Comparison Between UV Spectrophotometric and Capillary Electrophoresis Methods for Determination of Rabeprazole Sodium in Pharmaceutical Formulations

Cássia V. GARCIA *1, Andreas L. MENDEZ [2], Martin STEPPE [1] & Elfrides E.S. SCHAPOVAL [1]

[1] *Programa de Pós-graduação em Ciências Farmacêuticas - Faculdade de Farmácia,*
Universidade Federal do Rio Grande do Sul. Av. Ipiranga, 2752,
Lab 402, CEP 90610-000, Porto Alegre/RS – Brasil

[2] *Curso de Farmácia – Universidade Federal do Pampa. Uruguaiana/RS - Brazil*

SUMMARY. Rabeprazole sodium (RAB) is an anti-secretory agent which inhibits the enzyme H^+/K^+ ATPase, present in the stomach parietal cells. The aim of this work is to develop and validate a simple and fast ultraviolet spectrophotometric method (UV) for quantification of RAB in pharmaceutical formulation and compare it with a capillary electrophoresis (CE) one, previously validated. The UV technique was applied using water (pH 10.0) as diluent and the determinations were made at $\lambda = 291$ nm. The method showed good linearity (r = 0.9997) in the concentration range of 6.0 to 18.0 μg ml^{-1}. The intra- and interday precision data demonstrated the method has good repeatability (RSD = 0.52 and 0.82, respectively). Accuracy and specificity were also evaluated and results were satisfactory. The detection and quantitation limits were 0.32 and 0.95 μg ml^{-1}, respectively. Both methods demonstrated to be adequate for the intended purpose.

INTRODUCTION

Rabeprazole (±)-sodium 2-[[4-(3-methoxypropoxy)-3-methylpyridine-2-yl]methylsulfinyl]-1*H*-benzimidazole (RAB) is a proton pump inhibitor that covalently binds and inactivates the gastric parietal cell proton pump (H^+/K^+ ATPase). It has proven efficacy in healing, symptom relief and prevention of relapse of gastric ulcer, duodenal ulcer and gastro-oesophageal reflux disease [1]. Another great activity RAB has is against *Helicobacter pylori,* an organism strongly associated with peptic ulcer disease. There are several different mechanisms supposed for this, but they are not well understood [2,3]. Since it is an acid labile drug, it is commercialize in enteric coated tablets [4]. The structural formula of RAB is given in Figure 1.

The literature survey reveals a crescent number of publications related to RAB determination in the pharmaceutical dosage form. The methods applied were: liquid chromatography [5,6], voltametry [7], CE in aqueous media [8] and deriva-
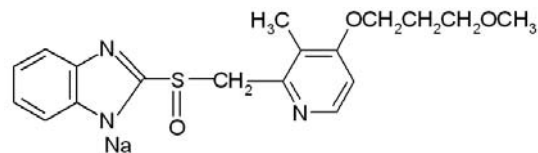

Figure 1. Chemical structure of Rabeprazole Sodium.

tive spectrophotometry [9]. The dissolution test of RAB tablets was also published [10], but descriptions on the drug have not appeared in any pharmacopeia up to now. The identification of six impurities in RAB bulk substance was performed by LC-MS and spectral data (IR, NMR) [11] and, recently, three photodegradation products were isolated and elucidated [12].

Although the work of El-Gindy *et al.* [5] comprises also a derivative ratio spectra method, very useful for stability indication, the method proposed in this work is simple, fast, could be applied in routine analysis without sophisticated tools and was not available yet.

KEY WORDS: Method validation, Quality control, Rabeprazole sodium, Spectrophotometry.

* Author to whom correspondence should be addressed. *E-mail:* cassiavgarcia@yahoo.com.br

The aim of this study is to develop and validate UV method for RAB determination in coated tablets. This method was compared to a CE one [8], which was previously validated with the same purpose. The validation procedures will follow the ICH and USP guidelines [13,14], evaluating the parameters specificity, linearity, precision, accuracy and detection and quantitation limits.

MATERIALS AND METHODS
Chemicals

RAB reference standard was supplied by Janssen-Cilag (Buenos Aires, Argentine). The coated tablets (Pariet®), containing 10 mg of RAB, were obtained commercially. The excipients of the pharmaceutical formulation were mannitol, hydroxypropyl cellulose, magnesium oxide, low-substituted hydroxypropyl cellulose, magnesium stearate, ethylcellulose, hydroxypropyl methylcellulose phthalate, diacetylated monoglycerides, talc, titanium dioxide, carnauba wax, and ferric oxide (red) as a coloring agent. All of them were obtained from different local distributors. Water was purified using Millipore® system and its pH was adjusted to 10.0 with ammonium hydroxide analytical grade (Grupo Química, Brazil).

Apparatus and conditions

A Shimadzu UV-160A double-beam spectrophotometer with 1 cm quartz cells and data processing capacity was used. The determinations were made at λ = 291 nm and scan speed of 480 nm min⁻¹. The Digimed potenciometer, model DM-20 (São Paulo, Brazil) was used to determine the water pH before the preparation of all solutions.

Specificity

The specificity of the method was evaluated through the analysis of a placebo mixture solution, prepared with the excipients of the pharmaceutical formulation in their usual concentration.

Linearity

Aliquots of a 100 µg ml⁻¹ solution of RAB reference standard were transferred to 25 ml volumetric flasks to obtain the final concentrations of 6.0, 8.0, 10.0, 12.0, 14.0, 16.0 and 18.0 µg ml⁻¹. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

Five tablets were transferred to 500 ml volumetric flask containing 50 ml of water pH 10.0 and shaken for 20 min in ultrasonic bath. The volume was completed with the same solvent. The solution was filtered using quantitative paper and also with nylon membrane (0.45 µm) before diluted to 12 µg ml⁻¹ (six replicates each day). New solutions were prepared in three days for inter-day precision evaluation.

Accuracy

The accuracy of the methods was evaluated through the recovery test. From a RAB standard solution of 25 µg ml⁻¹, aliquots of 2.0, 4.0 and 6.0 ml were taken and transferred to 25 ml volumetric flasks containing 3.0 ml of the sample solution at 100 µg ml⁻¹ (prepared as cited above). The volume was completed with water pH 10.0 (adjusted with ammonium hydroxide), obtaining the final concentrations of 14.0. 16.0 and 18.0 µg ml⁻¹, respectively. The concentrations reached were into the standard curve. Each solution was done in triplicate.

RESULTS AND DISCUSSION

In the beginning of the development of the method, different solvents were tested, such as water, methanol and ethanol. Water was considered the best since it had a great capability to disintegrate the tablets and the absorbance of the aqueous solutions of RAB was high. Besides, it does not offer toxicological risks and is not expensive. The adjust in pH was necessary since RAB is unstable under acid conditions.

The original UV spectrum was demonstrated in Figure 2. It is possible to observe the well-define peak at 291 nm. The specificity test demonstrated the placebo solution exhibited some interference in the wavelength of analysis, what
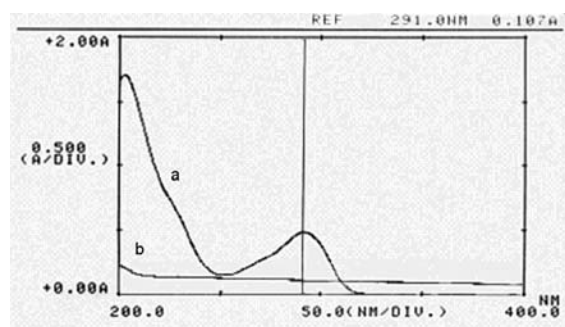

Figure 2. UV spectra of RAB reference standard (**a**) and placebo (**b**), without filtration, both in water (pH 10.0), concentration of 12 µg ml⁻¹.
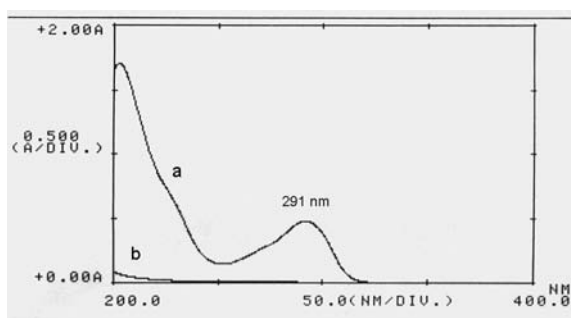
Figure 3. UV spectra of RAB reference standard (**a**) and placebo (**b**), both in water (pH 10.0), concentration of 12 µg ml^{-1}, after filtration with nylon membrane.

would impede the use the method. New investigations were performed and it was found that the UV method could be applied for this determination if the sample aqueous solution was filtered with nylon membrane of 0.45 µm (Fig. 3). Otherwise, the titanium dioxide, one of the excipients, which was in dispersion, would cause interference in the absorbance. So, it was necessary to adopt the filtration step, which did not represent a complex procedure. On the other hand, the centrifugation was not adopted since it would add another instrument in the analysis and it was not an objective.

The results from linearity are demonstrated in Table 1. The data were validated by means of analysis of variance (ANOVA), which demonstrated significative linear regression and no-significative linearity deviation ($p < 0.05$).

From this data, it was possible to calculate the detection and quantitation limits [13]. The results found were LOD = 0.32 µg ml^{-1}, LOQ = 0.95 µg ml^{-1}. These low values indicated the

Feature	UV
Regression equation	y = 0.042 x + 0.0008
Correlation coefficient (r)	0.9997
Linear range (µg ml^{-1})	6.0-18.0

Table 1. Results of the standard curve of UV spectrophotometric method (λ 291 nm) for RAB.

good sensitivity of the proposed method. The determinations of commercial samples showed excellent precision. The results obtained are listed in Table 2. All values for relative standard deviation are below 2.0%. In the sample preparation, it was not possible to use the powder of tablets, as usual, since the coating could not be powdered. So, it was adopted the solution obtained from five tablets, which were dissolved together in a volumetric flask. Using this number of units, the sampling was representative and the amount of excipients in suspension was not big enough to impede or delay the filtration step.

The evaluation of accuracy, made through the recovery test, showed a mean recovery of 99.65% (Table 3).

Comparison of methods

The CE method, previously validated [8], applied the capillary zone electrophoresis (CZE) separation technique, in which the capillary is filled with the running buffer solution (10 mM sodium tetraborate) and the ionic analytes are separated under high voltage (20 kV). The comparison between the UV and the CE methods was performed by t-Student test. It was found that the amounts of RAB determined using each

| Method | Amount (%) | | | | RSD |
	First day*	Second day*	Third day*	Mean	Inter-days
UV	102.40	101.42	102.27	102.26	0.82
	102.40	101.35	102.94		
	102.33	101.29	102.74		
	102.14	101.35	103.14		
	102.14	101.42	103.74		
	102.53	101.10	103.94		
Mean	102.32	101.32	103.13		
RSD	0.15	0.12	0.61		
CE mean (RSD)[8]	101.41 (1.89)	100.49 (1.47)	101.27 (1.82)	101.06	1.69

Table 2. Determination of commercial samples of RAB by UV spectrophotometric method (λ 291 nm, water as diluent) and CE. Electrophoretic conditions: sodium tetraborate buffer 10 mM (pH 9.0), voltage of 20 kV, UV detection – 291 nm [8] – precision test; *mean of three determinations.

Concentration (µg ml^{-1})		% of recovery [*]	Mean (%)
Added	**Found**		
2.0	1.99	99.50	
4.0	4.01	100.25	99.65
6.0	5.95	99.17	

Table 3. Recovery test for RAB by UV spectrophoto-metric method (λ 291 nm), using water pH 10.0 as diluent; [*] mean of three determinations.

method were not statistically different (t$_{calc}$ = 2,01 < t$_{tab}$ = 2,77, p < 0.05). It means that both methods could be used for the same purpose. A representative electropherogram of RAB is demonstrated in Figure 4.

Considering that chromatographic methods are more expensive, time consumer and need more steps, the proposed UV method is adequate for routine analysis and also cheaper, being a safe and sensitive alternative for quality control of RAB in tablets. Another important application could be the dissolution studies, which need rapid and accurate results. The CE method also has many advantages such as low consume of solvents, good efficacy and rapid analysis, but the instrumental are still not available in all laboratories, mainly for routine use, which may represent a barrier to apply the method.

CONCLUSIONS

The UV method was validated and demonstrated to be simple, linear, accurate, precise, specific and sensitive, which indicates its adequacy to pharmaceutical analysis, being equivalent to the CE one [8] for RAB determination in coated tablets.

Figure 4. Representative electropherogram of RAB in water pH 10.0 (20 µg ml^{-1}). Electrophoretic conditions: sodium tetraborate buffer 10 mM (pH 9.0), voltage of 20 kV, fused-silica capillary of 48 cm total length, UV detection – 291 nm [8].

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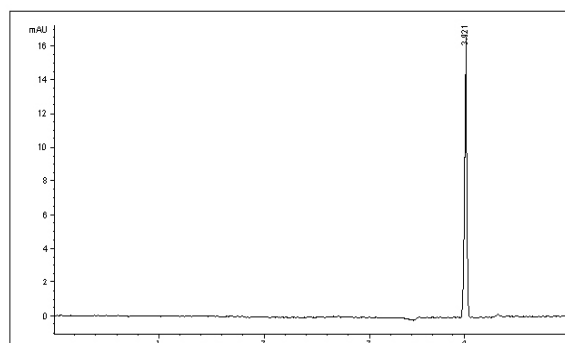2. Barth J. & W. Hahne (2002) *Aliment. Pharmacol. Ther.* **16**: 31-3.
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