CHEMOMETRIC DETERMINATION OF RABEPRAZOLE SODIUM IN PRESENCE OF ITS ACID INDUCED DEGRADATION PRODUCTS USING SPECTROPHOTOMETRY, POLAROGRAPHY AND ANODIC VOLTAMMETRY AT A GLASSY CARBON ELECTRODE

MARWA S. MONEEB
Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy,
University of Alexandria, Alexandria, 21521, Egypt

ABSTRACT

Chemometric stability indicating methods are presented for the determination of rabeprazole sodium in presence of its acid induced degradation products using spectrophotometry, differential pulse polarography and differential pulse anodic voltammetry at a glassy carbon electrode. The applied chemometric techniques are multivariate ones including classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS). A difference spectrophotometric (ΔA) method has also been applied. To develop the multivariate calibrations, a training set was used, consisting of 20 mixture solutions of rabeprazole sodium and its degradation products. These mixtures show percentage degradation ranging from 0.5-65%, 0.5-95% and 0.6-75 % for the spectrophotometric, polarographic and anodic voltammetric calibrations, respectively. The UV absorbances were recorded in 0.1 M NaOH within the wavelength range 220-340 nm at 2 nm intervals. The polarograms and anodic voltammograms were recorded in Britton- Robinson buffer (pH 8.0) within the potential range -500 to -1508 and 400 to 1192 mV at 6 mV intervals with a pulse amplitude of -100 and 50 mV, sweep rate of 15 and 10 mV s^{-1} and pulse interval of 0.4 and 0.6 s for the polarographic and anodic voltammetric methods, respectively. All the studied methods have been validated and successfully applied to the determination of rabeprazole sodium in tablet dosage form. The results were statistically compared to those obtained using a published HPLC method. No significant difference has been found.

Keywords: Rabeprazole sodium, Chemometrics, Differential pulse polarography, Differential pulse anodic voltammetry, Glassy carbon electrode, Difference spectrophotometry.

INTRODUCTION

Rabeprazole sodium, 2-[[[4-(3- Methoxypropoxy)- 3-methyl- 2- pyridinyl] methyl] sulfinyl]- 1H-benzimidazole sodium, is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the enzyme system of hydrogen/ potassium adenosine triphosphatase (H^+/K^+ ATPase) at the secretory surface of the gastric parietal cell (Olin, 2001; Martindale, 2005). Rabeprazole has been approved by the FDA, 1999 (Olin, 2001). It is indicated for the treatment or symptomatic relief of various gastric disorders such as gastric and duodenal ulcers, gastroesophageal reflux disease and pathological hypersecretory conditions including Zollinger- Ellison syndrome (Olin, 2001; Martindale, 2005).

A survey of the literature revealed very few methods for rabeprazole determination in pharmaceutical formulations or biological fluids. These methods included: non-aqueous capillary electrophoresis (Tivesten *et al*., 1999) and high- performance liquid chromatography with NMR (Yokoyama *et al*., 1998), ultraviolet (Garcia *et al*., 2004) or mass spectrometric (Zhang *et al*., 2004) detection. A differential pulse anodic voltammetric method at a glassy carbon electrode was also published for its determination in tablet dosage form (Radi *et al*., 2004). The determination was carried out in Britton- Robinson buffer (pH 8.0) as supporting electrolyte. The method provided a linearity range of 1.0×10^{-6} to 2.0×10^{-5} M with a detection limit of 4.0×10^{-7} M.

Rabeprazole is a substituted benzimidazole. Like most compounds of this class, it is decomposed in acid media to yield two main products, the sulfenamide and the benzimidazole sulphide (McClean *et al*., 1994). El- Gindy *et al*. (2003) developed stability indicating methods for the determination of rabeprazole in presence of its degradation products using HPLC, HPTLC- densitometry and first derivative of the ratio spectra. No other stability indicating methods were found in the literature for the determination of rabeprazole in presence of its degradation products. Therefore, the aim of the present work is to develop chemometric stability indicating methods for the determination of rabeprazole sodium in presence of its acid induced degradation products using spectrophotometry, differential pulse polarography and differential pulse anodic voltammetry at a glassy carbon

---

**Corresponding author:* Tel.: +20-3-4871-317; Fax: +20-3-4873-273, e-mail: marwamoneeb@yahoo.com

electrode. The applied chemometric techniques are multivariate ones including classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS). A difference spectrophotometric (ΔA) method has also been applied. The developed methods were validated and successfully applied to the determination of rabeprazole sodium in synthetic mixtures with its degradation products and in its tablet dosage form. The HPLC method (El-Gindy *et al*., 2003) was chosen as a comparative method in evaluating the developed methods.

Diverse chemometric methods have been applied to improve results in the multi- component analysis by different analytical techniques which have been mainly spectroscopic (El- Gindy *et al*., 2004; Dinç and Baleanu, 2002; Dinç and Üstündağ, 2002) and, more recently, electroanalytical (Moneeb, 2006; Lopez- de- Alba *et al*., 2002; Alonso- Lomillo *et al*., 2001). Classical least squares(CLS), sometimes known as K-matrix calibration, can be applied to simple systems where the concentration values of all the components present in the training samples are provided (Kramer, 1998; Kenneth *et al*., 1998). Principal component regression (PCR) is sometimes described as performing a least squares regression of the projections of the data onto the basis vectors of a factor space using inverse least squares (Kramer, 1998; Kenneth *et al*., 1998). Partial least squares (PLS) is a multivariate calibration method based on factor analysis. PLS method involves simultaneously the independent and the dependent variables in the data compression and decomposition operations (Kramer, 1998; Kenneth *et al*., 1998; Matthias Otto, 1999).

The (ΔA) method has been successfully used to eliminate interferences from dosage form excipients as well as from degradation products (Wahbi *et al*., 2002).

EXPERIMENTAL

Apparatus and software

The spectrophotometric measurements were performed using a Thermo Spectronic- Helios Alpha UV-Visible spectrophotometer interfaced to a personal computer loaded with "Vision 32 software" for spectral acquisition. The obtained spectral data can be saved to a floppy disk and retrieved when required.

The polarographic and the anodic voltammetric measurements were carried out using a Metrohm 693 VA processor. A Metrohm 694 VA stand was used in the dropping mercury electrode (DME) mode to perform the polarograhic measurements. For the anodic voltammetric measurements, the working electrode consisted of a glassy carbon electrode (disk diameter 2.0 ± 0.1 mm). The three electrodes system was completed by means of a Ag/AgCl (3M KCl) reference electrode and a Pt auxillary electrode.

The pH measurements were carried out by means of a Schott Gerate pH-Meter CG 820 at 25^{o}C.

The Chemometrics Toolbox 3.02 software (Kramer, 1995) was used for the CLS, PCR and PLS calculations.

Reagents

- Rabeprazole sodium was kindly supplied by Pharaonia Pharmaceuticals, New Borg El- Arab city, Alexandria, Egypt.
- Commercial Pariet® gastro- resistant tablets batch no: 6BSV400, Eisai Co., Ltd, Tokyo, Japan, for Janssen Pharmaceutica, Beerse, Belgium, were used. Each tablet was labeled to contain 20 mg rabeprazole sodium equivalent to 18.85 mg rabeprazole, in addition to tablet excipients consisting of mannitol, magnesium oxide, low-substituted hydroxypropyl cellulose, hydroxypropyl cellulose, magnesium stearate, ethylcellulose, hypromellose phthalate, diacetylated monoglycerides, talc, titanium dioxide, yellow iron oxide, red iron oxide, carnauba wax and ink (shellac food grade, red iron oxide, soya lecithin and antifoam DC 1510).
- Hydrochloric acid, sodium hydroxide, acetic, o-phosphoric and boric acids were of analytical grade.
- Britton- Robinson buffer (pH 8.0) (Lentner, 1984) (0.04 M each of acetic, o- phosphoric and boric acids, adjusted to the required pH with 0.2 M sodium hydroxide solution) was used as supporting electrolyte in the polarographic and anodic voltammetric measurements.
- Deionized water was used allover the polarographic and anodic voltammetric work.

Preparation of the acid induced degradation products

The procedure of El- Gindy *et al*. (2003) for preparation of the acid induced degradation products was followed. The prepared degradation products were tested for complete degradation using the HPLC method (El- Gindy *et al*., 2003). 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 to rabeprazole.

Standard solutions and calibrations

Stock standard solution (100 mg/100 ml) of rabeprazole sodium was prepared in 0.1 M sodium hydroxide for the spectrophotometric methods and in Britton- Robinson buffer (pH 8.0) for the polarographic and anodic voltammetric methods. Suitable dilutions were made using the same specified solvents. The solutions were found to be stable for at least 2 days when stored in tightly capped volumetric flasks, protected from light in the refrigerator.

To develop the chemometric multivariate calibrations, a training set was used, consisting of 20 mixture solutions in the possible combinations containing 4-40 μg ml^{-1} rabeprazole sodium and 0.2- 35 μg ml^{-1} degradation products; 0.6- 8 μg ml^{-1} rabeprazole sodium and 0.04- 40 μg ml^{-1} degradation products; and 0.3- 9 μg ml^{-1} rabeprazole sodium and 0.05- 8 μg ml^{-1} degradation products for the spectrophotometric, polarographic and anodic voltammetric calibrations, respectively. The training solutions of the spectrophotometric multivariate methods have been prepared in the presence of the different excipients according to the manufacturing formula at concentrations that can be found in the tablet dosage form.

To validate the developed calibrations, a validation set was used containing 20 synthetic mixtures in the range of 4.5- 40 μg ml^{-1} rabeprazole sodium and 0.3- 34 μg ml^{-1} degradation products; 0.6- 8 μg ml^{-1} rabeprazole sodium and 0.1- 40 μg ml^{-1} degradation products; and 0.3- 9 μg ml^{-1} rabeprazole sodium and 0.05- 8 μg ml^{-1} degradation products for the spectrophotometric, polarographic and anodic voltammetric calibrations, respectively.

The training and validation solutions were prepared using the above stock solutions.

For the (ΔA) method, solutions used for the calibration ranged from 7- 40 μg ml^{-1} rabeprazole sodium.

Sample preparation

Ten tablets were weighed and finely powdered. A portion of the powder equivalent to about 20 mg of rabeprazole sodium was accurately weighed, dissolved and diluted to 100 ml with 0.1 M sodium hydroxide for the spectrophotometric methods and with Britton- Robinson buffer (pH 8.0) for the polarographic and anodic voltammetric methods. The sample solutions were filtered. Further suitable dilutions were made using the same specified solvents.

Measurements

Spectrophotometric measurements

For the multivariate methods, the UV absorbances were recorded within the wavelength range 220- 340 nm at 2 nm intervals.

For the (ΔA) method, the absorbance difference spectra (rabeprazole sodium versus degradation products in 0.1 M NaOH) were recorded. The amplitude of the curve at 292 nm was measured.

Polarographic measurements

A 10 ml aliquot of standard (or sample) solution in Britton- Robinson buffer (pH 8.0) was transferred into the polarographic cell. The solution was de-aerated by purging with nitrogen for 5 min. The differential pulse polarograms were recorded from -500 to -1508 mV at 6 mV intervals with -100 mV pulse amplitude, 15 mV s^{-1} sweep rate and 0.4 s pulse interval.

Anodic voltammetric measurements

A 10 ml aliquot of standard (or sample) solution in Britton- Robinson buffer (pH 8.0) was transferred into the cell. The anodic potential sweep was carried out in the differential pulse mode. The voltammograms were recorded from 400 to 1192 mV at 6 mV intervals with 50 mV pulse amplitude, 10 mV s^{-1} sweep rate and 0.6 s pulse interval. To provide a reproducible active surface and improve the sensitivity and resolution of the voltammetric peaks, the working electrode was polished with 0.5 μm alumina powder on a polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and deionized water, and gently dried with a tissue paper.

RESULTS AND DISCUSSION

Spectrophotometric methods

The UV absorption spectra of rabeprazole sodium and its acid induced degradation products in 0.1 M NaOH display considerable overlap (fig. 1), that the application of the conventional spectrophotometry and the derivative technique (first, second and third) failed to resolve it.

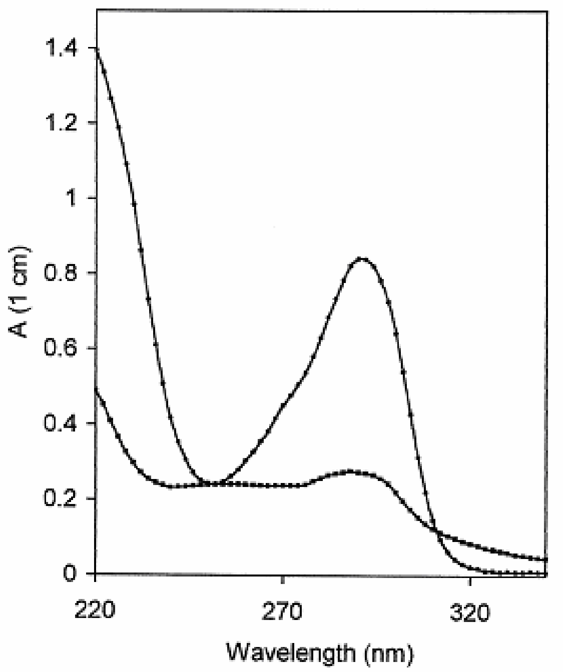

Fig. 1: Absorption spectra of 20 μg ml^{-1} rabeprazole sodium (-•-) and 10 μg ml^{-1} its degradation products (-▪-) in 0.1 M NaOH.

Interferences of dosage form excipients

The effect of the excipients present in the gastro-resistant tablet dosage form was examined. The compensation method has been applied to detect the presence of irrelevant absorption, due to excipients interference. The latter has been detected at the balance point. The irrelevant absorption was nearly rectilinear from about 260 to 320 nm.

Multivariate methods

To generate a valid multivariate calibration, the constructed training set should contain all expected components in the unknown samples (Kramer, 1998). In view of the fact that the compensation method revealed the presence of irrelevant absorption, due to excipients interference, all the training solutions have been prepared in the presence of the different excipients according to the manufacturing formula at concentrations that can be found in the tablet dosage form. The concentration of the added excipients as well as that of the degradation products must be included in the concentration matrix of the training set when applying the CLS calibration, otherwise erroneous results would be obtained. This is because CLS can be applied only to simple systems where the concentration values of all the components present in the training samples are provided (Kramer, 1998; Kenneth *et al.*, 1998). The advantage of factor based methods, PCR and PLS over CLS is that they do not require that the concentration values for all of the components present have to be provided. They can be used for very complex mixtures since only knowledge of constituents of interest is required (Kramer, 1998; Kenneth *et al.*, 1998; Matthias Otto, 1999).

Twenty mixtures of rabeprazole sodium and its degradation products standard solutions containing different proportions of them have been selected as the training set (table 1). Their composition has been designed in order to obtain maximum information from the calibration procedure. The training mixtures show percentage degradation ranging from 0.5 up to 65%. The number of calibration mixtures in the training set has been selected according to the rule of five. This rule states that using five times the number of samples as there are components provides enough samples to reasonably represent all possible combinations of different concentration values (Kramer, 1998). The number of components in the training mixtures has been estimated to be four, the intact rabeprazole sodium, its two degradation products and the dosage form excipients. The absorbance data matrix for this training set was obtained by recording the absorbances within the wavelength range 220-340 nm at 2 nm intervals. The multivariate calibrations were computed with the CLS, PCR and PLS algorithms using the correlation for the absorbance data matrix and the corresponding concentration data matrix of the training set.

(ΔA) method

Fig. 2 shows the (ΔA) spectrum (rabeprazole sodium versus its degradation products in 0.1 M NaOH). Of the various possibilities offered by the (ΔA) spectrum, the amplitude at 292 nm having maximum sensitivity has been selected for the quantitative analysis. The absorbance difference at 292 nm proved to be linearly related to rabeprazole sodium concentration over the concentration range 7- 40 µg ml^{-1}. Regression analysis has been carried out with correlation coefficient, r, (0.99989), intercept, a ± S_a, ($-1.01\times10^{-3} \pm 1.69\times10^{-3}$) and slope, b ± S_b, ($0.148 \pm 1.52\times10^{-3}$). The good linearity of the calibration graph and negligible scatter of the experimental points were clearly evident by the values of the correlation coefficient and the standard deviation around the slope and intercept.

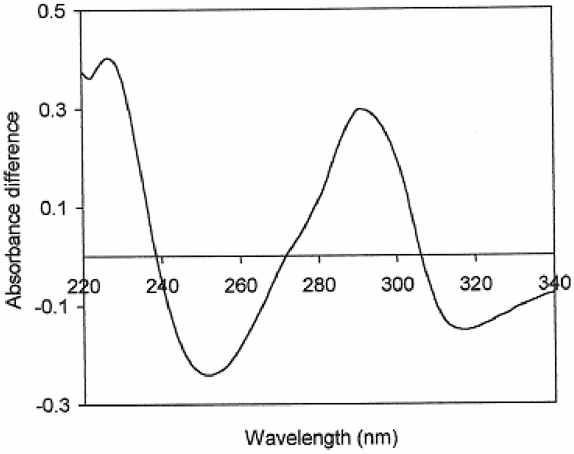

Fig. 2: Absorbance difference (ΔA) spectrum of 20 µg ml^{-1} rabeprazole sodium versus its degradation products in 0.1 M NaOH.

The spectra of the UV absorbing excipients which are commonly used for the formulation of gastro- resistant tablets are generally independent of pH. This has been proved in the present case. So the developed (ΔA) method could be used for the determination of rabeprazole sodium in its tablet dosage form. In order to check the applicability of the proposed (ΔA) method as stability indicating assay, rabeprazole sodium has been determined in ten mixtures containing different concentrations of the degradation products. These mixtures show percentage degradation ranging from 0.5 up to 65 %. The mean percentage recovery was found to be 100.1 ± 0.41 % (table 2). This satisfactory result indicates that the developed (ΔA) method is effective for the selective determination of rabeprazole sodium in presence of its acid induced degradation products with good accuracy and precision.

The acid induced degradation products of rabeprazole sodium exhibited different absorption spectra in 0.1 M NaOH and 0.1 M HCl. Unfortunately, the two spectra showed no isosbestic points. Accordingly, the (ΔA) method (rabeprazole sodium in 0.1 M NaOH versus its degradation products in 0.1 M HCl) could not be used for the selective determination of the drug in presence of its degradation products.

Polarographic and anodic voltammetric methods

The differential pulse polarograms of 2 μg ml^{-1} rabeprazole sodium and 4 μg ml^{-1} its degradation products in Britton- Robinson buffer (pH 8.0) in the potential range of -500 to -1508 mV are shown in fig. 3. Rabeprazole sodium shows a single cathodic peak at about -1028 mV. The degradation products show two peaks at about -1040 and -1316 mV.

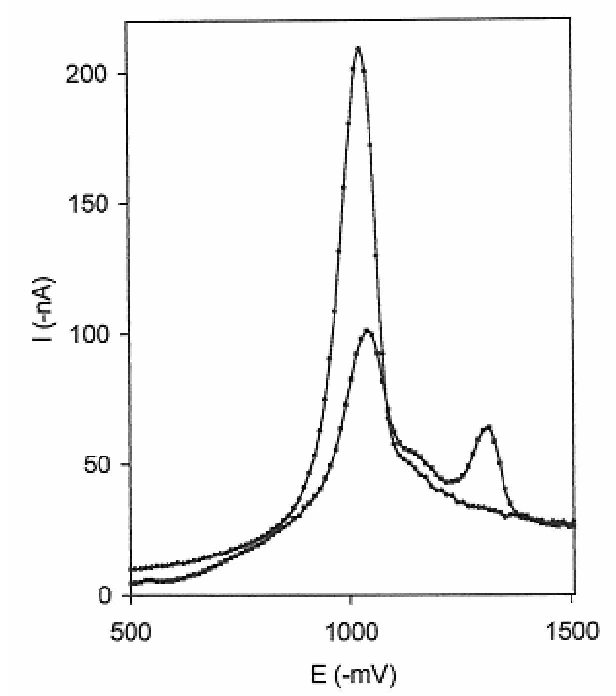

Fig. 3: Differential pulse polarograms of 2 μg ml^{-1} rabeprazole sodium (-••-) and 4 μg ml^{-1} its degradation products (-▪▪-) in Britton- Robinson buffer (pH 8.0).

The differential pulse voltammograms of 0.5 μg ml^{-1} rabeprazole sodium and 0.5 μg ml^{-1} its degradation products, at a glassy carbon electrode, in Britton-Robinson buffer (pH 8.0) in the potential range of 400 to 1192 mV are shown in fig. 4. Rabeprazole sodium shows a single anodic peak at about 826 mV. The degradation products show two peaks at about 790 and 1120 mV.

The strong overlap between the two polarograms in fig. 3 and the two voltammograms in fig. 4 can be seen. This fact shows the impossibility of the direct determination of rabeprazole sodium in presence of its degradation products using either polarography or anodic voltammetry at a glassy carbon electrode.

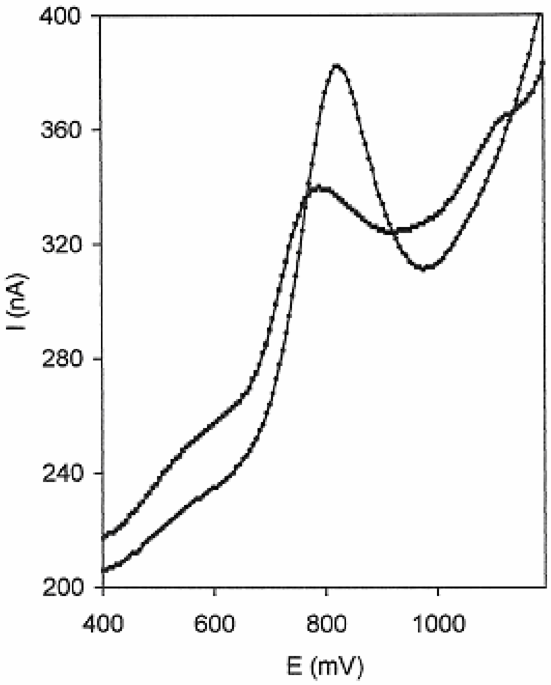

Fig. 4: Differential pulse voltammograms of 0.5 μg ml^{-1} rabeprazole sodium (-••-) and 0.5 μg ml^{-1} its degradation products (-▪▪-) in Britton- Robinson buffer (pH 8.0) at a glassy carbon electrode.

Effect of supporting electrolyte

The influence of various electrolytes on the analytical signal of rabeprazole sodium was tested in the pH range 6.0- 10.0. The best defined voltammograms, maximum peak current and relatively better separation of rabeprazole sodium and its degradation products voltammetric waves were obtained with Britton-Robinson buffer at pH 8.0 in both the polarographic and anodic voltammetric methods. Thus, it was selected as the optimum supporting electrolyte for quantitative analysis.

Optimization of measurement conditions

The optimum instrumental conditions were chosen from a study of the variation of the peak current with pulse amplitude, sweep rate and pulse interval. The best ratio of rabeprazole sodium peak to the degradation products and the background currents was obtained with -100 and 50 mV pulse amplitude for the polarographic and anodic voltammetric methods, respectively. The influence of sweep rate and pulse interval was investigated. Best results were obtained at sweep rate of 15 and 10 mVs^{-1} and at pulse interval of 0.4 and 0.6 s for the polarographic and anodic voltammetric methods, respectively.

Table 1: Composition of the training and validation sets of the spectrophotometric, polarographic and anodic voltammetric multivariate methods.

Mix no.	Spectrophotometric						Polarographic						Anodic voltammetric					
	Training set			Validation set			Training set			Validation set			Training set			Validation set		
	R[a]	D[a]	%[b]	R[a]	D[a]	%[b]	R[a]	D[a]	%[b]	R[a]	D[a]	%[b]	R[a]	D[a]	%[b]	R[a]	D[a]	%[b]
1	4	7.4	65	4.5	7.7	63	0.6	11.4	95	0.6	10	94	0.7	2.1	75	0.5	1.5	75
2	21	35	63	21.1	34	62	0.7	6.3	90	4	40	91	0.3	0.8	73	0.3	0.7	70
3	6	9	60	5.8	9	61	3.5	40	92	0.7	6	90	3	7	70	2.8	7.1	72
4	25	30	55	24	29	55	5.3	30	85	5	35	88	4.3	8	65	4	8	67
5	8	8	50	8.5	8.5	50	0.8	3	79	0.8	2.8	78	4	6	60	3.7	5.8	61
6	31	25	45	30	25	45	7	21	75	0.9	2	69	1	1.2	55	1	1.3	57
7	10	6.7	40	9.5	6.5	41	0.9	2.1	70	2	4	67	5	5	50	6	6	50
8	37	20	35	36.5	21	37	1	1.5	60	1	1.4	58	3.7	3	45	3.5	3	46
9	13	6.1	32	12	6	33	2.5	2.5	50	1.5	1.9	56	2	1.5	43	2	1.4	41
10	35	15	30	34	15	31	3	2	40	2	2	50	6	4	40	5.9	4	40
11	17	5.7	25	16	6	27	3.5	1.9	35	3	2.5	45	2.4	1.3	35	2.6	1.3	33
12	20	5	20	20	6	23	4	1.7	30	3	1.9	39	1.4	0.6	30	1.5	0.6	29
13	23	4	15	23	4	15	4.5	1.5	25	3.5	1.7	33	0.6	0.2	25	0.7	0.2	22
14	26	3.6	12	25	3.5	12	5	1.2	19	4.2	1.8	30	4.5	1.1	20	4.2	1	19
15	27	3	10	26.5	3	10	5.5	1	15	5	1.3	21	5.5	1	15	5.3	0.9	15
16	32	2.4	7	31	2.6	8	6	0.7	10	5.5	1	15	6.5	0.7	10	6.5	0.7	10
17	34	1.8	5	35	2	5	6.2	0.3	5	6	0.8	12	7	0.35	5	7.2	0.4	5
18	37	0.8	2	37	0.7	2	7	0.15	2	6.5	0.4	6	7.5	0.15	2	7.5	0.2	3
19	38	0.4	1	38	0.4	1	7.5	0.08	1	7.4	0.2	3	8	0.08	1	9	0.15	2
20	40	0.2	0.5	40	0.3	0.7	8	0.04	0.5	8	0.1	1	9	0.05	0.6	8.5	0.05	0.6

The spectrophotometric training solutions have been prepared in presence of the dosage form excipients

[a] R and D stand for rabeprazole sodium and its degradation products, respectively

[b] % is the percentage degradation = [degradation products (μg ml^{-1})/ (rabeprazole sodium (μg ml^{-1}) + degradation products (μg ml^{-1}))] x 100 %

Multivariate methods

To develop the chemometric multivariate calibrations, a training set was used, consisting of twenty binary mixture solutions in the possible combinations (table 1). These mixtures show percentage degradation ranging from 0.5-95% and 0.6- 75% for the polarographic and anodic voltammetric calibrations, respectively. The composition of these mixtures has been designed in order to obtain maximum information from the calibration procedure.

Usually, in voltammetric analysis, the analyte current is evaluated as the difference between each voltammogram and the background electrolyte voltammogram. In this multivariate work, there was no need to calculate the difference. The current data of the measured solutions was fed directly into the absorbance data matrix of the training set.

The number of calibration mixtures in each training set has been selected according to the rule of five (Kramer, 1998). The number of components in the training solutions has been estimated to be four, the intact rabeprazole sodium, its two degradation products and the background electrolyte. The current data matrix for each training set was obtained by recording the current values within the potential range -500 to -1508 and 400 to 1192 mV at 6 mV intervals for the polarographic and anodic voltammetric matrices, respectively. The multivariate calibrations were computed with the CLS, PCR and PLS algorithms using the correlation for the current data matrix and the corresponding concentration data matrix of the training set.

The concentration of the supporting electrolyte as well as that of the degradation products must be included in the

Table 2: Assay results of rabeprazole sodium in presence of its degradation products in laboratory made mixtures by the (ΔA) method.

Mixture No.	Rab[a]	Deg[a]	Percentage degradation[b]	Recovery (%)
	μg ml^{-1}			
1	20	37.1	65	99.9
2	20	24.4	55	100.2
3	20	16.4	45	100.7
4	20	10.8	35	100.0
5	20	6.7	25	99.8
6	20	3.5	15	100.1
7	20	2.2	10	99.3
8	20	1.1	5	100.4
9	20	0.4	2	100.6
10	20	0.1	0.5	100.0
Mean				100.1
±S.D.				0.41
R.S.D.				0.41

R.S.D., relative standard deviation

[a] Rab and Deg stand for rabeprazole sodium and its degradation products, respectively

[b] percentage degradation = [degradation products (μg ml^{-1})/ (rabeprazole sodium (μg ml^{-1}) + degradation products (μg ml^{-1}))] x 100 %

concentration matrix of the training set when applying the CLS calibration, otherwise erroneous results would be obtained.

Interferences of dosage form excipients

The effect of the excipients present in the gastro-resistant tablet dosage form was examined by determining 1 μg ml^{-1} rabeprazole sodium in presence of the different excipients at concentrations that can be found in the tablet dosage form. The mean percentage recoveries of six determinations were found to be 100.5 ± 1.21 %, 100.1 ± 1.03 % and 100.2 ± 1.14 % for the CLS, PCR and PLS polarographic calibrations, respectively; and 100.1 ± 0.99 %, 99.8 ± 0.95 % and 100.0 ± 0.93 % for the CLS, PCR and PLS anodic voltammetric calibrations, respectively. These results indicate that there is no interference from any excipients because they are electrochemically inactive at the selected potential ranges. Therefore, the proposed multivariate polarographic and anodic voltammetric methods can be used as selective methods for the determination of rabeprazole sodium in its tablet dosage form.

Validation of the spectrophotometric, polarographic and anodic voltammetric multivariate calibrations

The developed calibrations were validated by determining the content of rabeprazole sodium in the validation set mixtures. The composition of these validation mixtures is shown in table 1. These mixtures present percentage degradation ranging from 0.7-63%, 1-94% and 0.6-75% for the spectrophotometric, polarographic and anodic voltammetric calibrations, respectively. The mean percentage recoveries obtained are shown in table 3. These results contributed to the high accuracy and precision of the proposed multivariate methods.

STATISTICAL ANALYSIS

The predictive ability of a model can be defined using several validation diagnostics. These include the standard error of prediction (SEP), the mean squared error of prediction (MSEP), the root mean standard error of prediction (RMSEP) and the variance of prediction (s^2) (Kramer, 1998; Kenneth *et al.*, 1998).

$$SEP = [\textstyle\sum^{n}_{i=1} (C_i^{True} - C_i^{Predicted})^2 / (n-1)]^{½}$$

$$MSEP = \textstyle\sum^{n}_{i=1} (C_i^{True} - C_i^{Predicted})^2 / n$$

$$RMSEP = [\textstyle\sum^{n}_{i=1} (C_i^{True} - C_i^{Predicted})^2 / n\ ]^{½}$$

$$s^2 = \textstyle\sum^{n}_{i=1} (C_i^{Predicted} - C_i^{True} - bias)^2 / (n-1)$$

Where C_i^{True} is the true concentration, $C_i^{Predicted}$ is the predicted concentration and n is the total number of validation samples. The numerical values of SEP, MSEP, RMSEP and s^2 are indicated in table 4. The small values of the calculated validation diagnostics indicate the negligible error of prediction and the high predictive ability of the proposed methods.

Another way to validate the models and to examine the results is the predicted versus true concentration plot. In

Table 3: Assay results of rabeprazole sodium in presence of its degradation products in the validation mixtures by the developed multivariate methods.

Mixture No.[a]	Recovery (%)								
	Spectrophotometric			Polarographic			Anodic voltammetric		
	CLS	PCR	PLS	CLS	PCR	PLS	CLS	PCR	PLS
1	99.9	99.8	99.7	100.6	100.7	100.7	99.8	100.2	100.3
2	100.8	100.5	100.5	99.5	99.5	99.6	100.0	100.4	100.6
3	100.4	100.1	100.2	99.9	100.1	100.1	98.9	99.3	99.4
4	100.3	99.9	100.0	101.7	101.6	101.6	101.3	101.6	101.7
5	100.8	100.7	100.6	100.4	100.6	100.5	97.9	98.3	98.5
6	100.1	100.0	100.0	98.3	98.4	98.5	98.1	98.5	98.6
7	99.3	99.3	99.2	99.4	99.7	99.9	99.0	99.2	99.2
8	100.0	99.7	99.7	100.9	100.9	100.9	99.8	100.1	100.2
9	100.5	100.4	100.4	99.0	99.2	99.2	99.0	99.4	99.5
10	99.8	99.4	99.6	99.2	99.3	99.2	97.7	98.1	98.2
11	101.4	101.0	101.0	100.8	100.9	100.9	100.1	100.6	100.6
12	100.3	100.2	100.1	98.2	98.4	98.5	101.2	101.4	101.3
13	99.2	99.1	99.0	101.9	102.0	101.8	100.0	100.3	100.4
14	99.4	99.0	99.2	100.0	100.2	100.3	99.1	99.6	99.8
15	100.9	100.7	100.7	100.5	100.4	100.4	98.5	98.8	99.1
16	99.4	99.5	99.3	100.7	100.9	100.8	98.9	99.1	99.2
17	101.0	100.6	100.7	101.5	101.6	101.6	100.7	101.0	101.0
18	101.2	100.9	100.9	98.9	99.1	99.1	99.2	99.5	99.6
19	99.4	99.3	99.3	100.9	101.0	100.9	100.9	101.1	101.0
20	100.7	100.4	100.5	100.0	100.2	100.1	99.4	99.7	99.9
Mean	100.2	100.0	100.0	100.1	100.2	100.2	99.5	99.8	99.9
±S.D.	0.68	0.62	0.63	1.07	1.02	0.98	1.05	1.02	0.97
R.S.D.	0.68	0.62	0.63	1.07	1.02	0.98	1.06	1.02	0.97

R.S.D., relative standard deviation, [a]Validation mixtures described in table 1

this plot, points are expected to fall on a straight line with a slope of one and a zero intercept (Kenneth *et al*., 1998). The correlation coefficient, r, is calculated for each calibration to indicate the quality of fit of all data to a straight line. The regression analysis for these linear relationships has been carried out and the results are shown in table 4. The absence of bias has been proved by determining the confidence limits for the intercept, a, and the slope, b, at the 95% significance level (Miller and Miller, 2000). The upper and lower confidence limits are shown in table 4. For all the developed multivariate models, the 95% confidence interval of the intercept includes the ideal value of zero and that of the slope includes the ideal value of one. This indicates good fitness and absence of bias which confirms the trueness of the developed methods. Furthermore, no sample(s) appears to be unusually far from the line than the rest of the data.

Determination of the optimum number of factors to be used in the PCR and PLS calibrations

Determining how many factors to be used in the calibration is a key step in factor based techniques, PCR and PLS. Only those factors that contain analytical information must be kept. The discarded factors should contain only noise (Kramer, 1998; Kenneth *et al*. 1998; Haaland and Thomas, 1988 a; Haaland and Thomas, 1988 b; Espinosa- Mansilla *et al*., 1993). The Chemometrics Toolbox 3.02 software offers several indicators which could be used for determining the optimum number of factors (rank). The Cross- validation procedure leaving out one sample at a time, is used for this purpose (Kramer, 1998; Espinosa- Mansilla *et al*., 1995) and the predicted residual error sum-of-squares, PRESS is calculated.

$$PRESS= \sum_{i=1}^{n} (C_i^{True} - C_i^{Predicted})^2$$

Table 4: Statistical parameters of the validation mixtures

Statistical parameters	Methods								
	Spectrophotometric			Polarographic			Anodic voltammetric		
	CLS	PCR	PLS	CLS	PCR	PLS	CLS	PCR	PLS
SEP ($\times10^2$)	19.25	16.51	16.78	4.37	4.40	4.21	5.67	5.09	4.69
MSEP ($\times10^3$)	35.20	25.90	26.73	1.81	1.84	1.69	3.05	2.46	2.09
RMSEP($\times10^2$)	18.76	16.09	16.35	4.26	4.29	4.11	5.52	4.96	4.57
s^2 ($\times10^3$)	33.69	27.26	28.11	1.80	1.72	1.58	4.79	2.88	2.31
a ($\times10^3$)	-3.15	8.55	-2.89	-9.17	-9.55	-7.76	-11.79	-9.16	-7.84
Lower 95% [a]	-0.210	-0.180	-0.194	-0.045	-0.043	-0.041	-0.057	-0.054	-0.049
Upper 95% [a]	0.204	0.197	0.188	0.027	0.024	0.025	0.034	0.035	0.034
b	1.003	1.000	1.000	1.006	1.007	1.006	0.997	1.000	1.000
Lower 95% [b]	0.995	0.993	0.993	0.997	0.999	0.998	0.988	0.991	0.992
Upper 95% [b]	1.010	1.007	1.008	1.014	1.015	1.014	1.007	1.009	1.009
r	0.99988	0.99990	0.99989	0.99986	0.99987	0.99988	0.99982	0.99983	0.99986

SEP, standard error of prediction; MSEP, mean squared error of prediction; RMSEP, root mean standard error of prediction; s^2, variance of prediction; a, intercept; b, slope; r, correlation coefficient

[a] Lower and upper confidence limits for the intercept at the 95 % confidence level

[b] Lower and upper confidence limits for the slope at the 95 % confidence level

Table 5: Assay results of rabeprazole sodium in commercial Pariet® tablets using the developed methods

Mean found % ±S.D.[a]										
Spectrophotometric					Polarographic			Anodic voltammetric		HPLC[b]
	CLS	PCR	PLS	(ΔA)	CLS	PCR	PLS	CLS	PCR	PLS	
	99.8 ±0.58	100.2 ±0.50	100.1 ±0.52	100.4 ±0.36	99.9 ±0.95	100.0 ±0.82	100.1 ±0.79	99.7 ±1.01	99.9 ±0.99	99.9 ±0.97	100.1 ±0.53
t-	0.94	0.34	0	1.15	0.45	0.25	0	0.86	0.44	0.44	(2.23)[c]
F-	1.20	1.12	1.04	2.17	3.21	2.39	2.22	3.63	3.49	3.35	(5.05)[c]

[a] Mean of six determinations, [b] (El- Gindy *et al.,* 2003), [c] Theoretical t- and F-values at P = 0.05

Where C_i^{True} represents the true concentration, $C_i^{Predicted}$ denotes the predicted concentration and n is the total number of validation samples.

A better way for selecting the optimum number of factors involves the generation of a calibration for every possible rank. Each calibration is used to predict the concentrations for a set of independently measured, independent validation samples. Then the PRESS is calculated (Kramer, 1998). Another way to determine the optimum number of factors is the two-way F-test on reduced eigenvalues (REV's) according to the method of Malinowski (Kramer, 1998).

A rank of three factors was found to be the optimum system rank for the spectrophotometric, polarographic and anodic voltammetric PCR and PLS models according to the three studied criteria. The first factor is suggested to be due to rabeprazole sodium. The second is suggested to be due to the degradation products. The third is suggested to be due to the added dosage form excipients in the spectrophotometric calibrations; and due to the background electrolyte in the polarographic and anodic voltammetric calibrations. One factor instead of two is suggested to be due to the degradation products. So, the ratio of one degradation product to the other may be almost constant.

Determination of rabeprazole sodium in tablets

The developed spectrophotometric, polarographic, anodic voltammetric multivariate methods and the difference spectrophotometric (ΔA) method were applied to the determination of rabeprazole sodium in commercial Pariet® gastro- resistant tablets. Six determinations were made. Satisfactory results were obtained in a good agreement with the label claim (table 5). These results were statistically compared to those of the published HPLC method (El- Gindy *et al*., 2003) with regard to accuracy and precision using Student's t-test and the F-ratio test at 95% confidence level (table 5). The calculated values did not exceed the theoretical ones, indicating that there is no significant difference between the developed methods and the published HPLC method.

CONCLUSION

The developed methods can be used successfully to determine rabeprazole sodium in tablet dosage form and in presence of its acid induced degradation products. These methods have been validated and have been found to be accurate, precise and selective.

The polarographic and anodic voltammetric methods were found to be more sensitive and selective than the spectrophotometric methods. On the other hand, the spectrophotometric methods are more versatile, easier to apply and have the advantage of lower cost and rapidity.

The factor based methods (PCR and PLS) are suggested to be preferred than the CLS method as they do not require that the concentration values for all of the components present have to be provided in the concentration matrix. Only knowledge of constituents of interest is required.

The superiority of the spectrophotometric multivariate methods over the published derivative ratio (^{1}DD) method (El-Gindy *et al*., 2003) is manifested in being fast, easy, simple and do not require prerequisite for the successful application. The (^{1}DD) method requires that the spectrum of the investigated drug is divided by that of the degradation products to obtain a ratio curve. A suitable derivative order curve should be derived therefrom in order to correct for the constant term resulting from the above mentioned division to obtain the drug concentration. It is also necessary to test the influence of the variables: divisor concentration, $\Delta\ \lambda$ and smoothing function.

The developed polarographic and anodic voltammetric multivariate methods proved to be more selective (percentage degradation ranging from 0.5 up to 95% and 0.6 up to 75% for the two techniques, respectively) than the published HPLC and HPTLC methods (El-Gindy *et al*., 2003) (percentage degradation ranging from 9 up to 66.7%). Furthermore, the polarographic and voltammetric methods are simpler, faster, easier to apply and require less expensive instrumentation and running costs than the chromatographic methods.

The studied methods are simple, precise and affordable. They also require no complex pretreatment nor prior separation of the investigated drug. These methods represent good alternatives for the analytical determination of rabeprazole sodium and are suitable for quality control laboratories, where economy and time are essential.

REFERENCES

Alonso-Lomillo MA, Dominguez-Renedo O and Arcos-Martinez MJ (2001). Resolution of ternary mixtures of rifampicin, isoniazid and pyrazinamide by differential pulse polarography and partial least squares method. *Anal. Chim. Acta,* **449**(1-2): 167-177.

Dinç E and Baleanu D (2002). Spectrophotometric quantitative determination of cilazapril and hydrochlorothiazide in tablets by chemometric methods. *J. Pharm. Biomed. Anal.,* **30**(3): 715-723.

Dinç E and Üstündağ Ö (2002). Chemometric resolution of a mixture containing hydrochlorothiazide and amiloride by absorption and derivative spectrophotometry. *J. Pharm. Biomed. Anal.,* **29**(1-2): 371-379.

El-Gindy A, El-Yazby F and Maher MM (2003). Spectrophotometric and chromatographic determination of rabeprazole in presence of its degradation products. *J. Pharm. Biomed. Anal.,* **31**: 229-242.

El-Gindy A, El-Yazby F, Mostafa A and Maher MM (2004). HPLC and chemometric methods for the simultaneous determination of cyproheptadine hydrochloride, multivitamins and sorbic acid. *J. Pharm. Biomed. Anal.,* **35**(4): 703-713.

Espinosa-Mansilla A, Munoz de la Pena A, Salinas F and Martinez- Galera M (1993). Simultaneous determination of 2- furfuraldehyde, 5-hydroxymethylfurfuraldehyde and malonaldehyde in mixtures by derivative spectrophotometry and partial least squares analysis. *Anal. Chim. Acta*, **276**(1): 141-149.

Espinosa-Mansilla A, Salinas F and De Orbe Paya I (1995). Simultaneous determination of sulfadiazine, doxycycline, furaltadone and trimethoprim by partial least squares multivariate calibration. *Anal. Chim. Acta,* **313**(1-2): 103-112.

Garcia CV, Paim CS and Steppe M (2004). New liquid chromatographic method for determination of rabeprazole sodium in coated tablets. *J. AOAC. Int.,* **87**(4): 842-846.

Haaland DM and Thomas EV (1988a). Partial least squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. *Anal. Chem*., **60**(11): 1193-1202.

Haaland DM and Thomas EV (1988 b). Partial least-squares methods for spectral analyses. 2. Application to simulated and glass spectral data. *Anal. Chem.,* **60**(11): 1202-1208.

Kenneth RB, Randy JP and Seasholtz MB (1998). Chemometrics: A Practical Guide, John Wiley and Sons, Inc., New York, pp.201, 204, 243-245, 278-290.

Kramer R (1995). Chemometrics TOOLBOX for use with MATLAB, The Math Works. Inc., Natick.

Kramer R (1998). Chemometric Techniques for Quantitative Analysis, Marcel Dekker Inc., New York, pp.20, 51-142, 168, 169.

Lentner C (1984). Geigy Scientific Tables, 8th ed., Ciba-Geigy, 3: p.58.

Lopez-de-Alba PL, Lopez-Martinez L and de-Leon-Rodriguez LM (2002). Simultaneous determination of synthetic dyes Tetrazine, Allura red and sunset yellow by differential pulse polarography and partial least squares: A multivariate calibration method. *Electroanalysis,* **14**(3): 197-205.

Martindale (2005). The Complete Drug Reference, 34th ed., Sweetman SC editor, published by the Pharmaceutical Press, London, p.1285.

Matthias Otto M (1999). Chemometrics Statistics and Computer Application in Analytical Chemistry, Wiley-VCH, Weinheim, p.197.

McClean S, O'Kane E, Ramachandran VN and Smyth WF (1994). Differential pulse polarographic study of the degradation of H^+/K^+ ATPase inhibitors SK & F 95601 and omeprazole in acidic media and the subsequent reactions with thiols. *Anal. Chim. Acta,* **292**: 81-89.

Miller JN and Miller JC (2000). Statistics and Chemometrics for Analytical Chemistry, 4th ed., Pearson Education Limited, England, pp.127, 128.

Moneeb MS (2006). Polarographic chemometric determination of zinc and nickel in aqueous samples. *Talanta*, **70**: 1035-1043.

Olin BR (2001). Drug Facts and Comparisons, 55th ed., Burnham TH editor, Hebel SK publisher, Facts and Comparisons, A Wolters Kluwer Company, St. Louis, pp.1167, 1171.

Radi A, Abd El-Ghany N and Wahdan T (2004). Voltammetric behaviour of rabeprazole at a glassy carbon electrode and its determination in tablet dosage form. *Il FARMACO*, **59**: 515-518.

Tivesten A, Folestad S, Schonbacher V and Svensson K (1999). Non aqueous capillary electrophoresis for the analysis of labile pharmaceutical compounds. *Chromatographia,* **49**(Suppl. I): S7-S11.

Wahbi AM, Abdel- Razak O, Gazy A, Mahgoub H and Moneeb MS (2002). Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. *J. Pharm. Biomed. Anal*., **30**: 1133-1142.

Yokoyama Y, Kishi N, Ohe H, Tanaka M, and Asakawa N (1998). Fundamental processes for LC- NMR and its applications. *Chromatography,* **19**(4): 262-263.

Zhang Y, Chen XY, Gu Q and Zhong DF (2004). Quantification of rabeprazole in human plasma by liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta*, **523**(2): 171-175.