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Design, Characterization, Analytical HPLC Method Validation and Stability Studies of Racecadotril Capsules

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Abstract

This study aims to develop, characterize and validate analytical method and perform stability studies of Racecadotril capsules to treat acute diarrhea. Six formulations of Racecadotril 100mg Capsules were prepared with different excipients by varying their concentrations. The HPLC method was validated on analytical parameters recommended by ICH Q2R guidelines, including specificity, accuracy and recovery, precision, quantitation limit, detection limit, range, linearity, and robustness. Forced degradation studies were performed as per the Stability Indicating Method under various stress conditions. Accelerated stability studies were performed on three stability batches of best fit formulation of Racecadotril 100mg Capsules as per ICH guidelines. Among the six formulations of Racecadotril 100mg Capsule, F6 was the best fit with a comparatively good dissolution profile with 76.9% release in 60 minutes. The HPLC system was suitable as % R.S.D. was 0.619147%, within the acceptance criteria. Furthermore, parameters including specificity, accuracy and recovery, precision, quantitation limit, detection limit, range, linearity, and robustness lie within the acceptance criteria. The percent degradation of Racecadotril after photolytic (sunlight for 6 hr.), oxidative (3% H_2O_2), acidic (0.1N HCl) and necessary (0.1N NaOH) stress was found to be 6.5%, 5.8%, 11.4%, and 28.4%, respectively. The product remains unchanged after thermal stress. HPLC method was successfully validated for Racecadotril 100mg Capsule as per ICH Q2R guidelines.

Keywords: Racecadotril, Anti-diarrheal, HPLC, Stability Indicating Method, Accelerated Stability Studies

Introduction

According to several guidelines, the concomitant use of Racecadotril with oral rehydration solution is recommended for treating acute diarrhea in children (Eberlin, *et al.*, 2018). Racecadotril has an excellent tolerability over Loperamide in patients with acute diarrhea (Fischbach, *et al.*, 2016). It works by inhibiting enkephalinase-preventing, its degradation found abundantly in the intestinal villi. Enkephalins produce an anti-secretory effect via inhibition of cyclic adenosine monophosphate (cAMP). The inhibition of enzymes occurs when the parent drug (Racecadotril) is converted to its metabolite thiorphan in peripheral tissue membranes. The concentration level of enkephalin increases due to opioid receptor activation, which results in cAMP reduction. Ultimately electrolytes and water secretion reduced into the intestinal lumen (Schiller, 2017; Wajeeha, *et al.*, 2020). Maximum absorption occurs when the drug is administered orally at different doses, i.e., 30 mg, 100 mg, and 300 mg, and C*max* is achieved within 1

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hour. According to B.C.S., Racecadotril belongs to Class-II drug (high permeability, low solubility). Class-II drugs (B.C.S.) are further classified based on pKa as Class IIa (pKa<5), Class IIb (pKa>6.5), and Class IIc (Neutral Drug). The pKa of Racecadotril is 12.6, which means that drug falls in B.C.S. Class IIb can be predicted to have high solubility and dissolution rates at acidic pH in the stomach. The bioavailability is not affected by food and rapidly converts to its active metabolite Thiorphan results in inhibition of enkephainase enzyme with anti-secretory activity [5].

The method validation is an analytical procedure providing a means of performing analysis. The process must include sample, use of apparatus, the formula for calculation, generation of a calibration curve, reagent preparations and standard, etc. During method validation, all variables are evaluated and verified according to acceptance criteria [6]. According to ICH guidelines for method validation, the following analytical parameters: specificity**,** Accuracy and recovery, Precision, quantitation limitation, detection limitation, range and linearity are evaluated [7]. The RP-HPLC is a commonly used analytical method for Separation and impurities quantification; U.V. detectors are frequently coupled. Stress testing or SIM is done using HPLC to meet necessary regulatory specifications. Samples are prepared by applying forced degraded conditions that are more critical than any conditions for accelerated degradation (Ngwa 2010). Forced degradation is applied to a drug at acidic, basic, phytolytic, thermal, and oxidative conditions. The primary objective of producing a degradation product is to measure any change, which is likely to be delivered in any realistic drug storage condition [8-10]. The study aims to design and characterize a stable capsule solid dosage form of Racecadotril.

Materials and Methods

Chemicals

Racecadotril (E.P. Specification) manufactured by M/s Rampex Labs Pvt. India, kindly donated by M/s Vision pharmaceuticals, Islamabad, Pakistan. Maize Starch, Magnesium Stearate, Lactose Monohydrate, Talcum powder, Colloidal Silicon Dioxide, Microcrystalline Cellulose PH102, Mannitol, Methyl Cellulose, Crosscarmelose Sodium. All excipients used are of pharmaceutical grade. Acetonitrile (Labscan, India); Potassium Dihydrogen Phosphate (Honeywell, Pakistan); Phosphoric Acid (Panreac); Sodium Chloride; Hydrochloric Acid; Hydrogen peroxide; Sodium Lauryl Sulphate (S.L.S.); distilled water. All chemical/reagents used for analysis are of analytical grade.

Apparatus and Equipment

High Performance Liquid Chromatography (HPLC) (Hitachi, Japan); Dissolution apparatus (Electrolab, Pakistan); UV-Visible Spectrophotometer (Shimizdu, Japan); Fourier-Transform Infra-Red (FTIR) Spectroscopy (Quebec, GIK 9H4 Canada); Melting point apparatus (Stuart SMP10, Japan); Vacuum Desiccator (Glass Pyrex, Germany); Volumetric Flasks (50ml, 100ml) (Glass "A" Pyrex Japan.); Pipette 5 ml, 10 ml (Glass Pyrex, Germany); Beakers 100ml, 50ml, 1000ml; Measuring cylinder 10 ml, 50 ml, 100 ml (Glass Pyrex, Germany); pH meter; Analytical balance (PA 214 C, Ohaus corporation, USA).

Methods

HPLC method of analysis and validation

The method validation was performed in the quality control analytical section of Vision Pharmaceuticals – Islamabad, Pakistan.

Mobile phase preparation

For mobile phase A, 1 g of potassium dihydrogen phosphate was dissolved in 950 ml water, 2.5 pH was maintained with dilute phosphoric acid and finally made volume 1000 ml served as a phase while mobile phase B was acetonitrile.

Preparation of standard

20 mg of Racecadotril was taken in a volumetric flask of 50 ml, and final volume was made up with diluent (mobile phase A: mobile phase B)

Preparation of sample

10 capsules of Racecadotril were opened to serve a 20 mg content. The sample was transferred to a 50 ml volumetric flask and made up the final volume with diluent, i.e., mobile phase.

Chromatographic conditions and procedure on HPLC

HPLC was performed under ambient conditions; a modern HPLC system (Hitachi, Japan) with a U.V. detector was used to absorb 210 nm having a pump at 30 degrees. The Rheodyne injector was used for sample injection fitted with a loop of 10 µl. A reverse-phase column was used for separation with 25 cm × 4.6 mm and 5 µm packing L1. Buffer and acetonitrile were used as the mobile phase with a flow rate of 1.0 ml/min. The run time was 40 minutes with gradient elution [11-12].

Steps of method validation

According to ICH guidelines, the following validation parameters were assessed [7].

System suitability

It was checked by analyzing five replicate injections of sample and standard preparation. Standard deviation (SD) and relative standard deviation (RSD) was determined for peak areas and example and

traditional practice retention time. The acceptance criteria were less than 2%.

Accuracy The standard addition method was used for the determination of accuracy and recovery. Previously analyzed samples of Racecadotril were spiked at recovered concentration, standard deviation (S.D.), and relative standard deviation (R.S.D.) were found out for each concentration [13].

Linearity (calibration curve)

Solution was prepared at different concentration as (0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml, 0.6 mg/ml, and 0.7 mg/ml) for development of calibration curve. Baseline was monitored and these prepared concentrations were injected one by one for the calculation of peak area (millivolt/min). Graph was plotted between developed concentration (mg/ml) and peak area (millivolt/min) [14].

Robustness

It shows the variation in testing conditions. One sample was analyzed with the same mobile phase (Buffer: Acetonitrile, 50:50) as mentioned in the testing method, while other samples were analyzed using mobile phase having a slight change in composition (Buffer: Acetonitrile, 60: 40), change in pH 2.3, 2.6 and change in injection rate 1,5ml/min, 2ml/min. Standard deviation (SD) and relative standard deviation (RSD) were found out for different testing conditions [15].

Limit of quantitation and detection (sensitivity)

The limit of quantitation (L.O.Q.) and detection limit (L.O.D.) were determined by applying the following equation.

LOQ=10 × SD/Slope LOD=3 × SD/Slope

Precision

According to ICH guidelines, precision was determined at two different levels repeatability and intermediate accuracy. The assay was performed under the same operating condition after a short interval of time to assess repeatability, and various analysts performed an assay to find out intermediate precision. Standard deviation (SD) and relative standard deviation (RSD) was also derived.

Stability testing

Forced degradation studies by stability indicating methods (SIM)

All the analysis after stress studies were carried out through a validated testing method of HPLC under testing validated conditions. The primary goal of stress studies is the degradation of 5-10 % API. Further degradation can lead to the destruction of a relevant compound or the production of irrelevant products [7].

Photolytic degradation

Twenty capsules of Racecadotril were taken, weighed accurately, and content was emptied. Capsule powder was used, equivalent to 10 mg of Racecadotril. The powder was exposed under direct sunlight for 6 hours; then it was transferred to the volumetric flask of 50 ml. The final concentration of 100 mcg/ ml was obtained after dilution.

Oxidative degradation

Capsule powder was equivalent to Racecadotril (10 mg), 3% hydrogen peroxide (5 ml) was added to the above mixture and then kept at 70^0C for 10 min. Ambient temperature is applied to the above solution, diluted up to 50 ml by using diluent, and a final dilution of 100 mcg/ml was made. For the preparation of blank, 3% hydrogen peroxide (5 ml) was diluted with 50 mL of diluent. The same procedure was also applied for a placebo sample.

Thermal degradation

Capsule powder equivalent to 10 mg of Racecadotril is taken and kept in air oven for two hours at 100°C. The above powder was then transferred to the volumetric flask of 50 ml, the final concentration of 100 mcg/ml was obtained after a dilution concentration of 100 mcg /ml. The same procedure was also applied for a placebo sample.

Acid degradation

Capsule powder was used that is equivalent to Racecadotril (10mg), 0.1N HCl (5 ml) was added to the above mixture and then kept at 700C for 10min. The ambient temperature is applied to the above solution, 0.1N NaOH (5 ml) was added for neutralization and diluted up to 50 ml by using diluent. For the preparation of blank, 0.1N HCl (5 ml) and 0.1N NaOH (5 ml) were diluted up to 50 ml by using diluent. The same procedure is also applied for a placebo sample.

Base degradation

Capsule powder was used that is equivalent to Racecadotril (10 mg), 0.1N NaOH (5 ml) was added to the above mixture and then kept at 70^0C for 10 minutes. The ambient temperature is applied to the above solution, 0.1N HCl (5 ml) was added for neutralisation and diluted up to 50 ml by diluent. For the preparation of blank, 0.1N NaOH (5 ml) and 0.1N HCl (5 ml) was diluted up to 50 ml by using diluent. The same procedure is also applied to a placebo sample.

Pre-formulation studies

Identification by FTIR

FTIR Spectra for the drug was obtained on a FTIR spectrophotometer (Quebec, G.I.K. 9H4, Canada) in transmission mode, wave number region 4000-500 cm-1 was used.

Melting point: The melting point apparatus determined the melting point of Racecadotril API sample and standard.

Solubility: The solubility studies of Racecadotril were conducted to find appropriate solvents for the drug. Different solvents varying from polar nature to non-polar nature were used to determine solubility. Excess amount of the drug was added to 2 ml of each solvent, screw-capped vials were used. Prepared saturated solutions were kept in a reciprocating shaker water bath for 24 hours at 25 ± 1°C of temperature with 120 rpm for constant shaking. The solutions were filtered, and drug concentration in solution was determined using a U.V. spectrophotometer (Shimadzu, Japan) at 231 nm. The procedure was repeated three times with the same steps to find out reproducible results.

Loss on drying:

The loss on drying was measured on 1 g of powder by drying at 60ºC in vacuum desiccators at for four (4) hours (B.P. 2013), said loss shall not be more than 0.5% [16]

Assay of API: HPLC was performed under ambient conditions; Modern HPLC system(Hitachi, Japan) with a U.V. detector was used at absorbance of 210 nm having pump at a temperature 30oC. Rheodyne injector was used for sample injection fitted with a loop of 10-µL. A reverse phase column was used for separation with a measurement of 25 cm × 4.6 mm and 5 µm packing L1. Buffer and acetonitrile was used as mobile phase with a flow rate of 1.0 mL /min. The run time was 40 minutes with gradient elution.

Bulk density: Bulk densities for powder blends and granules were found out by method, described in U.S.P. (United States Pharmacopoeia) monograph (U.S.P. 2007). The sample was weighed accurately in grams and poured in measuring cylinder of 100ml. Calculation of bulk densities was done as given in equation 1

$$\text{Bulk density} = Ws/Vo$$

"Ws" is sample weight, and" Vo" is unsettled sample volume. The unit used for bulk density is g/cm3.

Preparation of Racecadotril 100mg Capsules: Six different formulations (assigned as F1 to F6) were prepared by varying the proportion of excipients as given in table 1. The quantity of drug used was 100 mg for all formulations. All ingredients was passed Through sieve no 30 individually and mixed for 10 to 15 minutes. Then weighed 200 mg of mixture accurately and filled in capsule shell no 30 having a green body and white cap for further characterization.

Characterization of Racecadotril capsules: Prepared formulations were tested by various physicochemical parameters, including Average weight, weight variation, loss on drying, disintegration, percent assay, dissolution, and compared with Pharmacopoeia standards.

Tapped Density: Tapped densities for powder blends and granules were measured by method 2, described in U.S.P. (United States Pharmacopoeia) monograph (U.S.P. 2018). As the bulk density was measured in section 2.2.3.6.A, the same sample was used to determine tapped density. To tapping rate was adjusted at 250 taps per minute at a fixed height (3+0.2 mm), then allowed it to fall from above said height by its weight. The volume of sample was determined after 500 taps. Equation 2 represented the tapped density of the sample.

Tapped density: Ws/Vf

Angle of response: It is a common method to find outflow properties of powders. It is an internal angle between the heap of powder surface and its horizontal axis. The funnel was inserted above 2 cm of the flat base then the powder was allowed to release from the funnel base. The angle of repose (α) was calculated by applying the following equation 3:

$$\alpha = \tan^{-1}(h/r)$$

"h" is cone height, and "r" is the base radius.

Compressibility index and Hausner's ratio: The extent of powder to flow can be determined by using Carr's index/ compressibility index and Hausner's ratio. It was calculated by bulk density and tapped density. It is calculated using the formula given in the U.S.P. (United States Pharmacopoeia) monograph.

$$CI\ (\%) = (\rho t - \rho b / \rho t) \times 100$$

"CI" is Carr's index, "ρt" is tapped density, "ρb" is bulk density.

If bulk solid compressibility greater, the flow property will be less, therefore assess properties like uniformity in shape and size, surface area deformability, moisture content and cohesion of any the material. Alternatively, H.R. was calculated using the measured values of bulk density and tapped density as mentioned in the USP monograph 2005 [17].

Moisture content:

Moisture content was determined by moisture Balance (Sartorius, japan). 1g of sample was weighed and put on a heating pan of moisture balance for 2 minutes at 105^0C temperature. Its limit must not exceed more than 3%.

Average Weight: Twenty capsules were selected randomly and individually weighed using analytical balance (PA 214 C,Ohaus Corporation, U.S.A.) and average weight determined.

Disintegration time test: Disintegration tester apparatus (121-L Galvano scientific, Pakistan) was used with a single capsule placed in each tube of instrument, then disk was inserted. The assembly was suspended in 1000 ml beaker of distilled water. The

temperature was maintained at 37±0.5ºC throughout the operating procedure.

Dissolution

Dissolution rate was determined by using U.S.P. dissolution apparatus 1 (Electrolab, Pakistan). Six capsules were added individually to basket of dissolution apparatus containing 900 ml of 3% S.L.S. as dissolution media .the speed was set at 50 rpm for 60 minutes at temperature 37ºC + 0.5ºC. 10 ml of sample were drawn after every 15 minutes from each basket and amount of percent dissolution were determined.

Assay

Assay of Raceacdotril capsules were performed by HPLC (Hitachi, Japan). Modern HPLC Hitachi system was used with detector at 210 nm absorbance with pump at temperature 30ºC. Samples were injected by means of a Rheodyne injector fitted with a 10 µL loop. Reverse phase column 25 cm × 4.6 mm, 5 µm packing L1 was used for separation. The mobile phase was buffer and Acetonitrile (A.C.N.) at a flow rate of 1.0 ml /min. The elution was done in a gradient manner with run time 40 minutes.

Comparative dissolution

Comparative dissolution was performed according to guidelines recommended by F.D.A. Comparative profiles of both test and reference was prepared and analyzed.

Preparation of stock solution

3% Sodium lauryl sulphate (S.L.S.) was prepared as stock solution. 3g of S.L.S. was taken and dissolved in distilled water (100 ml). 12 L of stock solution was prepared and each basket was filled with 900 ml of S.L.S.

Preparation of sample

Weigh 6 capsules of Racecadotril 100 mg and transfer individually in each of 6 dissolution baskets, suspended in vessel containing 900 ml of 3% S.L.S, equilibrated at 37± 0.5ºC.

Weigh 6 capsules of Hidracec 100 mg (Abott laboratries) and transfer individually in each of 6 dissolution baskets, suspended in vessel containing 900 ml of 3% S.L.S, equilibrated at 37± 0.5ºC.

Table 1. Composition of different formulations of Racecadotril 100 mg capsules

SNo	Ingredients	F1	F 2	F3	F4	F5	F6	Role of ingredient
1	Racecadotril	100	100	100	100	100	100	Active
2	Maize Starch	15		10			30	Lubricant Disintegrant
	Stearic acid	2	2	1				Lubricant
3	Mg Stearate				5	5	1	Lubricant
4	Talcum powder	3	3	5				Glidant
5	Colloidal silicon dioxide	0.5			3	1	0.5	Glidant
6	Lactose Monohydrate	50	50		50	45	66.5	Diluent
7	Microcrystalline cellulose pH 102	31.5	30	75				Diluent
8	Mannitol					10		Diluent
9	Methyl cellulose				45	40		Disintegrant
10	Crosscarmelose sodium		15	10				Disintegrant
Total fill weight per capsule		200	200	200	200	200	200	

Preparation of reference

10 mg of working/reference standard was accurately weighed, put it to 100 ml volumetric flask then final volume was made with S.L.S. solution to dissolve the solution and sonicated it. 10 ml of stock solution was transferred to 25 ml volumetric flask and diluted it with S.L.S. solution to make final volume.

Dissolution testing

Repeated as stated above and readings for both sample and ref solutions in a 1 cm cell on a U.V/Visible spectrophotometer at 232 nm using S.L.S. solution as blank. Percent absorption was calculated by following formula.

$$\%age = \frac{\text{Absorbance of sample} \times \text{conc. of std.} \times 100}{\text{Absorbance of standard} \times \text{conc. of sample.}} \times \text{label claim}$$

Limits: N.L.T. 70% should release after 60 minutes.

Statistical analysis for comparison of dissolution profiles

Model independent approach was applied to compare the dissolution data of two products. According to recommended F.D.A. guidelines, dissolution data profile or equivalence can be determined. This approach is directly based on statistical calculation of difference factor (f1) and similarity factor (f2) to provide simple way of comparison between dissolution data of two products.

The difference factor (f1) measures the variance in percentage by comparing the dissolution curves of sample and standard formulations at each time point.

The relative comparison of said curves can be obtained by using following formula.

F1= {[åt=1n|Rt-Tt|]/ [åt=1nRt]} •100

The factor of similarity (f2) measures the resemblance in percentage by comparing the logarithmic reciprocal square root transformation of the sum of squared error of dissolution curves of sample and standard formulations at each time point

F2=50•log {[1+ (1/n) åt=1n (Rt-Tt)2]-0.5•100

where "n" is the time points number, "Rt" is the value of reference batch dissolution (prechange) at the time t," Tt" is the value of the test batch dissolution (postchange) at the time t. If values of f1 are closer to 0 and values of (f2) are closer to 100 the curves will be similar that ensures sameness or equivalence between two comparative curves.

Accelerated Stability Study

Capsules prepared by optimized F6 formulation were stored in stability testing chamber at temperature 40±2ºC and relative humidity 75±5% for a period of six months and samples were drawn at prescribed time points i.e. zero, one, two, three and six months. The physical tests like color, average weight, moisture content, disintegration time and chemical test including dissolution were determined [18].

Results and Discussion

Method Validation

System suitability

It was checked by giving five (05) replicate injections of Racecadotril working standard solution the average/mean peak area was 11204623. Standard Deviation was determined for peak areas as well as the retention time (R.T.) of standards were recorded as described in table 2. The acceptance criteria for system suitability on percent R.S.D. was less than 2%.

Table 2. System suitability test (SST).

Ref. standard	Peak Area (milli volt/ml)	Mean Peak Area	S.D.*	R.S.D. (%)**
01	11243686	11204623	69373	0.62
02	11097937			
03	11172360			
04	11242053			
05	11267079			

*S.D., Standard Deviation; **R.S.D. (%), Percent Relative Standard Deviation

Accuracy

Accuracy and recovery was measured by method of standard addition. Three levels of solutions (50, 100 and 150%) of the nominal analytical concentrations were prepared and results were analyzed as Recovered concentration, standard deviation (SD) and relative standard deviation (RSD) Table 3.

Linearity (calibration curve)

Calibration curve prepared between concentration (mg/ml) and peak area (millivolt/min), and slope, intercept and coefficient of regression was shown in figure 1.

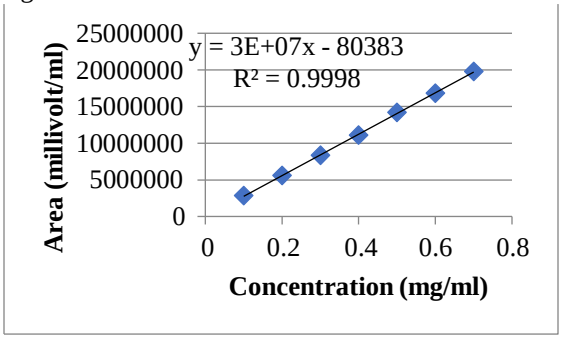

Fig 1. Calibration curve of Racecadotril by HPLC assay

Robustness

Assay was performed at different testing condition. One sample was analyzed with the same mobile phase (Buffer: Acetonitrile, 50: 50) as mentioned in the testing method while other sample was analyzed using mobile phase having a slight change in composition (Buffer: Acetonitrile, 60: 40), change in pH 2.3, 2.6 and change in injection rate was 1.5 ml/min, and 2ml/min. Standard deviation (S.D.) and relative standard deviation (R.S.D.) was find out for results of different testing conditions (table 4) [19].

Limit of detection and quantitation (sensitivity)

The limit of detection (L.O.D.) and limit of quantitation (L.O.Q.) were calculated as 0.610715 mg and 2.035715 mg.

Precision

HPLC Assay was performed according to guidelines of ICH results of repeatability and intermediate precision was within limit (90-110 %) so method is precise. Standard deviation (S.D.) and relative standard deviation (R.S.D.) were given in table 5.

Forced degradation studies

Forced degradation studies were performed for Racecadotril capsules 100 mg to conclude the stability-indicating method. The degradation was performed to stress conditions to evaluate the planned approach for separation of drug from the product of degradation. The results showed more degradation under base stress than acid stress, oxidative and photolytic degradation to the Racecadotril capsule 100mg while thermal stress remained unaffected. Table 6 indicates the percent degradation of all stress conditions.

Pre-formulation studies

Identification by FTIR

Table 3. Accuracy and recovery of Racecadotril HPLC assay at 50%, 100% and 150% standards.

Rep	Concentration (mg/mL)					
	At 50%		At 100%		At 150%	
	% assay	Conc. Recovered	% assay	Conc. Recovered	% assay	Conc. Recovered
1.	51.3	0.2052	101.0	0.404	151.9	0.6076
2.	50.8	0.2032	101.1	0.4044	151.4	0.6056
3.	51.0	0.2040	101.8	0.4072	151.1	0.6044
Mean	51.033	0.204	101.300	0.405	151.47	0.606
SD*	0.252	0.001	0.436	0.002	0.404	0.002
RSD (%)**	0.493	0.493	0.430	0.430	0.267	0.267

*SD, Standard Deviation; **RSD, Relative Standard Deviation

Table 4. Results of robustness at different test conditions

Sample	Test Conditions		Conc. (%)
1	Change in mobile phase ratio	Buffer : Acetonitrile, 50: 50	100.8
2		Buffer : Acetonitrile, 60: 40	101.3
3	Change in pH	2.3	101.1
4		2.6	100.1
5	Change in injection rate (ml/minute)	1.5	100.4
6		2	100.3
Average		100.66	
SD		0.476	
RSD%		0.4729	

Table 5. Results of repeatability and intermediate precision of Racecadotril HPLC Assay

Sample	Conc. % (At same conditions)	Conc. % (At same conditions, different analyst)
1	101.1	100.8
2	100.4	101.3
3	101.3	100.2
4	101.0	101.1
5	100.8	100.3
6	101.8	100.4
Average	101.07	100.68
SD	0.472	0.454
RSD%	0.4669%	0.4504%

SD, standard deviation; RSD%, percent relative standard deviation

The I.R. spectrum of the reference (figure 2) matches the Racecadotril API.

Melting point

The Racecadotril API sample and Racecadotril working standard melts at 79ºC in melting point apparatus.

Solubility studies

The figure 3 shows solubility profiling of active pharmaceutical ingredient of Racecadotril in water, 0.1 N HCl, 1% sodium lauryl sulphate (S.L.S.), 3% sodium lauryl sulphate (S.L.S.), and buffer pH 7.4. The data shows that Racecadotril has maximum solubility in 3% S.L.S. and minimum solubility in water.

Loss on drying

The loss on drying of Racecadotril API sample was 0.26 % w/w. The results comply with the acceptance criteria, i.e., NMT 0.5%.

Assay

The assay of Racecadotril active pharmaceutical ingredient (API) was validated via the HPLC method. The average peak area for Racecadotril reference standard was 11382446 with Standard deviation (S.D.) of 116576 and 1.024173% as percent relative standard deviation (R.S.D.) figure 4. The peak areas for Racecadotril samples were 11427488 and 11410165 figure 6. Compared to the reference standard, the average assay of Racecadotril API was 99.995%. The assay results came within the acceptable limits, i.e., 102.0% on a dried basis. Also, the principal peak's retention time obtained in the sample solution matches that of the standard solution as obtained in the assay.

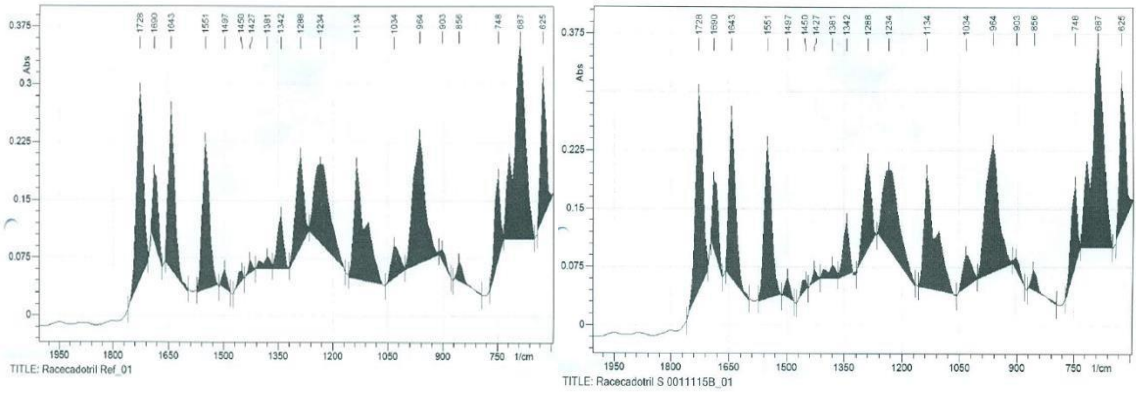

Fig 2. FTIR Spectra of Racecadotril working reference standard (Left) and API sample (right).

Table 6. Forced degradation study of Racecadotril capsule 100 mg.

S. No.	Stress Condition	S. weight (mg)	Racecadotril Area	% Assay	% Degradation
1	Photolytic (sunlight for 6 hr.)	36.8	10686236	93.5	6.5
2	Oxidative (3% H_2O_2)	36.4	10656142	94.2	5.8
3	Thermal (100°C for 2 hr.)	37.0	11555950	100.5	--
4	Acidic (0.1N HCl)	36.7	10100346	88.6	11.4
5	Basic (0.1N NaOH)	36.6	8138595	71.6	28.4

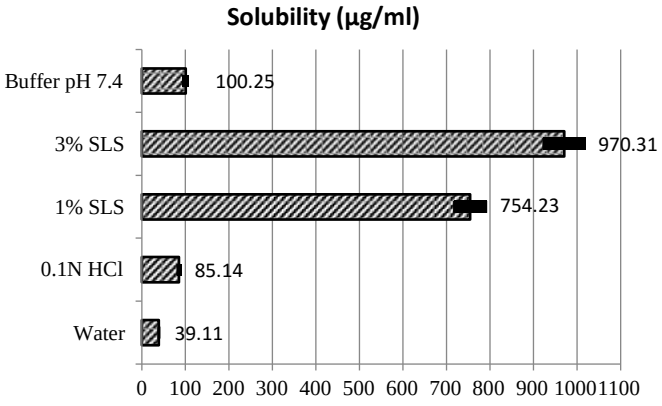

Fig 3. Graphical representation of solubility trend of Racecadotril

Table 7. Summary of flow properties of Racecadotril API

Sr No	Flow properties	Results	Interpretation
1	Bulk Density (g/cm3)	1.21±0.02	N/A*
2	Tapped Density (g/cm3)	1.94±0.02	N/A*
3	Angle of repose (θ)	45.54±2.95	Poor flow
4	Carr's index (%)	38.02±0.41	Very poor flow
5	Hausner's ratio	1.61±0.32	Very poor flow

*N/A - not applicable

Flow properties

The flow properties of the Racecadotril API sample are summarized in Table 7. The interpretation was made and it was found that the overall flow of Racecadotril API was low.

Characterization of formulations

Test formulations (F1 to F6) were characterized through the following parameters, i.e., average weight, moisture content, disintegration time, assay, and dissolution.

Average weight The average weight of F1, F2, F3, F4, F5 and F6 were 202.75, 202.97, 202.15, 201.65, 202.75 and 203.2, respectively. All the values were expressed in milligrams "mg." The acceptance criteria were 190 mg to 210 mg, and all test formulations conform to the prescribed limits.

Moisture content

The moisture contents of F1, F2, F3, F4, F5, and F6 were 1.4, 1.2, 1.5, 1.2, 1.4, and 1.2, respectively.

Table 8. Dissolution profiles of six (06) formulations (F1-F6) of racecadotril 100mg capsules.

Formulations	Time points (Min)	Percentage Dissolution (%)						
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**Mean**
F1	15	59.4	60.4	60.8	58.4	62.4	64.5	60.98
	30	70.4	68.5	71.3	67.5	67.3	69.4	69.07
	45	68.5	75.7	75.9	75.7	62.4	64.5	70.45
	60	68.2	74.4	70.6	75.7	73.5	77.4	73.30
F2	15	51.4	54.9	53.3	52.2	54.4	51.5	52.95
	30	61.2	63.4	64.1	63.9	61.4	64.7	63.12
	45	62.6	57.1	61.5	56.7	63.4	61.3	60.43
	60	75.4	77.2	75.2	74.8	76.3	74.5	75.57
F3	15	68.5	69.5	68.7	58.4	62.4	64.5	65.33
	30	70.4	67.6	71.3	68.5	70.1	67.5	69.23
	45	68.5	75.7	75.9	75.7	62.4	64.5	70.45
	60	68.2	74.4	70.6	75.7	73.5	77.4	73.30
F4	15	58.3	65.7	68.7	58.4	62.4	64.5	63.00
	30	63.4	72.2	71.3	71.8	75.1	74.5	71.38
	45	68.5	72.3	68.5	70.4	62.4	64.5	67.77
	60	68.2	70.4	66.5	70.5	73.5	70.4	69.92
F5	15	68.5	65.7	68.7	60.5	62.4	66.5	65.38
	30	69.8	72.2	67.5	68.5	70.1	70.5	69.77
	45	68.5	69.6	71.1	70.5	62.4	64.5	67.77
	60	69.6	74.4	70.6	75.7	73.5	77.7	73.58
F6	15	72.2	65.7	68.7	65.0	62.2	62.8	66.1
	30	70.4	72.2	75.3	71.8	75.1	74.5	73.2
	45	75.5	75.7	75.9	75.7	75.7	75.9	75.8
	60	76.1	77.8	77.4	75.7	76.5	78.2	76.9

Table 9. Dissolution profile of test (Racecadotril capsules 100mg) and reference (Hidrasec capsules 100mg) products

Sr #	Time of sample withdrawal (min)	Percent dissolution obtained (test) %						
		S1	S2	S3	S4	S5	S6	Mean
1	15	72.2	65.7	68.7	65.0	62.2	62.8	66.1
2	30	70.4	72.2	75.3	71.8	75.1	74.5	73.2
	45	75.5	75.7	75.9	75.7	75.7	75.9	75.8
4	60	76.1	77.8	77.4	75.7	76.5	78.2	76.9
Reference								
1	15	73.3	66.3	70.0	67.3	71.0	70.2	69.7
2	30	80.0	81.3	78.4	78.8	75.5	76.3	78.4
3	45	75.3	76.9	75.7	81.1	75.5	75.3	76.6
4	60	78.8	79.2	77.2	81.8	78.2	77.2	78.6

Disintegration time

The disintegration time of F1, F2, F3, F4, F5, and F6 was 16, 16, 15, 16, 17, and 16 minutes. All values are expressed as minutes.

Assay

The assay results of F1, F2, F3, F4, F5 and F6 were found to be 99.7, 98.2, 97.6, 97.9, 99.2, and 99.5, respectively.

Dissolution

Results of dissolution profiles of all test formulations were summarized in table 8. It was observed that F6 showed better drug release pattern as compared to F1-F5. The average release of Racecadotril test product was 66.1%, 73.2%, 75.8%, and 76.9% at 15, 30, 45, and 60 minutes, respectively. Similarly, the average release of Racecadotril reference product was 69.7%, 78.4%, 76.6%, and 78.6% at 15, 30, 45, and 60 minutes, respectively.

Comparative dissolution results

The percentage drug release from test product of racecadotril capsules 100mg (table 9) and reference product Hidrasec capsules 100mg was compared at 15, 30, 45, and 60 minutes.

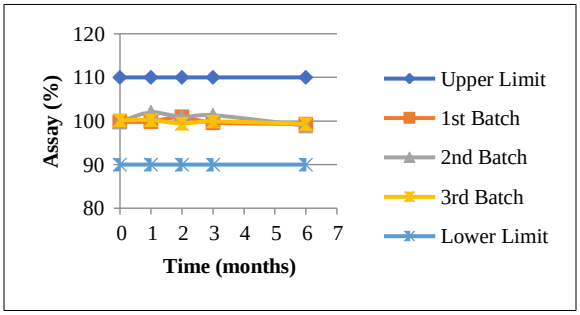

Fig 4. Comparison of assay results of batches at various time

Statistical analysis of compared dissolution profiles

The Model Independent approach statistically compared the test product's dissolution profile (Racecadotril capsules 100 mg) and reference product (Hidrasec capsules 100 mg). The calculated the difference (f1) and similarity (f2) factors as 3.73% and 75.32%, respectively by a software (DD-Solver). Both the factors (f1 and f2) are well within the acceptable criteria.

Accelerated stability study

The table 10 represents the summary of results obtained after testing set parameters (physical appearance, moisture content, dissolution, and assay) at set time points on batches kept in the chamber for 6 months under accelerated stability study conditions of Zone-IVa, i.e., temperature 40°C± 2°C and relative humidity 75% ±5%.

Table 10. Summary of accelerated stability study data.

Stability Batch	Parameters Tested	Acceptance criteria	Initial (T0)	1st month	2nd month	3rd month	6th month
1st Batch	Physical appearance	White powder fill in green body and white cap shell	Complied	Complied	Complied	Complied	Complied
	Moisture content	Not more than 3%	1.2	1.8	2.1	2.06	2.68
	Dissolution	Must comply	81.6	N/P	N/P	79.4	75.4
	Assay	90-110%	99.83	99.87	101.0	99.61	99.3
2nd Batch	Physical appearance	White powder fill in green body and white cap shell	Complied	Complied	Complied	Complied	Complied
	Moisture content	Not more than 3%	1.2	2.3	1.9	2.1	2.62
	Dissolution	Must comply	86.4	N/P	N/P	79.4	75.6
	Assay	90-110%	99.83	102.01	100.9	101.34	98.9
3rd Batch	Physical appearance	White powder fill in green body and white cap shell	Complied	Complied	Complied	Complied	Complied
	Moisture content	Not more than 3%	1.8	1.7	1.72	2.3	2.35
	Dissolution	Must comply	83	N/P	N/P	79	74.8
	Assay	90-110%	100.2	99.4	99.91	99.34	98.3

The data shows that all the batches kept under accelerated stability study conditions (40°C±2°C and 75% ±5%) were found stable with no significant difference was recorded in values of parameters assessed during Six (06) months. Figure 5 shows graphical comparison between assay results of batches at various time points kept under temperature 40°C±2°C and relative humidity 75% ±5%, and there was no significant difference.

Conclusions

Capsule formulation Racecadotril100mg was successfully prepared. According to ICH guidelines and the stability-indicating method, the method was validated by HPLC, indicating that the product was designed to bear environmental and accelerated stress conditions. The best suitable formulation proceeded further for comparative dissolution studies that showed the prepared formulation meets all criteria.

Three batches of the formulations were examined for accelerated stability conditions shows that capsules are stable.

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