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Spectrophotometric and chromatographic determination of rabeprazole in presence of its degradation products

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Abstract

Three methods were presented for the determination of rabeprazole (RA) in presence of its degradation products. The first method was based on high performance liquid chromatographic (HPLC) separation of RA from its degradation products on a reversed phase, ODS column using a mobile phase of methanol–water (70:30, v/v) and UV detection at 284 nm. The second method was based on HPTLC separation followed by densitometric measurement of the spots at 284 nm. The separation was carried out on Merck HPTLC sheets of silica gel 60 F 254, using acetone–toluene–methanol (9:9:0.6 v/v) as mobile phase. The third method depends on first derivative of the ratio spectra (^{1}DD) by measurement of the amplitudes at 310.2 nm. Moreover, the proposed HPLC method was utilized to investigate the kinetics of the oxidative and photo degradation processes. The pH-rate profile of degradation of RA in Britton–Robinson buffer solutions within the pH range 3–11 was studied. In addition, the activation energy of RA degradation was calculated in Britton–Robinson buffer solution pH 7.

Keywords: Rabeprazole; HPLC; HPTLC-densitometry; First derivative of the ratio spectra; Kinetics of degradation; pH-rate profile; Arrhenius plot

1. Introduction

Rabeprazole (RA), 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulfinyl]-1H-benzimidazole, is a proton-pump inhibitors that suppress gastric acid secretion by specific inhibition of the gastric H^+, K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell [1]. RA is not official in any pharmacopoeia. The literature survey reveals a non-aqueous capillary electrophoresis method for analysis of RA [2]. A system for online sample preparation with column-switching HPLC, gradient elution and detection by NMR has been developed for analysis of RA. The process took about 1.5 h [3]. Another HPLC method was reported for determination of RA and its metabolites in human plasma using Inertisil C_8 column at 40 °C with acetonitrile −0.1 M phos-

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phate buffer of pH 7 (28:72, v/v) as a mobile phase [4].

The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [5]. Acidic, oxidative and photolytic stability are required. RA is a substituted benzimidazole, which can be easily degraded. No method has been reported in the literature for the determination of RA in presence of its degradation products. Therefore, it was thought necessary to study the stability of RA towards acidic, oxidative and photo degradation processes. The aim of this work was to develop stability indicating methods for determination of RA in presence of its degradation products using HPLC, HPTLC-densitometry and first derivative of the ratio spectra (^{1}DD). Furthermore, the developed HPLC method was used to investigate the kinetics of the oxidative and photo degradation processes, and to calculate the activation energy for RA degradation.

2. Experimental

2.1. Instrumentation

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A Degasser, Rheodyne 7725i injector with a 20 μl loop and a SPD-10AVP UV–VIS detector, separation and quantitation were made on a 250×4.6 mm (i.d.) VP-ODS Shim-pack column (4.6 μm particle size). The detector was set at $\lambda = 284$ nm. Data acquisition was performed on class-VP software.

A Camag TLC Scanner 3 (Switzerland) was used. Data collections and data analysis were conducted using on-line computer with Camag TLC software (CATS 4). The samples were applied to the plates using Camag Linomat IV. HPTLC plates (20×10 cm^2, aluminum plates precoated with silica gel 60 F_{254}) were purchased from E. Merck (Darmstadt, Germany). Twin-trough glass development chamber, 20×20 cm^2 (Camag) was used. The experimental conditions of measurements were $\lambda = 284$ nm, mode: absorbance/reflectance, slit dimensions: 0.45×4 mm^2.

A double-beam shimadzu (Japan) UV–VIS spectrophotometer, model UV-1601 PC connected to an IBM compatible computer and a HP600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}. The absorption spectra of a test and reference solutions were recorded in 1-cm quartz cells over the range 340–210 nm.

Pharmaceutical grade of RA (Quimica Sintetica S.A. CHEMO, Switzerland) was used. It certified to contain 99.9%. The water for HPLC was prepared by double glass distillation and filtration through 0.45-μm membrane filter. The methanol used was HPLC grade (BDH, Poole, UK). Acetone, toluene, sodium hydroxide, hydrogen peroxide, hydrochloric, phosphoric, acetic and boric acids were analytical grade.

Commercial Pariet tablets (Eisai Co., Ltd, Tokyo, Japan for Janssen Pharmaceutica, Beerse, Belgium) were used. Each tablet contains 20 mg RA sodium, in addition to tablet excipients consisting of mannitol, magnesium oxide, low-substituted hydroxypropyl cellulose, magnesium stearate, ethylcellulose, hypromellose phthalate diacetylated monoglycerides, talc, titanium dioxide, yellow iron oxide, carnauba wax and ink (shellac food grade, red iron oxide, soya lecithin and antifoam DC 1510).

2.2. Chromatographic conditions

The mobile phase of HPLC was prepared by mixing methanol and water in a ratio of 70:30 v/v. the mobile phase was filtered using a 0.45-μm disposable filter (Millipore, Milford, MA) and degassed by ultrasonic vibrations prior to use. The samples were also filtered using 0.45-μm disposable filters. The flow rate was 2 ml min^{-1}. All determinations were performed at ambient temperature. The injection volume was 20 μl.

For optimal sensitivity of HPTLC method, solutions of the testing samples and standard

were applied to the HPTLC plates as bands rather than spots. Bands were 4 mm long and 10 μl of sample was applied to each band. The bands are separated by a distance of 8 mm apart and 10 mm from the bottom of the plate. The development chamber was saturated with mobile phase. The HPTLC plate was developed in the ascending way with acetone–toluene–methanol (9:9:0.6 v/v) as a mobile phase. After developing over a distance of 8 cm, the HPTLC plate was air dried and scanned at 284 nm. The scan length and width were adjusted to cover the entire band.

2.3. Preparation of the degradation products

2.3.1. Preparation of the acidic degradation products

Accurately weighed 100 mg of RA were dissolved in 80 ml 0.1 N hydrochloric acid. The solution was heated in a water bath at 40 °C for 1 h. Subsequently, the solution was neutralized with 1 N sodium hydroxide and quantitatively transferred to 100 ml volumetric flask. The volume was completed to 100 ml with distilled water.

2.3.2. Preparation of the oxidative degradation products

Accurately weighed 100 mg of RA were dissolved in 90 ml distilled water. Subsequently, 1 ml of hydrogen peroxide 33.3% v/v was added and the solution was left at room temperature for 24 h. The solution was quantitatively transferred to 100 ml volumetric flask and the volume was completed to 100 ml with distilled water.

2.3.3. Preparation of the photolytic degradation products

Accurately weighed 100 mg of RA were dissolved in 100 ml distilled water. The solution was exposed to electrical light of tungsten lamp (40 W) for 7 days.

The prepared acidic, oxidative and photolytic degradation products were tested for complete degradation using the HPLC system described above. Two peaks at retention time 2.7 and 9.3 min. were observed. While no peak was observed at retention time 5.2 min corresponding for RA.

2.4. Standard solutions and calibration

Stock standard solutions were prepared by dissolving 100 mg of RA in 100 ml of water (for

Table 1
Characteristic parameters for the regression equations of HPLC and ^{1}DD methods for determination of RA

Parameters	HPLC	^{1}DD
Calibration range (μg ml^{-1})	4–20	10–30
Quantitation limit (μg ml^{-1})	0.076	0.058
Detection limit (μg ml^{-1})	0.025	0.019
Regression equation (Y)[a]		
Slope (b)	65.07×10^{3}	88.32×10^{-2}
Standard deviation of the slope (S_b)	8.17×10^{2}	8.23×10^{-3}
Relative standard deviation of the slope (%)	1.25	0.93
Confidence limit of the slope [b]	64.31×10^{3}–65.82×10^{3}	87.52×10^{-2}–89.12×10^{-2}
Intercept (a)	11.13×10^{2}	-1.70×10^{-2}
Standard deviation of the intercept (S_a)	10.06×10^{3}	17.11×10^{-2}
Confidence limit of the intercept [b]	(-8.19×10^{3})–10.42×10^{3}	(-18.33×10^{-2})–14.93×10^{-2}
Correlation coefficient (r)	0.9999	0.9999
Standard error of estimation	4.36×10^{3}	5.04×10^{-2}

[a] $Y = a + bC$, where C is the concentration of RA in μg ml^{-1} and Y is the peak area or ^{1}DD amplitude for HPLC and ^{1}DD methods, respectively.
[b] Ninety five percent confidence limit.

Table 2
Characteristic parameters for the second order polynomial regression equation of the HPTLC-densitometric method for determination of RA

Parameters	
Calibration range (μg/spot)	0.5–2.5
Quantitation limit (μg/spot)	0.31
Detection limit (μg/spot)	0.10
Regression coefficient 1	2.86×10^2
Standard deviation of the coefficient 1	0.15×10^2
Confidence limit of the coefficient 1[a]	2.59×10^2–3.13×10^2
Regression coefficient 2	-0.44×10^2
Standard deviation of the coefficient 2	0.05×10^2
Confidence limit of the coefficient 2[a]	(-0.35×10^2)–(-0.53×10^2)
Intercept	1.15×10^2
Standard deviation of the intercept	0.10×10^2
Confidence limit of the intercept [a]	0.97×10^2–1.33×10^2
Correlation coefficient	0.9999
Standard error of estimation	2.18

[a] Ninety five percent confidence limit.

HPLC method), methanol (for HPTLC method) or 0.1 N sodium hydroxide (for ^{1}DD method). The standard solutions within the linearity range were prepared using the specified solvent (Tables 1 and 2).

2.4.1. For HPLC method

Triplicate 20 μl injections were made for each concentration and chromatographed under the conditions described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph.

2.4.2. For HPTLC method

Ten microliters of each standard solution was applied to the HPTLC plate. Triplicate applications were made for each solution. The plate was developed using previously described mobile phase. The peak heights were measured for the calibration.

2.4.3. For ^{1}DD

The UV absorption spectra of standard solutions of RA were divided by the UV absorption spectrum of 6 μg ml^{-1} of degraded RA (in 0.1 N sodium hydroxide as a solvent). The first derivative was calculated for the obtained spectra with $\Delta\lambda = 4$ nm and scaling factor = 40. The ^{1}DD obtained were smoothed with 8 experimental points. The amplitudes at 310.2 nm were measured and found to be proportional to the concentration of RA.

2.5. Sample preparation

Ten tablets were weighed and finely powdered. A portion of the powder equivalent to about 15 mg of RA was accurately weighed, dissolved and diluted to 100 ml with water (for HPLC method), methanol (for HPTLC method) or 0.1 N sodium hydroxide (for ^{1}DD method). The sample solution was filtered.

2.5.1. For HPLC and ^{1}DD methods

Further dilutions of the sample solution were carried out with the specified solvent to reach the linearity range specified for each method (Table 1). The general procedures for HPLC and ^{1}DD methods described under calibration were followed and the concentration of RA was calculated.

2.5.2. For HPTLC method

Ten microliters of the sample solution were applied to the HPTLC plate. The plate was developed in the previously described chromatographic conditions. The concentration of RA in the sample was determined by multilevel calibration developed on the same plate under the same conditions, using second order polynomial regression equation.

2.6. Kinetic investigation

Accurately weighed 50 mg of RA were dissolved in 250 ml Britton–Robinson buffer solution pH 7 [6]. Separate 10 ml aliquots of this solution were transferred into separate stoppered conical flasks. The flasks were placed in a thermostatic oven at

different temperatures (60, 50, 40, 35, 30 °C) for different time intervals. At the specified time interval the contents of the flasks were transferred into 100 ml volumetric flasks and diluted to volume with mobile phase of HPLC. Aliquots of 20 μl of each solution were chromatographed under the HPLC conditions described above and the concentration of the remaining RA was calculated at each temperature and at time interval.

2.7. Kinetic investigation of oxidative degradation

Accurately weighed 50 mg of RA were dissolved in 200 ml distilled water. Subsequently, 2.5 ml of hydrogen peroxide 33.3% v/v were added and the volume was completed to 250 ml with distilled water. The solution was kept at ambient temperature for different time intervals. At the specified time interval, 10 ml of the solution was diluted to 100 ml with the mobile phase of HPLC. Aliquots of 20 μl of each solution were chromatographed under the HPLC conditions described above.

2.8. Kinetic investigation of photolytic degradation

Accurately weighed 50 mg of RA were dissolved in 250 ml distilled water. The solution was exposed to electrical light of tungsten lamp (40 W) for different time intervals. At the specified time interval, 10 ml of this solution was diluted to 100 ml with the mobile phase of HPLC. Aliquots of 20 μl of each solution were chromatographed under the HPLC conditions described above.

2.9. pH Rate profile

Accurately weighed 50 mg of RA were dissolved in 250 ml of Britton–Robinson buffer solutions [6]. The pH values of the buffer solutions used for measurement of the pH-rate profile of the degradation of RA are within pH 3–11 in one unit pH intervals. The pH values of these buffer solutions were checked before and after the reaction, and were unchanged. The ionic strength of these buffer solutions was adjusted with sodium chloride. Separate 10 ml aliquots of the buffer solution containing 200 μg ml^{-1} RA were transferred into separate stoppered conical flasks. The flasks were placed in a thermostatic oven at 40 °C for different time intervals. At the specified time interval the contents of the flasks were neutralized to pH 7.0 using hydrochloric acid or sodium hydroxide solutions. The contents of the flasks were transferred into 100 ml volumetric flasks and diluted to volume with mobile phase of HPLC. Aliquots of 20 μl of each solution were chromatographed under the HPLC conditions described above and the concentration of the remaining RA was calculated at each pH value and time interval.

3. Results and discussion

3.1. HPLC method

The developed HPLC method has been applied for the determination of RA in presence of its acidic, oxidative and photolytic degradation products. To optimize the HPLC assay parameters, the mobile phase composition was studied. A satisfactory separation was obtained with a mobile phase consisting of methanol–water (70:30, v/v) at

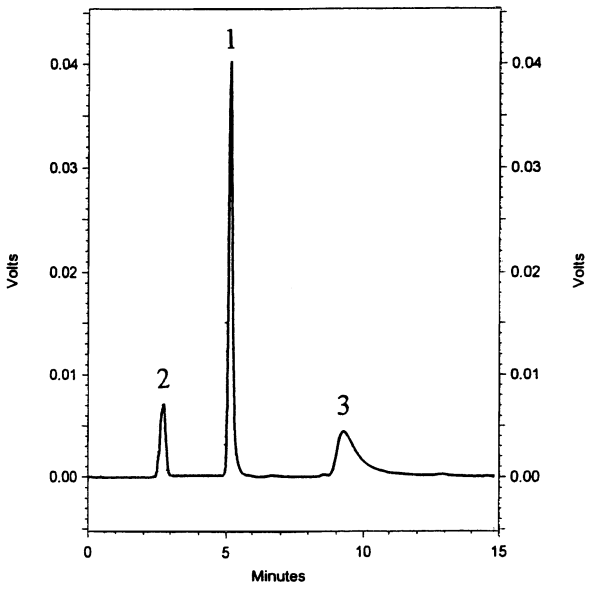

Fig. 1. Typical HPLC chromatogram of 20 μl injection of synthetic mixture of 10 μg ml^{-1} RA (1) and 10 μg ml^{-1} its degradation products (2,3).

ambient temperature. Increasing methanol concentration to more than 90% led to inadequate separation of RA and its degradation products. At lower methanol concentration ($<45\%$) separation occurred but with excessive tailing and increased retention time. Quantitation was achieved with UV detection at 284 nm based on peak area.

The acidic, oxidative and photolytic degradation processes for RA are similar and give 2 degradation products. The specificity of the HPLC method is illustrated in Fig. 1 where complete separation of RA and its two degradation products was noticed. The average retention time $\pm$S.D. for RA and its two degradation products were found to be 5.2 ± 0.008, 2.8 ± 0.005 and 9.3 ± 0.009 min, respectively, for ten replicates.

To determine the linearity of HPLC detector response, calibration standard solutions of RA were prepared as described in the text. Linear correlation was obtained between peak area versus concentration of RA. Characteristic parameters for regression equation of the HPLC method and correlation coefficient obtained by least squares treatment of the results were given in Table 1.

3.2. HPTLC method

Experimental conditions such as mobile phase, wavelength of scanning and slit dimensions were optimized to provide accurate, precise and reproducible results for determination of RA in presence of its degradation products. The chosen slit dimensions were 0.45×4 mm^2 and the wavelength of scanning was chosen to be 284 nm. The greatest differences between the R_f values of three compounds (0.60 for RA and 0.30, 0.78 for its degradation products), with minimum tailing were obtained by using the mobile phase consisting of acetone-toluene-methanol in ratio of 9:9:0.6 v/v, respectively. The specificity of the HPTLC method is illustrated in Fig. 2 where complete separation of RA and its two degradation products was noticed. The shape of calibration curves in thin layer chromatography is generally inherently non-linear due to scattering of light. Calibration curves generally comprise a pseudolinear region at low sample concentration and then departure from linearity begins at higher sample concentrations. The extent of individual ranges of the calibration curves is frequently very different for different substances. In some instances, the pseudolinear range may be adequate for most analytical purposes, in others no reasonable linear range exist [7]. Scattering of light is highly dependent on the type of the TLC plate, measuring wavelength, measuring mode, molar absorptivity and concentration of the sample. The use of HPTLC plates is therefore advantageous since they are less scattering than conventional TLC plates [8].

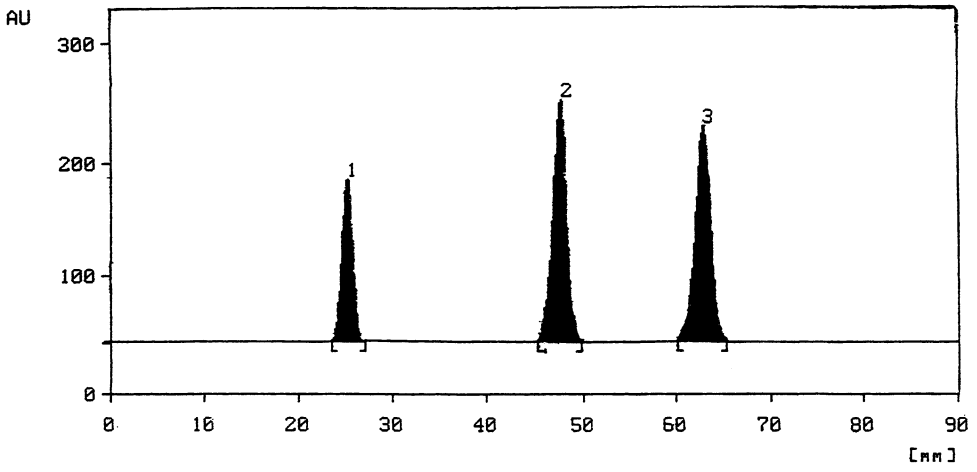

Fig. 2. Typical HPTLC chromatogram of 0.5 μg/spot RA (2) and 0.5 μg/spot its degradation products (1,3).

Several transformation techniques have been suggested to linearize the normally non-linear calibration curve by conversion of the sample concentration and/or signal into reciprocals, logarithms or squared terms [7,9]. However, errors in the original data are also transferred in the above methods leading to inhomogeneous variances in the transformed data [7], hence with the ready availability of computers in laboratories, there is increasing interest in applying non-linear curve fitting techniques to experimental data [10].

The *Y*-intercept for linear regressions of HPTLC methods are often not zero as would be expected with HPLC methods. Unlike HPLC methods, for which linearity of detector response over a wide range of concentrations of analyte can be obtained, the calibration curve of UV detector response versus a wide range of concentration for HPTLC often does not follow linear regression but rather polynomial regression. With HPTLC, the analyte interact with the layer surface of the stationary phase where scattering and absorption tend to take place, especially with high concentration of analyte [11]. These combined processes are not adequately described by Beer–Lambert law, but the Kubelka Munk model [12].

The relationship between the concentration of RA and peak height of its spot was investigated. The linear relationship was tested and found to be unaccepted due to the poor correlation. The second order polynomial fit was found to be more suitable. The calibration graph was constructed in the range of 0.5–2.5 μg/spot for RA. The second order polynomial regression equation was found to be: $h = 115.46 + 286.48C - 44.31C^2$, where, h is the peak height of the spot and C is the concentration of RA in μg/spot. The characteristic parameters of the second order polynomial regression equation of the RA are shown in Table 2.

3.3. ^{1}DD method

The UV absorption spectra of RA and its degradation products in 0.1 N sodium hydroxide as a solvent display considerable overlap (Fig. 3), that the application of the conventional spectrophotometry and the derivative technique (first, second, third) failed to resolve it. However, this

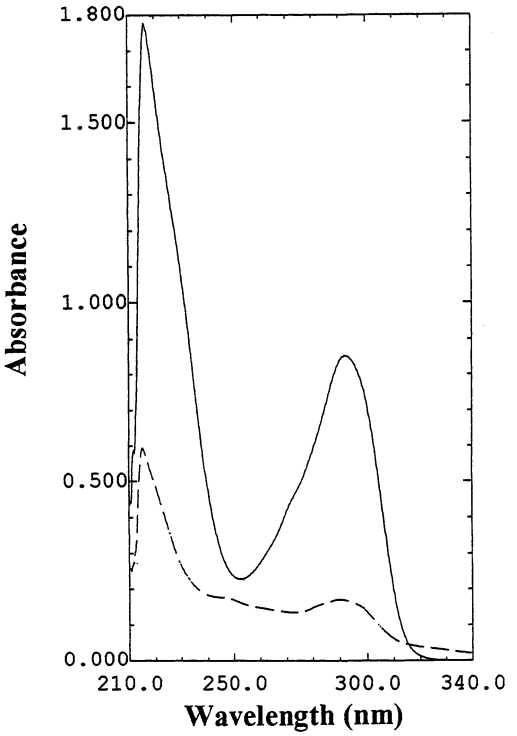

Fig. 3. UV absorption spectra of 20 μg ml^{-1} of RA (—) and 6 μg ml^{-1} of its degradation products (---) in 0.1 N sodium hydroxide as a solvent.

spectral overlapping was sufficiently enough to demonstrate the resolving power of the ^{1}DD method to be used as a stability indicating method. The UV absorption spectra of acidic, oxidative and photo degradation products are similar.

The main advantage of the derivative of the ratio spectra method may be the chance of doing measurements in correspondence of peaks, hence there is a potential for greater sensitivity and accuracy. While the main disadvantages of the zero crossing method in derivative spectrophotometry for resolving a mixture of components with overlapped spectra are the risk of small drifts of the working wavelengths and the circumstance that the working wavelengths generally do not fall in correspondence of peaks of the derivative spectrum. This may be particularly dangerous when the slope of the spectrum is very high with consequent loss of accuracy and precision, and the working wavelength is proximity of the base of the

spectrum, which causes poor sensitivity [13]. In the present case, the above circumstances are present.

To optimize the ^{1}DD method for determination of RA in presence of its degradation products, its is necessary to test the influence of the variables: divisor concentration, $\Delta\lambda$ and smoothing function. All these variables were studied. The influence of the $\Delta\lambda$ for obtaining the ^{1}DD was tested and $\Delta\lambda =$ 4 nm was selected as optimum value. A correct choice of the divisor concentration is fundamental for several reasons. Among these, in the wavelength range where the absorbance of the spectrum used as divisor is zero or below the base line, the noise of ratio spectra is greatly increased. Hence, a certain overlap of spectra in the working wavelength region is actually desirable, to avoid an increase of the error. If the concentration of divisor is increased or decreased, the resulting derivative ratio values are proportionality decreased or increased with consequent variation of both sensitivity and linearity range. From several tests, the best results in terms of signal to noise ratio, sensitivity and repeatability followed using 6 μg ml^{-1} of degraded RA as divisor. Due to the extent of the noise levels on the ratio spectra, a smoothing function was used and eight experimental points were considered as suitable.

In this method, the UV absorption spectra of RA were divided by 6 μg ml^{-1} of degraded RA. The first derivative was calculated for the ratio spectra obtained with $\Delta\lambda = 4$ nm. These spectra were smoothed with eight experimental points due to the high noise of the signals obtained [14], (Fig. 4). The ^{1}DD showed several peaks at different wavelengths that permits the determination of RA in presence of its degradation products. The ^{1}DD peak at 310.2 nm was chosen for maximum sensitivity and for elimination of the background interference resulted from tablet excipients. The concentration of RA was proportional to the ^{1}DD amplitude at 310.2 nm in the concentration range 10–30 μg ml^{-1}. The characteristic parameters for

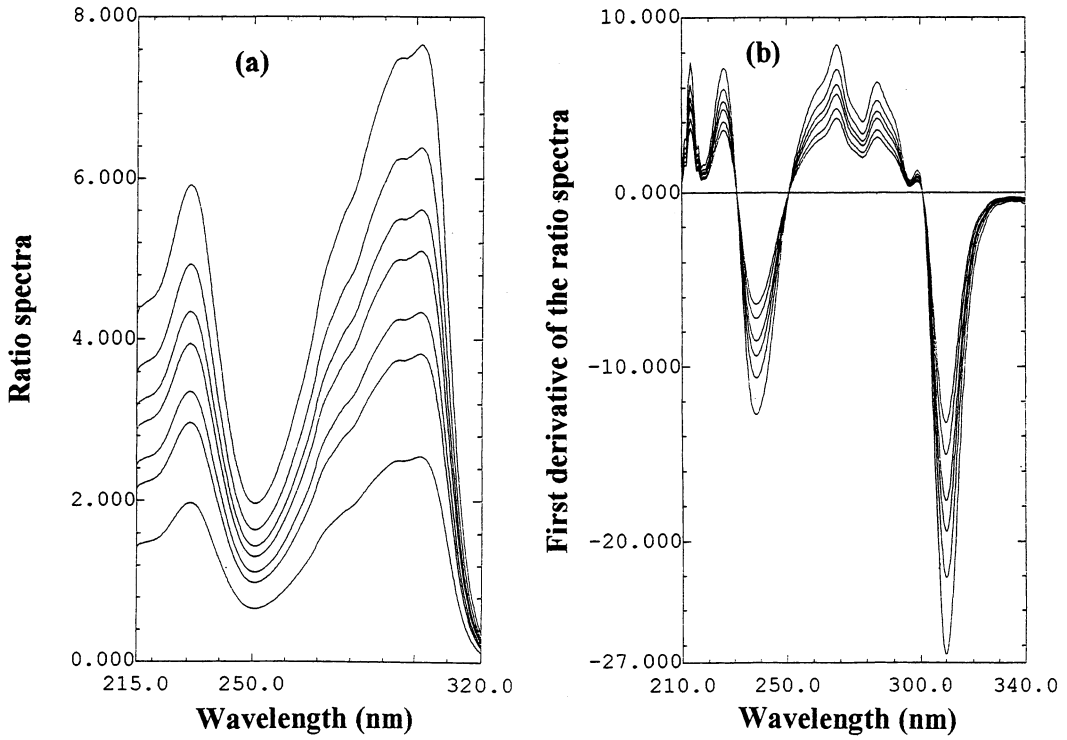

Fig. 4. Ratio spectra (a) and ^{1}DD (b) for different concentrations (10, 15, 17, 20, 22, 25, 30 μg ml^{-1}) of RA, using 6 μg ml^{-1} of degraded rabeprazole as a divisor.

Table 3
Determination of RA in synthetic mixtures and commercial tablets using the proposed methods

	Mean found ± S.D.[a]			
	HPLC	HPTLC	^{1}DD	Published HPLC [4]
Synthetic mixtures (acidic deg.)	99.8 ± 0.49	99.8 ± 0.81	99.7 ± 0.57	
Synthetic mixtures (oxidative deg.)	100.1 ± 0.41	99.7 ± 0.72	99.9 ± 0.49	
Synthetic mixtures (photolytic deg.)	100.2 ± 0.34	99.9 ± 0.83	100.1 ± 0.52	
Commercial tablets	99.8 ± 0.44	99.7 ± 0.71	100.0 ± 0.51	99.9 ± 0.60
t	0.36	0.57	0.34	(2.18)[b]
F	1.86	1.40	1.38	(4.28)[b]
Recovery[c]*	99.7 ± 0.62	99.7 ± 0.81	100.1 ± 0.41	
Recovery [c]**	100.2 ± 0.42	100.3 ± 0.55	100.0 ± 0.55	
Recovery [c]***	100.0 ± 0.51	100.1 ± 0.63	99.9 ± 0.43	

[a] Mean and SD for seven determinations, percentage recovery from the label claim amount.
[b] Theoretical values for *t* and *F*.
[c] For standard addition of different concentrations of rabeprazole and its acidic (*), oxidative (**) and photolytic (***) degradation products.

regression equation of ^{1}DD method are given in Table 1.

3.4. Tablet analysis

The three proposed methods were applied to the determination of RA in commercial tablets. Seven replicates determinations were made. Satisfactory results were obtained in a good agreement with the label claims (Table 3). These results were compared with those of the published HPLC method using C_8 column at 40 °C with acetonitrile-0.1 M phosphate buffer of pH 7 (28:72, v/v) as a mobile phase [4]. Statistical comparison of the results was performed with regard to accuracy and precision using Student's *t*-test and the *F*-ratio at 95% confidence level (Table 3). There is no significant difference between the proposed HPLC, HPTLC, ^{1}DD methods and published HPLC method with regard to accuracy and precision. Expired batch of Pariet tablets stored at ambient temperature under normal conditions was analyzed by the proposed HPLC and HPTLC methods. The degradation products of RA were found (Fig. 5).

3.5. Kinetic investigation

The kinetic of degradation of RA was investigated in Britton–Robinson buffer solution pH7, hydrogen peroxide and after exposure to electrical light. A regular decrease in the concentration of intact RA with increasing time intervals was observed and each degradation process followed pseudo first order kinetics. The apparent first order degradation rate constant and the half-life at ambient temperature were calculated and found to be 0.930 h^{-1}, 0.744 h for oxidative degradation process and 0.029 h^{-1}, 23.690 h for photolytic degradation process, respectively.

The influence of temperature on the degradation process of RA in Britton–Robinson buffer solution pH 7 is shown in Fig. 6. At the selected temperatures (60, 50, 40, 35 and 30 °C), the degradation process followed pseudo first order kinetics. From the slopes of the straight lines it was possible to calculate the apparent first order degradation rate constant and the half-life at each temperature (Table 4). Plotting log K_{obs} values versus $1/T$, the Arrhenius plot (Fig. 7) was obtained, which was found to be linear in the temperature range 30–60 °C. The activation energy was found to be 10.55 kcal $mole^{-1}$.

The pH-rate profile of degradation of RA in Britton–Robinson buffer solutions was studied at 40 °C using the proposed HPLC method (Fig. 8). Britton–Robinson buffer solutions were used throughout the entire pH range in order to avoid possible effects of different buffer species. The pH-

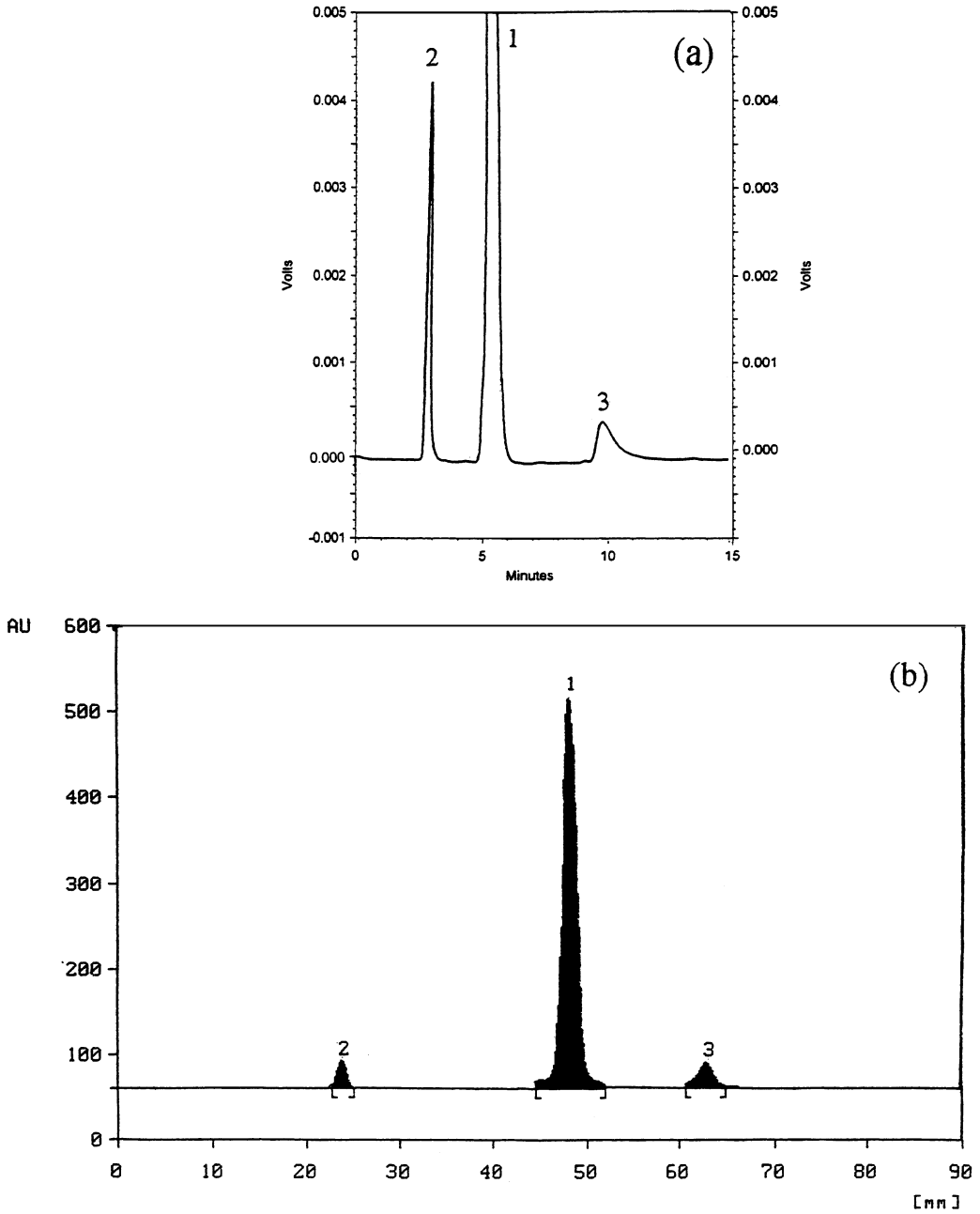

Fig. 5. Typical HPLC (a) and HPTLC (b) chromatograms of expired Pariet tablets containing RA (1) and its degradation products (2,3).

rate profile of RA was investigated at pH between 3 and 11, since the decomposition rate of RA at pH lower than 3 was too fast while at pH higher than 11 was too slow to obtain reliable kinetic data. The apparent first order degradation rate constant and the half-life were calculated for each

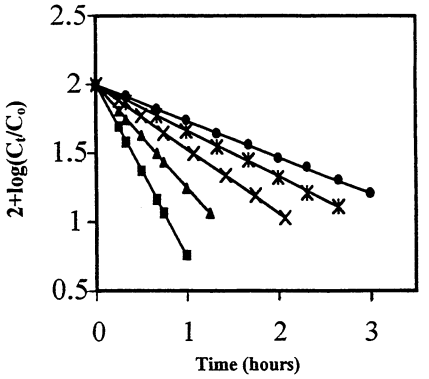

Fig. 6. Pseudo first-order plots for the degradation of rabeprazole in Britton–Robinson buffer solution pH7 at various temperature using HPLC method. Key: 60 (■); 50 (▲); 40 (x); 35 (*); and 30 °C (●); C_t, concentration at time t, and C_0, concentration at time zero.

Table 4
Degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for rabeprazole in Britton–Robinson buffer solution pH7 (determined by HPLC method)

Temperature (°C)	K_{obs} (h^{-1})	$t_{1/2}$ (h)
60	2.871	0.241
50	1.738	0.399
40	1.069	0.648
35	0.766	0.905
30	0.595	1.164

pH value (Table 5). RA was found to be rapidly degraded in acid medium and is more stable in alkaline medium.

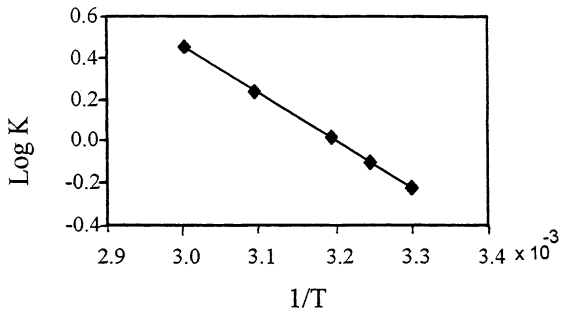

Fig. 7. Arrhenius plot for the degradation of rabeprazole in Britton–Robinson buffer solution pH 7 using HPLC method.

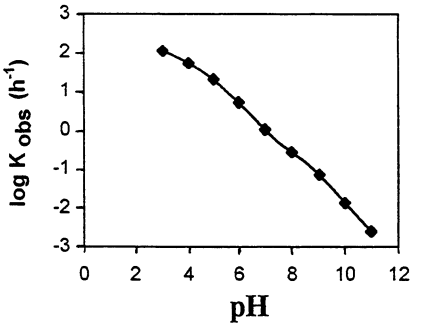

Fig. 8. pH-rate profile for the decomposition of rabeprazole at constant ionic strength and 40 °C.

Table 5
Degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for rabeprazole in Britton–Robinson buffer at different pH values and a temperature of 40 °C

pH	K_{obs} (h^{-1})	$t_{1/2}$ (h)
3	116.674	5.939×10^{-3}
4	54.912	0.013
5	20.686	0.034
6	5.565	0.125
7	1.069	0.648
8	0.289	2.394
9	0.074	9.403
10	0.014	49.330
11	2.533×10^{-3}	273.556

3.6. Validation of the methods

3.6.1. Linearity

The linearity of the proposed methods was evaluated by analysing a series of different concentrations of RA. According to the ICH [15], at least five concentrations must be used. In this study seven concentrations were chosen, ranging between 4–20 and 10–30 μg ml^{-1} for HPLC and ^{1}DD methods, respectively. Each concentration was repeated three times, the repeated runs were genuine repeats and not just repetitions at the same reading; this approach will provide information on the variation in peak area and ^{1}DD values between samples of same concentration. The assay was performed according to experimental conditions previously established. The linearity of the calibration graphs and adherence of the system to Beer's law were validated by the high value of the

correlation coefficient and the intercept value which was not statistically ($P < 0.05$) different from zero (Table 1).

3.6.2. Precision

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels for RA. The data for each concentration levels were evaluated by one-way ANOVA. A 8 days × 2 replicates design was performed. Statistical comparison of the results was performed using the P-value of the F-test. Table 6 shows three univariate analyses of variance for each concentration level. It can be seen from the table, that since the P-value of the F-test is always greater than 0.05, there is no statistically significant difference between the mean results obtained from one level of day to another at the 95% confidence level.

3.6.3. Range

The calibration range was established through consideration of the practical range necessary, according to the RA concentration present in pharmaceutical product, to give accurate, precise and linear results. The calibration range of the three proposed methods are given in Tables 1 and 2.

3.6.4. Detection and quantitation limits

According to ICH recommendations [15] the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and given in Tables 1 and 2.

3.6.5. Selectivity

Methods selectivity was achieved by preparing different mixtures of RA within the linearity range concentration and its acidic, oxidative and photolytic degradation products within ratio ranged from 1:0.1 to 1:2 for RA and its degradation products, respectively. The synthetic mixtures were analysed according to the previous procedures described under the three proposed methods. Satisfactory results were obtained (Table 3), indicating the high selectivity of the proposed

Table 6
Analysis of variance for repeatability and intermediate precision for rabeprazole by the proposed methods

Conc. level		Source of variation	Sum of squares			Degree of freedom	Mean squares			F-ratio[a]			P-value		
HPLC and ^{1}DD (μg ml^{-1})	HPTLC (μg/spot)		HPLC	^{1}DD	HPTLC		HPLC	^{1}DD	HPTLC	HPLC	^{1}DD	HPTLC	HPLC	^{1}DD	HPTLC
10	1	Between	1.362	1.190	0.784	7	0.194	0.170	0.112	0.276	0.216	0.174	0.946	0.971	0.983
		Within	5.645	6.270	5.145	8	0.706	0.784	0.643						
		Total	7.007	7.460	5.929	15									
15	1.5	Between	0.991	1.544	2.077	7	0.142	0.221	0.297	0.228	0.219	0.462	0.967	0.970	0.840
		Within	4.963	8.074	5.144	8	0.620	1.009	0.643						
		Total	5.954	9.618	7.221	15									
20	2	Between	1.219	1.936	0.321	7	0.174	0.276	0.046	0.311	0.368	0.085	0.930	0.897	0.990
		Within	4.477	6.010	4.290	8	0.559	0.751	0.537						
		Total	5.696	7.946	4.610	15									

[a] F-critical = 3.5.

methods for determination of RA in presence of its degradation products.

3.6.6. Accuracy

This study was performed by addition of known amounts of RA and its acidic, oxidative and photolytic degradation products to a known concentration of the commercial tablets (standard addition method). The resulting mixtures were assayed and results obtained for RA were compared with expected results.

Also, spiked placebos were prepared according to the manufacturing formula. The spiked placebos were tested at five levels: 50, 75, 100, 125 and 150% of label claim for RA. Assays were performed in duplicate on two samples at five levels. This was repeated with a second standard, sample preparation and analyst on different days.

The excellent recoveries of standard addition method (Table 3) and spiked placebos (Table 7) suggest that good accuracy of the three proposed methods and there is no interference from the degradation products of RA and excipients which are present in tablets.

3.6.7. Robustness

The robustness of a method is its ability to remain unaffected by small change in parameters. To determine robustness of the proposed methods, experimental conditions such as percentage of organic strength of the mobile phase and strength of sodium hydroxide were purposely altered and chromatographic, ^{1}DD characteristics were evaluated. Variation of the organic strength of the mobile phases by $\pm 2\%$ did not have a significant effect on chromatographic resolution in HPLC and HPTLC methods. Variation of strength of sodium hydroxide by $\pm 5\%$ did not have a significant effect on ^{1}DD amplitude in spectrophotometric method.

Table 7
Accuracy of the proposed methods determined by the recovery of rabeprazole from placebo tablets spiked with rabeprazole

Level (%)	% Recovery		
	HPLC	^{1}DD	HPTLC
50	100.1	100.2	100.9
50	100.5	100.1	100.5
75	99.8	100.4	99.3
75	100.2	100.7	100.4
100	100.4	100.5	100.8
100	99.6	99.9	99.2
125	100.3	100.8	100.1
125	100.1	99.4	99.1
150	99.5	100.6	100.4
150	99.8	99.5	99.4
Mean	100.03	100.21	100.01
S.D.	0.34	0.49	0.69

3.6.8. Stability

The stability of RA standard and sample solutions were evaluated. The solutions were stored in tightly capped volumetric flasks, protected from light, on a laboratory bench and in the refrigerator. Recovery of these solutions was checked for 10 h in interval of 1 h against freshly prepared solutions. The solutions kept on the laboratory bench and in the refrigerator were found to be stable for 5 h.

4. Conclusion

The proposed HPLC, HPTLC and ^{1}DD methods provide simple, accurate and reproducible quantitative analysis for the determination of RA in pharmaceutical tablets, without any interference from the excipients, and in the presence of its acidic, oxidative and photolytic degradation products. The two chromatographic methods were found to be more specific and selective than the ^{1}DD method. While the ^{1}DD method has the advantage of lower cost, rapid and environmental protecting. The HPTLC method is simple and uses a minimal volume of solvents, compared to the HPLC method. The proposed methods were found to be easier than the published methods for the determination of RA. The three proposed methods were completely validated and suitable for quality control laboratories, where economy and time are essential.

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