the dissolution method is linear between concentrations of LOQ-140% of the label value (Figure S2) (see supporting information).

3.6. Accuracy, Precision and Intermediate Precision

The accuracy parameter was evaluated using 12 measurements at four different concentration levels, starting from the LOQ and at 80%, 100%, and 120% concentrations of the specification limit. In the preparation of sample solution, the amount of active substance added corresponding to the relevant concentration was changed, keeping the placebo amount constant. The above-mentioned dissolution conditions were studied. In the accuracy study, 3 samples were prepared for each level and 3 repeated measurements were taken and the results are given in Table 5.

Table 5. Accuracy results

Parameter	Acceptance Criteria	Accuracy Level (%)	Mean Recovery (%)
Accuracy	98% - 102%	LOQ	101.07
		80	100.51
		100	100.05
		120	99.63

Device precision was evaluated with 6 repetitive injections of the standard solution, method precision was evaluated with 6 different sample results, and intermediate precision was measured with 6 sample results performed on different days by different analysts with different devices.

Acceptance criteria and results for precision parameters are given in Table 6 and the suitability of the method has been validated.

Table 6. Precision Results

Parameter	Acceptance Criteria	Results (%)
System precision	RSD% ≤ %2.0	0.05
Method precision	RSD% ≤ %10.0 (*n=6)	1.04
Intermediate precision	RSD% ≤ %10.0 (n=6) RSD %≤ %10.0 (n=12)	1.44 1.42

*n= number of samples

3.7. Robustness

Robustness is a significant criterion in the method validation and used as an internal validation to see if reliable experimental results are produced regardless of method parameters. Modifications applied as a result of the analytical technique are the use of different lot numbers of columns, changes in column temperature (±2°C), and experiments with different wavelengths (±2 nm). The absolute variation of the solution area from the initial value was calculated as more than 2% at both 208 nm and 212 nm in the wavelength change and it was found to exceed the acceptance criteria. Thus, it has been observed that the method is sensitive to the wavelength change.

At the same time, standard and sample solutions were kept in room and refrigerator conditions checked at different times to examine the solution stability. Standard and sample solutions were evaluated to be stable under room and refrigerator conditions for 48 hours.

3.8. Difference and Similarity Factors

Statistical analyses were performed to test the significance of the results obtained from the dissolution profiles for the reference product and the developed product. The difference factor (f_1) and similarity factor (f_2) specified in internationally accepted guidelines (FDA and EMA) were calculated and the two products were compared [11]. The f_1 value between 0 and 15 and the f_2 value between 50 and 100 indicate that the two dissolution profiles are similar, as stated in FDA regulations.

A simple model-independent approach uses a difference factor (f_1) and a similarity factor (f_2) to compare dissolution profiles. The difference factor (f_1) calculates the percentage (%) of difference between the two curves at each time point and is a measurement of the relative error between the two curves. In equations 1 and 2, n is the number of time points, R_t is the dissolution value of the reference (pre-change) batch at time t, and T_t is the dissolution value of the test (post-change) batch at time t [13-16].

$$f_1 = (\sum_{t=1}^{n}|R_t - T_t| / \sum_{t=1}^{n}|R_t|) \times 100 \quad (1)$$

The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percentage (%) of dissolution between the two curves [13-16].

$$f_2 = 50 \times \log\{[1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \times 100\} \quad (2)$$

The graphs of the results from the sample and reference product profile studies performed with 4 different media are given in Figure 2. The f_1 and f_2 values obtained in studies with those media are given in Table 7 in detail.

Table 7. The f_1 and f_2 values of the studies carried out in different mediums and for the test reference product

Dissolution medium	f_1 value	f_2 value
pH 4.5	49.5	40.3
pH 6.8	47.3	40.0
pH 1.2	45.8	41.1
Brij 35	10.4	51.0

3.9. Measurement Uncertainty Assessment

The uncertainty parameter was determined as uncertainty from purity of standard ($u_{standard}$), weighing ($u_{weighing}$), precision ($u_{precision}$), accuracy ($u_{accuracy}$) and calibration curve (u_{curve}) of the applied method. GUM methodology was applied in accordance with the EURACHEM CITAC and ISO Guide 35 for the estimation of the uncertainty measurement [17-21]. The combined uncertainty ($u_{Combined}$) was calculated as follows:

$$u_{Combined} = \sqrt{(u_{standard})^2 + \left(u_{weighing}\right)^2 + \left(u_{precision}\right)^2 + \left(u_{accuracy}\right)^2 + (u_{curve})^2} \quad (3)$$

$$u_{Combined} = \sqrt{(0.001)^2 + (0.0001)^2 + (0.425)^2 + (0.002)^2 + (1.610)^2}$$

$$u_{Combined} = 1.67$$

The expanded uncertainty ($u_{Expanded}$) calculated using a coverage factor of 2 giving a confidence level of approximately 95% was calculated as follows:

$$u_{Expanded} = u_{Combined} \times k \quad (4)$$

$$u_{Expanded} = 3.33$$

4. Conclusions

The pharmaceutical industry and regulatory authorities focus on appropriate drug dissolution for newly developed dosage forms. Quantitative analyses and mathematical evaluations obtained in dissolution tests are the parts of these studies. In this study, comparative studies are provided for the generically produced Racecadotril-containing sachet with the reference product.

For Racecadotril which is slightly soluble in water, a new medium with better solubility has been proposed, except for three different pH mediums simulating the gastrointestinal tract. Studies conducted between dissolution media containing 60 mM Brij 35 and other pH media have proven that dissolution with a more non-ionic surfactant is over 60%. Considering the organic solvent restrictions in simulated media, a new medium has been added to the literature for Racecadotril as a result of this study.

The results of the statistical study performed to prove the similarity between the reference product are given in Table 7, and it was found that the f_1 (10.4) and f_2 (51.0) values obtained with Brij 35-containing medium met the acceptance criteria. In this study, the dissolution medium we proposed showed its potential for being used for Racecadotril for the first time in laboratories where daily routine analyses are carried out in the pharmaceutical industry and the analytical method was validated with appropriate parameters for ICH. In addition, Brij-35 used in this study is an easily available and inexpensive surfactant produced without the use of organic solvents with green chemistry. Thus, the analysis showed that it has the potential to reduce costs.

In addition, the Racecadotril Sachet product belonging to the World Medicine Pharmaceutical Industry company was used as a sample in the study and it was found that the method was applicable for the sachet form of the drug. This is the first study in terms of the testability of the same form for the newly developed dissolution medium in different doses and different drug forms, and it has been interpreted that it can be studied according to the wide product forms in the industry.

Acknowledgements

The authors are thankful to the management of World Medicine İlaç San. ve Tic. A.Ş (İstanbul, Türkiye), for supporting this work. The authors would like to acknowledge that this paper is submitted in partial fulfillment of the requirements for a master's degree at Düzce University.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

ORCID
Onur Çakır: 0000-0002-6686-371X
Halil İbrahim Uğraş: 0000-0002-1633-967X
Yavuz Selim Toksöz: 0000-0002-0227-8060
Müge Güleli: 0000-0002-1539-3228
Cem Çalışkan: 0000-0003-2345-0242

References

[1] A.A.H. Sathali and V. Selvaraj (2012). Enhancement of solubility and dissolution rate of racecadotril by solid dispersion methods, *J. Curr. Chem. Pharm. Sci.* **2**, 209-225.
[2] G. Singhvi, G. Gampa, N. Yadav, V. Kumar and R. Ukawala (2013). Design and evaluation of rapidly disintegrating tablets of racecadotril with enhanced in-vitro dissolution achieved by solid dispersion technique, *Indian J. Pharm. Educ. Res.* **47**, 56-63.
[3] P. Tank, A. Zanwar, A.K. Seth and S. Kumar (2012). Development of new analytical methods for quantitative estimation of racecadotril as an active pharmaceutical ingredient by uv spectrophotometer, *Int. J. Pharm. Sci. Res.* **3**, 1495-1497.
[4] J. Deng, S. Staufenbiel, S. Hao, B. Wang, A. Dashevskiy and R. Bodmeier (2017). Development of a discriminative biphasic in vitro dissolution test and correlation with in vivo pharmacokinetic studies for differently formulated racecadotril granules, *J. Control. Releas.* **255**, 202-209.
[5] S. L. Prabu, T. Singh and D. Kumar (2009). Development and validation of a dissolution method with uv analysis for coumarin tablets, *J. Pharm. Res.* **8**, 213-215.
[6] USP-NF ⟨1092⟩ The dissolution procedure: development and validation, official as of 01-Dec-2020.
[7] S. Sharma, S. Kori and A. Parmar (2015). Surfactant mediated extraction of total phenolic contents (TPC) and antioxidants from fruits juices, *Food Chem.* **185**, 284-288.
[8] J. Chen, W. Deng, X. Li, X. Wang, Y. Xiao (2019). Hexafluoroisopropanol/Brij-35 based supramolecular solvent for liquid-phase microextraction of parabens in different matrix samples, *J. Chromatogr. A.* **1591**, 33-43.
[9] S. Ghosh and S.P. Moulik (1998). Interfacial and micellization behaviors of binary and ternary mixtures of amphiphiles (tween-20, brij-35, and sodium dodecyl sulfate) in aqueous medium, *J. Colloid Interface Sci.* **208**, 357-366.
[10] The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), Q2 (R1) validation of analytical procedures: text and methodology, Current Step 4 version, Parent Guideline dated 27 October 1994.
[11] Guideline on The Investigation of Bioequivalence, European Medicines Agency (EMA), London, 20 January 2010, Doc. Ref.: CPMP/EWP/QWP/1401/98 Rev. 1/ Corr.
[12] EP 11.0, current monograph 5.17 Recommendations on Methods for Dosage Forms Testing.
[13] Guidance for Industry Dissolution Testing of Immediate Release Solid Oral Dosage Forms, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), August 1997.
[14] J.W. Moore and H. Flanner (1996). mathematical comparison of curves with an emphasis on in vitro dissolution profiles. pharmaceutical technology, *Pharm. Technol.* **20**, 64-74.
[15] E. Vranić, A. Mehmedagić and S. Hadzović (2002). Mathematical methods for quantification and comparison of dissolution testing data, *Bosn J Basic Med Sci*. **2**, 49-52.
[16] Y. Wang, R.D. Snee, G. Keyvan and F.J. Muzzio (2015). Statistical comparison of dissolution profiles, *Drug Dev. Ind. Pharm.* **42**, 796-807.

[17] B. Magnusson and U. Omemark (2014). Eurachem guide: the fitness for purpose of analytical methods a laboratory guide to method validation and related topics, 2nd ed. ISBN 978-91-87461-59-0. Available from www.eurachem.org

[18] A. Kul (2022). Simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate and paracetamol by HPLC-PDA in pharmaceutical dosage forms, *J. Chem. Metrol.* **16**(**2**), 102-110.

[19] ISO Guide 35 (2017): Reference materials-guidance for characterization and assessment of homogeneity and stability 4th ed.

[20] BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML (1993). Guide to the expression of uncertainty in measurement. 1st ed.

[21] S.L.R. Ellison, A. Williams (2012). Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement, 3rd ed. ISBN 978-0-948926-30-3 Available from www.eurachem.org