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DEVELOPMENT OF STABILITY INDICATING HPTLC METHOD FOR RACECADOTRIL FROM BULK DRUG AND ITS DOSAGE FORM

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ABSTRACT

A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of racecadotril in bulk drug and its dosage form was developed and validated. The method employed Merck HPTLC aluminum sheets of silica gel $60F_{254}$ as the stationary phase. The solvent system consisted of chloroform : methanol (9.8 : 0.2 v/v) This system was found to give compact spots for racecadotril (R_f 0.61 ± 0.02). Racecadotril was subjected to acid and alkali hydrolysis, oxidation and photodegradation, and the degraded products were well separated from pure drug. Densitometric analysis of racecadotril was carried out in the remission - absorbance mode at 232 nm. The linear regression analysis data for the calibration plots showed good linear relationship with coefficient of regression value, $r^2 = 0.9994$ in the concentration range of 100-1000 ng per spot. The mean value of correlation coefficient, slope and intercept were 0.9995 ± 1.88, 0.352 ± 0.02 and 5.205 ± 0.05, respectively. The limits of detection and quantitation were 200 and 600 ng per spot, respectively. The method was validated as per ICH guidelines for precision, recovery and robustness. Racecadotril samples on being degraded with hydrogen peroxide showed additional peaks at R_f 0.006 and 0.64 while the drug on being subjected to acidic and basic hydrolysis showed additional peaks at R_f 0.50 and 0.18, respectively. This indicates that the drug is susceptible to acid-base hydrolysis degradation, oxidation and photochemical degradation. Statistical analysis proves that the method is reproducible and selective for the estimation of the said drug. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Key words: Racecadotril, HPTLC, degradation, precision, recovery, robustness

INTRODUCTION[1,2]

Racecadotril [acetorphan: N-[(R, S)-3-acetylmercapto-2-benzylpropanoyl]-glycine,

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benzyl ester, a novel drug for the treatment of acute diarrhoea owes its mechanism of action as a true antisecretory agent. No stability indicating HPTLC method for determination of racecadotril in pharmaceutical dosage form has been reported till now. The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of active substances.The objective of the present work was to elucidate the inherent stability characteristics of the drug substance. Susceptibility to oxidation is one of the required tests. The hydrolytic and photolytic stability are also required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. A very viable alternative for stability-indicating analysis of racecadotril in bulk drug and its dosage form is High-Performance Thin-Layer Chromatography (HPTLC). The advantage of HPTLC is that several samples can be run simultaneously by using small quantity of mobile phase unlike HPLC; thus, lowering analysis time and cost per analysis.

The objective of the present work was to develop an accurate, specific, reproducible, and stability indicating method for the determination of racecadotril in bulk drug and its dosage form in the presence of its degradation products and related impurities as per ICH guidelines.

EXPERIMENTAL

Materials and method

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on precoated silica gel aluminium plate 60 F – 254, (20 × 10 cm) with 250 μm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai, using a Camag Linomat V applicator (Switzerland). A constant application at the rate of 0.1 μL/s was employed and space between two bands was 6 mm.

The slit dimension was kept at 5 × 0.45 mm and 20 mm/s scanning speed were employed. Each track was scanned thrice and baseline correction was used. 10 mL of the mobile phase consisting of chloroform : methanol: (9.8 **:** 0.2 v/v); was used. Linear ascending development was carried out in the presaturated 20 x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). Dimensions: length × width × height = 12 × 4.7 × 12.5 cm. The optimized chamber saturation time with mobile phase was 20 min at room temperature (25^{o}C ± 2) at relative humidity of 60 % ± 5. The length of chromatogram run

was 80 mm. Subsequent to the development; HPTLC plates were dried in a current of air with the help of an air dryer.

Densitometric scanning was performed on Camag TLC scanner III in the remission-absorbance mode at 232 nm for all measurements and operated by WinCATS software. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm (Fig. 1).

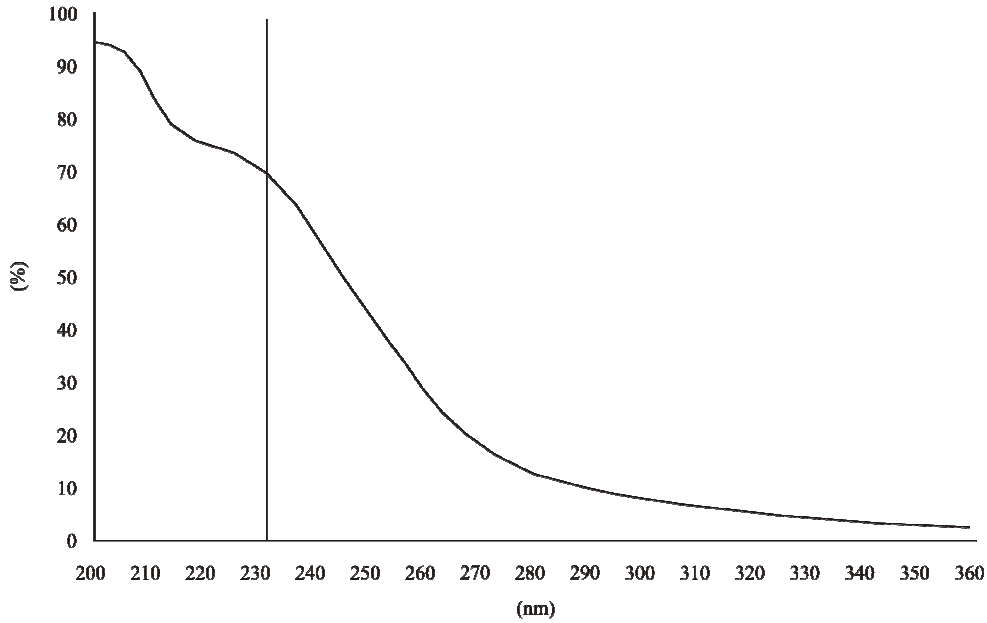

Fig. 1: Spectra of standard drug measured from 190 to 450 nm

RESULTS AND DISCUSSION

Optimization of method

The method was optimized by using different ratios of organic solvents. The mobile phase consisting of chloroform : methanol (9.8 : 0.2 v/v) gave good resolution with R_f value of 0.61 ± 0.02. (Fig. 2) Racecadotril was obtained, when densitometric scanning was performed at 232 nm. The spot appeared more compact and the peak shape was symmetrical. Well-defined spots of standard along with its degradation products were obtained, when the chamber saturation time was optimized at 20 min at room temperature.

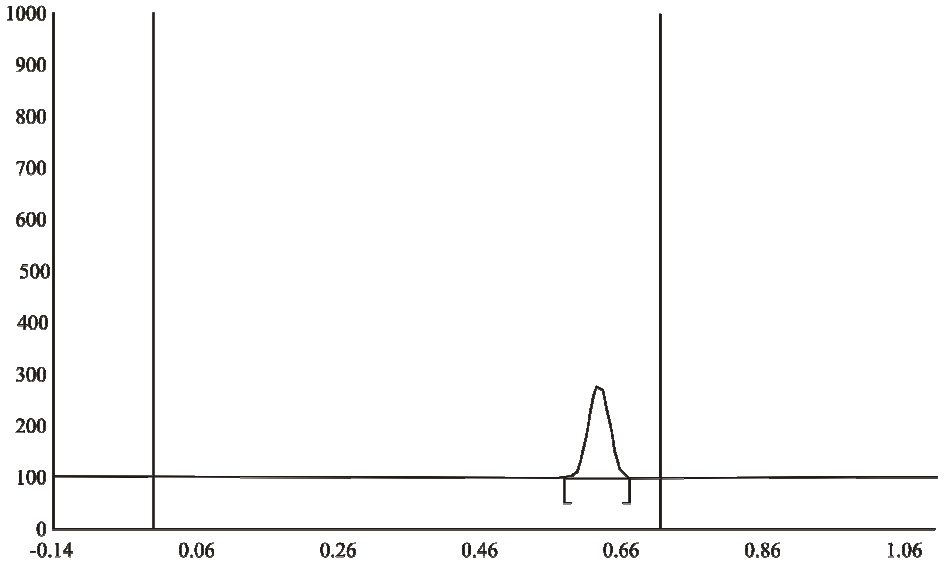

Fig. 2. Densitogram of standard drug (100 ng); peak 1 (R_f : 0.61 ± 0.02) measured at 232 nm, mobile phase chloroform : methanol (9.8 : 0.2 v/v)

Validation of the method[3-5] (Table 2)

Lineartity

Racecadotril showed linearity in concentration range of 100 -1000 ng per spot (r^2 = 0.9994 ± 1.88). Linearity was evaluated by determining 10 standard working solutions containing 100-1000 ng per spot twice in triplicate**.** (Fig. 3 , Table 1)

Table 1. Linear regression data for calibration curve (n = 6) a 95 % confidence limit

Linearity range	100-1000 ng/spot
r^2 ± SD	0.9995 ± 1.88
Slope ± SD	0.352 ± 0.02
Intercept ± SD	5.205 ± 0.05
Confidence limit of slope[a]	0.342 - 0.36
Confidence limit of intercept[a]	4.85 - 5.56
SE of estimation	0.77

Precision

The repeatability of sample application and measurement of peak areas were expressed in terms of % RSD and were found to be 1.68 and 1.13, respectively, which shows that the proposed method provides acceptable intra and inter-day variation of racecadotril.

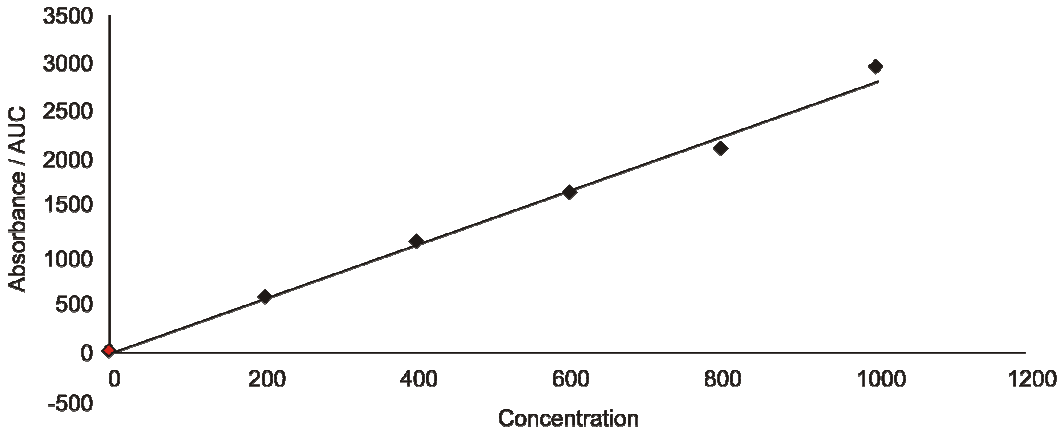

Fig. 3. Calibration curve for racecadotril

Robustness

The standard deviation of peak areas were calculated for each parameter and % RSD was found to be less than 2%. The low values of % RSD indicated the robustness of the method.

LOD and LOQ

The LOD and LOQ were found to be 200 ng/spot and 600 ng/spot, respectively for racecadotril.

Table 2. Summary of validation parameter

Parameter HPTLC densitometry	
Linearity range	100-1000 ng/spot
Correlation coefficient	0.9995 ± 1.88

Cont…

Parameter HPTLC densitometry	
Limit of detection	200 ng/spot
Limit of quantitation	600 ng/spot
Recovery	100.11 ± 0.95
Precision (% RSD)	-
Repeatability of application	1.58
Repeatability of measurement	1.08
Inter–day (n = 6)	1.63
Intra–day (n = 6)	1.54
Robustness	Robust
Specificity	specific

Specificity

Assessment of the peak purity was performed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot. i.e., r (start, middle) = 0.9994 and r (middle, end) = 0.9995. Good correlation (r = 0.9995) was obtained between standard and sample spectra of racecadotril.

Accuracy

It was performed by standard addition technique. Racecadotril from pharmaceutical dosage after spiking with additional drug showed afforded recovery in range of 99.17-101.45 %.

Analysis of marketed formulation

A single spot at R_f 0.61 for racecadotril was observed in the densitogram of the drug samples extracted from capsules. There was no interference from the excipients commonly present in the capsule. The drug content was found to be 99.17 % ± 1.68 (% RSD of 0.52) for racecadotril. Therefore, it may be inferred that degradation of racecadotril was not occurred in the marketed formulations that were analyzed by this method. The low % RSD value indicated the suitability of this method for routine analysis of racecodotril in pharmaceutical dosage form.

Stability - indicating property[6]

The densitogram of the acid degraded sample showed one additional peak at R_f = 0.50 along with Racecadotril peak R_f = 0.62, while in alkali degraded sample showed additional peak at R_f = 0.18.

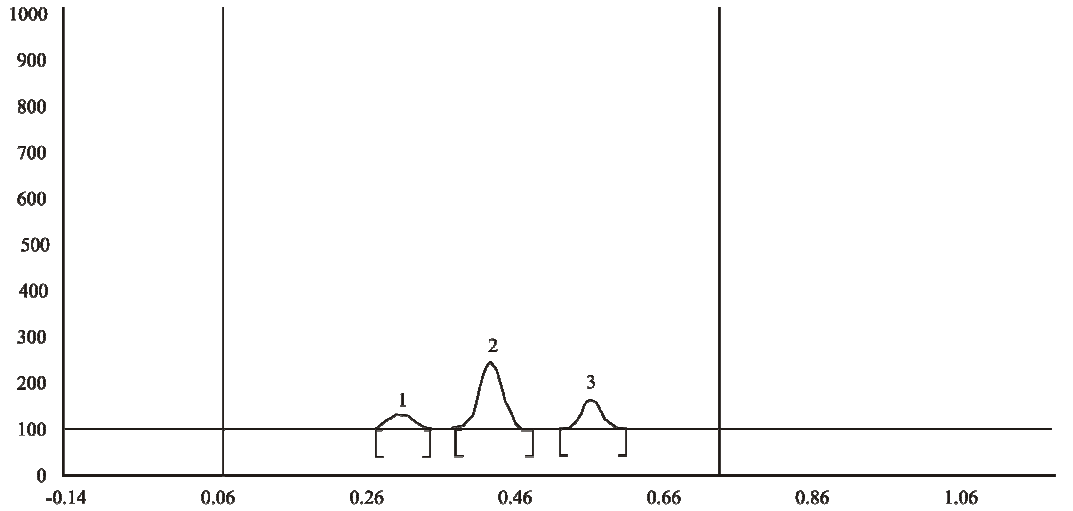

Fig. 4: Densitogram of acid (1N HCl, reflux for 2 h, temp 70ºC) treated drug; peak 1 (degraded) (R_f : 0.35), peak 2 (degraded) (R_f : 0.47), peak 3 (Standard) (R_f : 0.625)

The sample degraded with 6 % and 50 % w/v hydrogen peroxide showed two additional peaks at R_f value of 0.006 and 0.64. (Fig. 4) The spots of degraded products were well resolved from the drug spot. The photo degraded sample showed no additional peak and no change in peak area of standard, when drug solution was left in day light for 360 hr. The drug was degraded, when exposed to UV irradiation for 360 h and showed one additional peak at R_f value 0.45. The samples degraded under dry heat conditions showed one additional peak in HPTLC at R_f 0.45 and peak area of standard remains unchanged. Under wet heat conditions, the peak area of the parent drug remains unchanged and showed one additional peak at t_r 0.5 min. This indicates that the drug is susceptible to acid-alkali hydrolysis, dry and wet heat degradation, oxidation as well as UV degradation**.** (Table 3)

Table 3. Degradation of racecadotril using HPTLC method

Condition	Time (h)	% Recovery	R_f value of degradation product
Acid 1 N HCl, reflux (70 ºC)	2	3.97	0.35, 0.47
Base 1 N NaOH, reflux	2	-	0.18
H_2O_2 6 % w/v, reflux	2	40.75	0.006,0.64
H_2O_2 50 % w/v, reflux	2	6.25	0.006
Dry Heat (80 ºC)	6	100	0.45
Wet heat, reflux (100 ºC)	2	100	0.5
Day light (25 ºC)	360	100	-
UV light	360	83.95	0.45

CONCLUSION

The proposed HPTLC method provides simple, accurate, reproducible and stability indicating for quantitative analysis for determination of racecadotril in pharmaceutical capsules, without any interference from the excipients and in the presence of its acidic, alkaline, oxidative and photolytic degradation products. This method was validated as per ICH guidelines. The HPTLC method uses a minimal volume of solvents, compared to HPLC method. Statistical tests indicate that the proposed HPTLC reduce the duration of analysis and appear to be equally suitable for routine determination of racecadotril in pharmaceutical formulation in quality control laboratories, where economy and time are essential. This study is a typical example of development of a stability-indicating assay, established following the recommendations of ICH guidelines. It is one of the rare studies where forced decomposition was done under all different suggested conditions and the degradation products were resolved. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities and also in stability studies. It is proposed for the analysis of the drug and degradation products in stability samples in industry. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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