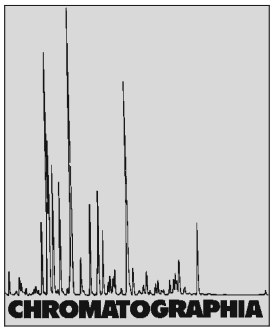
RP-LC Analysis and Hydrolytic Degradation Profile of Racecadotril



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Abstract

A sensitive, stability-indicating liquid-chromatographic method for analysis of racecadotril in the presence of its degradation products has been developed and validated. Efficient chromatographic separation was achieved on a C_{18} column with a simple isocratic mobile phase—60:40 methanol–water. Quantification was by photo-diode array (PDA) detection at 220 nm. The linearity of the method was excellent over the range 1–32 μg mL^{-1}. The method was sensitive, with low limits of detection (20 ng mL^{-1}) and quantification (100 ng mL^{-1}). The recovery of the method was consistently good (98.7–100.9%), with low (<1%) intra-day and inter-day relative standard deviation. Robustness studies confirmed that peak area was unaffected by small changes in temperature, and mobile phase composition and flow rate. Both alkaline and acidic hydrolytic degradation were performed in methanolic solution. In alkaline medium the drug was degraded immediately; it was degraded within 90 min in acidic medium. The validated, stability-indicating, method was used for analysis of racecadotril in pharmaceutical dosage form and also to reveal the hydrolytic degradation profile of the racecadotril.

Keywords

Column liquid chromatography
Forced degradation
Hydrolytic degradation
Stability-indicating method
Racecadotril

Introduction

Racecadotril, (±)-benzyl 2-(2-(acetylthiomethyl)-2-methyl-3-phenylpropanamido) acetate, is a new anti-diarrheal drug [1]. In peripheral tissue membranes racecadotril is converted into thiorphan, which inhibits the enzyme enkephalinase. Enkephalin concentration is increased as a result of this, leading to activation of opioid receptors and a decrease in cAMP level. This in turn results in reduced secretion of water and electrolytes into the intestinal lumen [2–9].

Racecadotril and its metabolite, thiorphan in human plasma have been analyzed by LC with UV detection after solid-phase extraction [10] and racecadotril in capsules has been determined by RP-LC with gemfibrozil as internal standard [11]. Three unknown impurities in racecadotril bulk drug at levels below 0.5% have been detected by simple reversed-phase isocratic LC. The structures of these impurities have been proposed on the basis of molecular ion information [12]. Quantitative analysis of racecadotril, in raw material and in capsules has been achieved by UV spectroscopy and RP-LC [13]. A liquid chromatographic–tandem mass spectrometric method for quantification of thiorphan in human plasma, using lisin-

opril the internal standard, has been developed and validated [14]. There is, however, no report of methods for determination of the degradation profile of racecadotril.

LC is a routine analytical technique for analysis of drugs. It has proved to be a very useful technique because of its low operating cost and the need for minimum sample clean-up [15]. The objective of the work discussed in this paper was to develop a stability-indicating LC method for analysis of racecadotril in the bulk product and in marketed formulations, and to establish the hydrolytic degradation profile of the drug under both acidic and alkaline conditions. An accurate, specific, repeatable, and stability-indicating method, which can also be used for analysis of racecadotril in the presence of its degradation products and for assessment of the purity of the bulk drug and the stability of dosage forms of the drug is described in this paper.

ICH guidelines requires stress testing to be performed to assess the inherent stability of the active substance [16]. A variety of tests of, for example, hydrolytic stability, susceptibility to oxidation, and thermal stability must be conducted [17]. An ideal stability-indicating method is one that quantifies not only the drug compound alone but also resolves its degradation products [18]. Forced degradation studies are used to facilitate the development of an analytical method, to obtain better understanding of the stability of active pharmaceutical ingredients and drug products, and to provide information about degradation pathways and degradation products [19].

Experimental

Chemicals and Materials

Racecadotril was kindly supplied as a gift by Ajanta Pharma, Hydrabad, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. Racecadotril capsules (Redotil; Dr. Reddy's Laboratories) were purchased locally.

Chromatography

LC was performed with Shimadzu LC-10ATvp instrument with a Shimadzu SPD-M10 AVP UV–visible diode-array detector. Compounds were separated on a 250 mm × 4.60 mm i.d., 0.5 µm particle size, 100 Å Luna 5-4, C_{18} column (Phenomenex) protected by a Phenomenex security guard column (universal fit). The mobile phase was 60:40 methanol–water at a flow rate of 1.5 mL min^{-1}. Before use the mobile phase was filtered through a Ufipore N_{66} Nylon-6,6 0.45 µm membrane filter membrane filter (Pall Life Sciences). All samples were also filtered before injection (Hamilton 702NR microliter syringe). The detection wavelength used for monitoring of racecadotril and its degradation products was 220 nm.

Method Validation

Linear Range and Calibration

A fresh stock solution (1,000 µg mL^{-1}) of racecadotril of was prepared in 50% aqueous methanol and standard solutions (six replicates) containing 1, 2, 4, 8, 16, and 32 µg mL^{-1} were prepared from the stock solution by dilution with the same solvent. Chromatograms were recorded (Fig. 1a, b) and average peak area was plotted against concentration.

Accuracy

Apparent recovery was determined to confirm the suitability and accuracy of the method. Standard dilutions (6 µg mL^{-1}) were analyzed and then spiked with 4, 6, and 8 µg mL^{-1} of the drug and re-analyzed. The experiment was repeated six times.

Precision

The repeatability of peak area was determined by performing six replicate injections of 2, 4, and 8 µg mL^{-1} solutions to determine inter-day variation and analyst-to-analyst variation.

Robustness

Mobile phase composition was varied by 2%, mobile phase flow rate was changed from 1.4 to 1.6 mL min^{-1}, and column temperature was varied by 5% and the effects on the results were examined. The robustness of the method was checked at three different concentrations—4, 6, and 8 µg mL^{-1}.

LOQ and LOD

To estimate the limits of detection (LOD) and quantitation (LOQ), diluent was injected six times and the signal-to-noise ratio (S/N) was determined. LOD and LOQ were regarded as the amounts for which S/N was 3:1 and 10:1, respectively.

Specificity

The specificity of the method was ascertained by analyzing drug standard solution and samples of equivalent concentration (8 µg mL^{-1}). The identity of the peak in the sample was confirmed by comparison of the retention time and UV spectrum of the peak from the sample with those of the peak from the standard. Peak purity for the drug was assessed by comparing UV spectra acquired at the peak start, peak apex, and peak end.

Stability in Sample Solution

A solution of concentration 8 µg mL^{-1} was stored on a laboratory bench at room temperature in a tightly capped volumetric flask, protected from light, and analyzed after 1, 2, 6, 12, and 24 h to seek the presence of any peak other than that of the drug.

Analysis of Dosage Form

Powder from Racecadotril capsules equivalent to 100 mg racecadotril was sonicated for 30 min with 25 mL methanol to ensure complete extraction of the drug. The volume was then diluted to 50 mL and the solution was filtered through a 0.45-µm membrane filter and

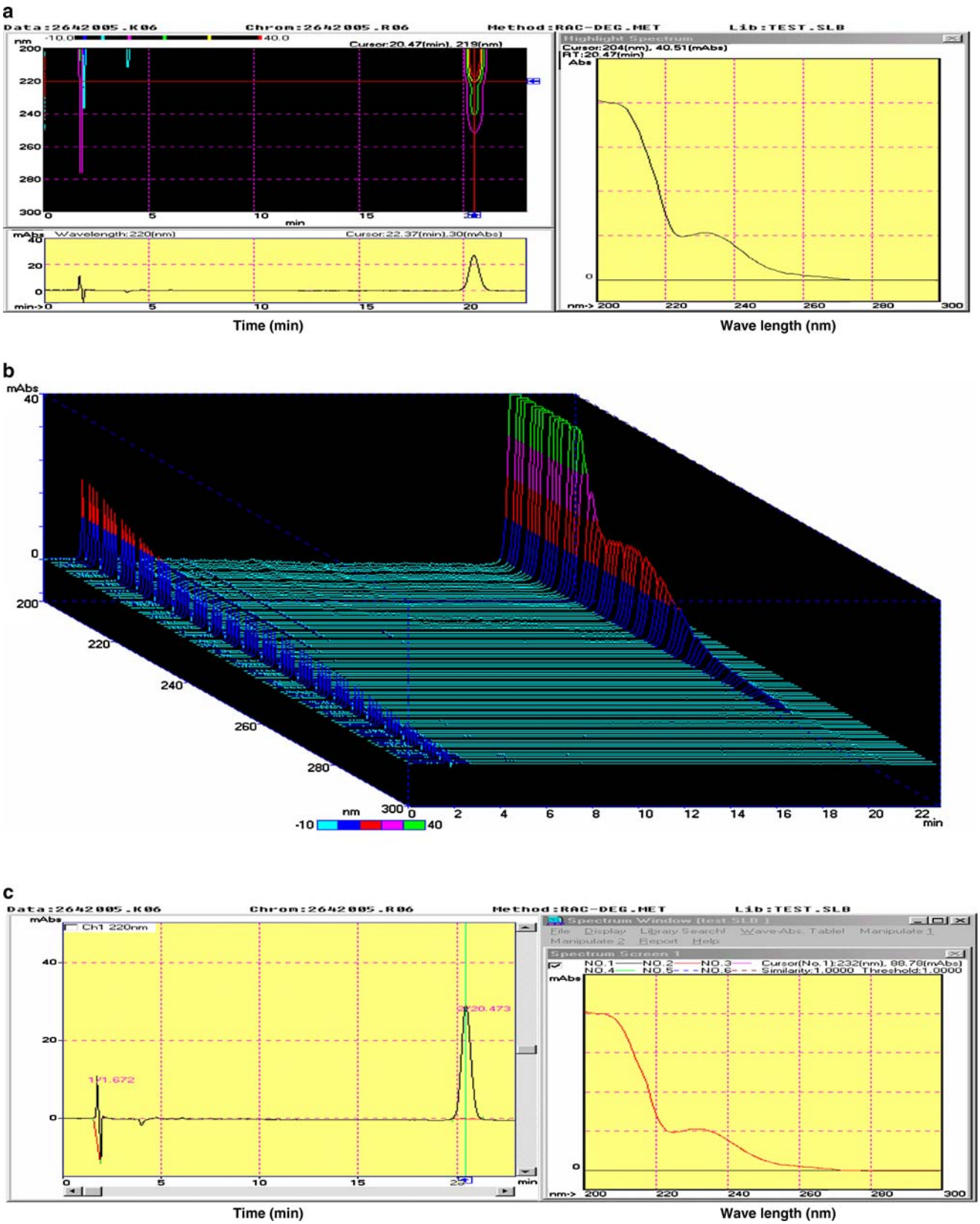

Fig. 1. **a** Chromatogram from a standard, contour plot, and absorption spectrum at 20.47 min; **b** three-dimensional view of the chromatogram; **c** representative chromatogram from analysis of racecadotril capsules

Table 1. Validation data for racecadotril

Method characteristic	Value ± SD, ±RSD (%)[a]
Linearity	1–32 μg mL^{-1}
Regression equation	Peak area = $23735.35X - 18.02$[b]
Correlation coefficient	$r^2 = 0.9999 \pm 0.0021$, 0.0002
Response ratio	2377.778 ± 4.267, ±0.1794
Range	2–12 μg mL^{-1} ± 3.82, ±0.0003
Accuracy	100.12 ± 0.917, ±0.0092
Precision	
Repeatability	99.92 ± 0.319, ±0.0032
Intermediate precision	
Inter-day	99.98 ± 0.147, ±0.0015
Analyst-to-analyst	99.97 ± 0.121, ±0.0012
Robustness	
Flow rate (1.4–1.6 mL min^{-1})	100.02 ± 1.25, ±0.247
Mobile phase composition (±2%)	99.45 ± 1.34, ±0.323
Temperature (±5%)	100.12 ± 1.64, ±0.053
LOQ	100 ng mL^{-1} ± 1.86, ±0.075
LOD	20 ng mL^{-1} ± 2.76, ±0.086
Specificity	Ascertained by analyzing standard drug and samples of equivalent concentration
Stability in sample solution	
Response ratio	2377.778 ± 4.267, ±0.1794

[a] Mean of six replicates
[b] X is concentration

diluted to 8 μg mL^{-1} for analysis. The experiment was repeated six times.

Forced Acidic and Alkaline Degradation

HCl (0.02 M, 50 mL) and NaOH (0.02 M, 50 mL) were added separately to methanolic stock solutions of racecadotril (1,000 μg mL^{-1}, 50 mL) and the mixtures were kept at room temperature for 1 h in the dark (to exclude possible degradative effect of light). Samples were withdrawn after 0, 15, 30, and 60 min, neutralized by addition of NaOH or HCl (0.01 M), diluted to 10 μg mL^{-1} with 60:40 methanol–water, and analyzed as described above.

Result and Discussion

Optimization of the Mobile Phase

The LC procedure was optimized with the objective of developing a stability-indicating assay. After performing both acidic and alkaline degradation of the drug, neutralized dilutions were injected and chromatographed with different mobile phases. Initially, 50:50 acetonitrile–methanol was investigated. Resolution of the drug peak was good but peaks of the degradation products were not resolved satisfactorily. To improve the resolution of the peaks of the degradation products water–methanol–acetonitrile 40:30:30 and 50:30:20 and methanol–water 40:60, 50:50, and 60:40 were evaluated as mobile phases. The best result was obtained by use of 60:40 methanol–water, which enabled good resolution of the drug from its degradation products. At a flow rate of 1.5 mL min^{-1} the retention time of racecadotril was 20.5 ± 0.2 min. Well-defined peaks were obtained when the column was equilibrated with mobile phase for 20 min at room temperature.

Method Validation

Linear Range and Calibration

Response was a linear function of concentration over the range 1–32 μg mL^{-1}; of this range, 2–12 μg mL^{-1} was used as the working range of the method. Peak area and concentration were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient (Table 1). The linearity of the calibration plots was confirmed by the high value of the correlation coefficients ($r^2 = 0.9999 \pm 0.0021$). % RSD for the correlation coefficients was <2.

Accuracy

When the method was used for extraction and subsequent analysis of the drug in the dosage form after spiking with 50, 100 and 150% of the drug, recovery was 98.7–100.9%, standard deviation 0.917 (% RSD ± 0.0092).

Precision

The repeatability of sample injection and measurement of peak area, expressed as RSD (%) are listed in Table 1. Repeatability and intermediate precision at three different concentrations (2, 4, and 8 μg mL^{-1}) for both within-day and day-to-day analysis were always <2%. These low values of the RSD showed the repeatability and intermediate precision of the method were good.

Robustness

The standard deviation of peak areas was calculated for each set of conditions and found to be <2%. The low values of RSD obtained after introduction of small deliberate changes in the conditions (Table 1) indicate the method is robust.

LOQ and LOD

The LOD for a S/N ratio of 3:1 was 20 ng mL^{-1} (RSD ± 0.086%) and the LOQ for a signal to noise ratio of 10:1 was 100 ng mL^{-1} (RSD ± 0.075%). These results indicated the sensitivity of the method was adequate.

Specificity

Comparison of the retention time and UV spectrum of the peak from the sample with those of the peak from the standard, and assessment of peak purity for the drug confirmed the specificity of the method.

Stability in Sample Solution

The drug was stable in the mobile phase, and the response ratio of the drug was

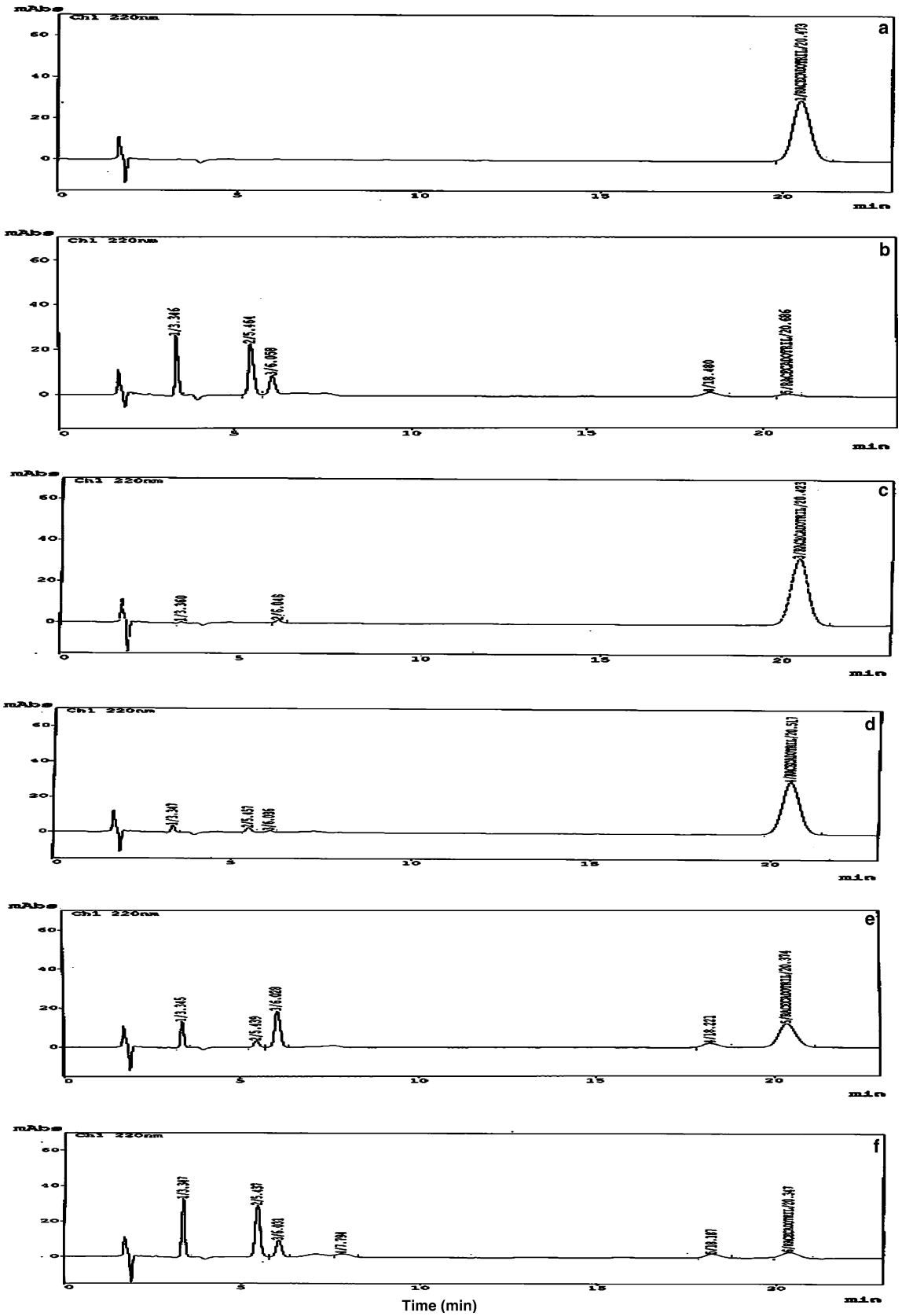

Fig. 2. Chromatograms obtained from racecadotril before treatment with acid or alkali (**a**), after treatment with 0.01 M NaOH for 0 min (**b**), and after treatment with 0.01 M HCl for 0 (**c**), 15 (**d**), 30 (**e**), and 60 min (**f**)

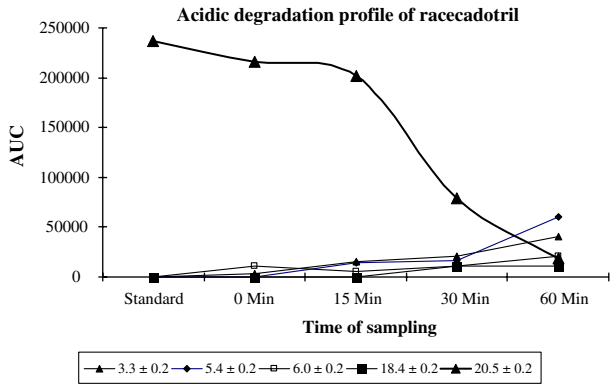

Fig. 3. Acidic degradation profile of racecadotril

2377.778 (RSD 0.1794%). It was therefore inferred that analysis of the drug could be performed any time after preparation of the standards and samples (Fig. 2a).

Analysis of the Dosage Form

There was no interference from excipients commonly used in the capsules (Fig. 1c). The drug content of the capsules was 99.88–100.13%, RSD 0.0010%. No degradation products were evident when commercial capsules where analyzed by this method. The good performance of the method indicated it was suitable for routine analysis of racecadotril in pharmaceutical dosage forms.

Hydrolytic Degradation Profile

Alkaline Degradation

Alkaline degradation of racecadotril was performed in 1:1 methanol–0.01 M sodium hydroxide because the drug was insoluble in sodium hydroxide solution. Racecadotril was highly susceptible to attack by sodium hydroxide. Hydrolysis began immediately after addition of sodium hydroxide and was complete after 5 min at room temperature. At this time four peaks (retention times 3.3, 5.4, 6.0, and 18.4 min) were apparent (Fig. 2b) and that of racecadotril was absent. At subsequent times the areas of the two peaks at retention times 3.3 and 5.4 min increased and the other two disappeared from the chromatogram.

Acidic Degradation

Acidic degradation of racecadotril was performed in 1:1 methanol–0.01 M HCl at room temperature because the drug was insoluble in hydrochloric acid. Acidic degradation of racecadotril started immediately on addition of HCl producing four degradation products (retention times 3.3, 5.4, 6.0, and 18.4 min) and the drug was completely degraded after 90 min. The degradation products of retention time 3.3 and 6.0 min appeared in the chromatogram obtained immediately after addition of the acid (Fig. 2c) and their concentration increased as time passed. A degradation product of retention time 5.4 min appeared after 15 min (Fig. 2d) and a degradation product of retention time 18.4 min appeared after 30 min (Fig. 2e, f). Thus racecadotril rapidly degrades in 1:1 methanol–0.01 M HCl (Fig. 3).

Conclusion

A new, sensitive, and stability-indicating LC method for analysis of racecadotril has been successfully developed. Simple isocratic elution and an easy extraction procedure enabled rapid and cost-effective analysis of the drug. The method was accurate and precise with consistently good recovery. It also enabled good resolution of the drug from its degradation products, impurities, and formulation excipients. The drug was rapidly degraded under alkaline conditions (degradation was complete within 5 min of addition of 0.01 M NaOH. In acidic medium (0.01 M HCl) the drug was completely degraded after 90 min. Thus, racecadotril is highly susceptible to hydrolytic degradation. The validated stability-indicating method can be used for routine analysis of racecadotril as the bulk drug and in the pharmaceutical dosage form, and other quality-control samples obtained during product development.

References

1. Matheoson AJ, Noble S (2000) Drugs 59(4):829–835. doi:10.2165/00003495-200059040-00010
2. Scand PD (2002) J Gastroenterol 37(6):656–661
3. Vetal JM, Barard H, Fretault N, Lecomte JN (1999) Aliment Pharmacol Ther 13(6):21–26
4. Lecomte JM (2000) Int J Antimicrob Agents 14(1):81–87. doi:10.1016/S0924-8579(99)00152-1
5. Alam NH, Ashraf H, Khan WA, Karim MN, Fuchs GJ (2003) Gut 52(10):1419–1423. doi:10.1136/gut.52.10.1419
6. Salaza LE, Santisteban PJ, Chea WE, Gutierrez M (2000) N Engl J Med 343(7):463–467. doi:10.1056/NEJM200008173430703
7. Schwartz JC (2000) Int J Antimicrob Agents 14(1):75–79. doi:10.1016/S0924-8579(99)00151-X
8. Primi MP, Bueno LP, Berared H, Lacomte JM (1999) Aliment Pharmacol Ther 13(6):3–7. doi:10.1046/j.1365-2036.13.s6.3.x
9. Rao SG (2000) J Indian Med Assoc 100(8):530–538
10. Fan X, Lingli Y, Guili X (2008) J Chromatogr B Analyt Technol Biomed Life Sci 861:130–135. doi:10.1016/j.jchromb.2007.11.038
11. Prabhu SL, Singh T, Joseph A, Kumar CD, Shirwaikar A (2007) Indian J Pharm Sci 69(6):819–821
12. Reddy KM, Babu JM, Sudhakar P, Sharma MS, Reddy GS, Vyas K (2006) Pharmazie 61(12):994–998
13. Rao PS, Nappinnai M (2007) Asian J Chem 19(5):3697–3702
14. Yu X, Jinchang H, Fei L, Shu G, Qingxiang G (2007) J Chromatogr B Analyt Technol Biomed Life Sci 852:101–107. doi:10.1016/j.jchromb.2006.12.041
15. Steven WB (2006) Trends Analyt Chem 25(8):758–767. doi:10.1016/j.trac.2006.05.012
16. International Conference on Harmonization (2000) Note for guidance on stability

testing of new drug substance and products. In: International conference on harmonization of technical requirement for registration of pharmaceutical for human use, Geneva
17. Pathare DB, Jadhav AS, Shingare MS (2007) Drug Dev Ind Pharm 33:551–557. doi:10.1080/03639040601134140
18. Daraghmeh NN, Al-Omari MM, Sara Z, Badwan AA, Jaber AMY (2002) J Pharm Biomed Anal 29:927–937. doi:10.1016/S0731-7085(02)00216-9
19. Karen MA, Akemi A, Roland B, Janice E, Todd DH, Wei K et al (2007) Adv Drug Deliv Rev 59:29–37. doi:10.1016/j.addr.2006.10.006