

## Bioavailability study of Coated Erythromycin Stearate Tablets in Rabbits

Luciane CALIL MYLIUS \* and Elfrides E.S. SCHAPOVAL

Post-Graduate Course in Pharmaceutical Sciences, Faculty of Pharmacy,  
Federal University of Rio Grande do Sul, Av. Ipiranga, 2752,  
CEP:90610-000, Porto Alegre, RS, Brasil

**SUMMARY.** Variation in bioavailability and the lack of equivalence in medications from different laboratories, which contain the same substance, are among the most important problems for the biopharmaceutical control of drugs. Unstable at acid pH, erythromycin is one of the drugs whose form of presentation affects its pharmacological activity. In the present investigation, we compared the behavior of 250 mg coated erythromycin stearate tablets from three different pharmaceutical laboratories. Water, 0.1 N hydrochloric acid, and pH 7.5 sodium phosphate buffer were used for the study. The dissolution pattern was similar to the three laboratories, with differences in the released percentage at each tested time. The samples from one of the laboratories were used as reference for the study in all parameters. Bioavailability was determined in rabbits using a complete crossover design. The animals received a single dose of the drug. The serum concentrations were determined by a microbiological assay and the pharmacokinetic parameters area under the serum concentration curve (AUC), maximum concentration peak ( $C_{max}$ ) and time of maximum concentration peak ( $t_{max}$ ) were compared by analysis of variance (ANOVA) and by the Tuckey test. In the present study, in rabbits, the tablets from two of the laboratories were found not to be bioequivalent to the tablets from the reference laboratory but were bioequivalent to each other. The *in vitro-in vivo* correlation determined by the Pearson correlation coefficient between absorbed and dissolved fractions was significant for the samples of the three laboratories.

**RESUMEN.** "Estudio de la Biodisponibilidad de Comprimidos Recubiertos de Estearato de Eritromicina en Conejos". Las variaciones en la biodisponibilidad, junto con la falta de equivalencia entre los medicamentos producidos por distintos laboratorios, constituyen uno de los problemas más importantes en el control biofarmacéutico de los medicamentos. Siendo inestable en pH ácido, la eritromicina es uno de los fármacos en los que el tipo de formulación interfiere en su actividad farmacológica. Por esta razón se estudió comparativamente el comportamiento *in vitro* de los comprimidos recubiertos del estearato de eritromicina (250 mg) provenientes de tres laboratorios farmacéuticos distintos, utilizando tres líquidos de disolución: agua, ácido clorhídrico 0,1 N y tampón fosfato de pH 7,5. El patrón de disolución fue semejante para los tres laboratorios, siendo distinto el porcentaje disuelto en cada uno de los tiempos ensayados. Las muestras de uno de los laboratorios fueran utilizadas como referencia para el estudio de todos los parámetros. Se estudió la biodisponibilidad, en conejos, según el planeamiento en dibujos cruzados completos, en dosis única y las concentraciones séricas fueron determinadas por el método microbiológico de difusión en agar. Los parámetros farmacocinéticos  $AUC_{0-10}$ ,  $C_{máx}$ , y  $t_{máx}$  fueron comparados a través del análisis de varianza (ANOVA) y del Test de Tuckey. Fue detectado en conejos que los comprimidos de dos de los laboratorios no son bioequivalentes en relación al laboratorio de referencia, mas son bioequivalentes entre sí. La correlación *in vitro - in vivo* fue determinada por el coeficiente de Pearson, que fue significativo para las muestras de los tres laboratorios.

**KEY WORDS:** Bioavailability, Bioequivalence, Erythromycin stearate.

**PALABRAS CLAVE:** Biodisponibilidad, Bioequivalencia, Estearato de eritromicina.

\* Author to whom correspondence should be addressed

## INTRODUCTION

The pharmaceutical control of medications, in addition to guaranteeing that drugs comply with physicochemical parameters of quality, is also used to determine the therapeutic efficacy of the same drug present in different formulations.

Erythromycin, an antibiotic used against Gram-positive microorganisms, can be commercialized as a free base, salt or ester. The base and the salt, by being unstable at acid pH, are absorbed in the upper part of the intestine <sup>1</sup>. To assure higher blood concentrations, the drug should be administered in the form of coated tablets that dissolve in the duodenum <sup>2</sup>. Physiological factors such as the presence of food, and intestinal motility and transit may affect the absorption of the drug, with a consequent variation in bioavailability <sup>3,4</sup>.

Highest serum concentrations of the base or stearate is 0.3-0.5 ( $\mu\text{g/ml}$ ), four hours after administration. It rapidly reaches bactericidal concentrations in all tissues and body fluids, except in the brain. It is usually eliminated in the active form from urine and bile, but part of the drug is also metabolized by demethylation <sup>2</sup>. Concerning the importance of the inherent factors in pharmaceutical forms and formulations, and their absorption in the gastrointestinal tract, the present study was undertaken to analyze and compare the bioavailability of coated erythromycin stearate tablets from three different pharmaceutical laboratories in rabbits.

## MATERIALS AND METHODS

The study was conducted by the use of coated erythromycin stearate tablets containing 250 mg erythromycin base, from three pharmaceutical laboratories called A, B and C. Laboratory A was considered to be reference.

The standard used was erythromycin stearate (CIBRAN), anhydrous. The culture media were N° 1, 3 and 11 of Grove & Randall.

For the construction of the standard curve, the equivalent of 62.5 mg of erythromycin base was weighed analytically and diluted in methanol to a concentration of 2.5 mg/ml. This concentration was used to prepare a 0.1 mg/ml solution with sodium phosphate buffer, pH 8.0. This solution was used to construct a standard curve at concentrations of 0.0156, 0.0625, 0.25, 1.0 and 4.0 ( $\mu\text{g/ml}$ ).

The *in vitro* dissolution test was performed using a flow cell apparatus (Desaga 147060) at  $37 \pm 1$  °C. The dissolving fluids were used in the following order according to the disintegration test recommended for coated tablets: water, 5 min; 0.1 N hydrochloric acid, pH 1.0-3.0, 60 min; sodium phosphate buffer, pH 7.5, up to 8 hours after the experiment. The samples were collected each 2 hours.

Erythromycin concentration was determined in samples (dissolution liquids and serum) by the microbiological method of agar diffusion, in N° 11 culture medium using *Micrococcus luteus* ATCC 9341. The microorganism was replicated in a test tube in media N° 1 and 3 for 24 hours in an oven at  $35 \pm 1$  °C. At the time of the assay, the suspension in N° 3 medium was diluted to 2% in N° 11 medium, maintained at 45-48 °C and used for the preparation of plates. Twelve plates were used for the samples from each laboratory and the concentrations were equal to the standard curve.

The bioavailability study was performed using 12 healthy New Zealand rabbits weighing 2.9 to 3.9 kg. The animals were kept in a climatic room at 22-25 °C.

The tablets were administered to them by the oral route in about 20 ml of water. Approximately 3 ml of blood were obtained from the marginal veins of the ears at 0.25, 0.5, 1, 2, 4, 6, 8, and 10 hours after administration, and it was placed in an oven or in a waterbath at 35 °C for coagulation. Afterwards, the serum was separated for the drug level determination.

The bioavailability of tablets A, B and C was determined in sequential experiments using a complete crossover design at 1 week intervals.

### **Pharmacokinetics analysis**

The non-compartmental data were obtained using an Excel spreadsheet, specially developed to do the analysis. The area under serum concentration time curve (AUC) was calculated by trapezoidal rule. Extrapolation from the last measurement point to infinity was done as  $C_{p_x} / k_e$ . Time of maximum concentration ( $t_{max}$ ) and maximum plasma concentration ( $C_{max}$ ) were extracted directly from the observed data points. Half life ( $t_{1/2}$ ) was calculated as  $t_{1/2} = 0,693/K_e$  and mean residence time (MRT) was calculated as  $MRT = AUC_{\infty} / AUMC_{\infty}$ . For the area under the first moment curve (AUMC) it was used the trapezoidal rule  $c_{px} * t_x$  versus  $t_x$  - pairs. Extrapolation from the last measurement point to infinity was done from:  $AUMC_t = t_x * c_{px} / K_e + c_{px} / K_e^2$ .

The terminal elimination rate constant ( $K_e$ ) was determined for each subject from the terminal slope of semi-logarithmic plots of plasma concentration-time profiles.

The sub-routine used automatically selects the best coefficient of determination, using the last three to seven data points without removing any data point in the middle of the selected range.

### **Statistical analysis**

Data were analyzed by the Lilliefors test for normality and by the Bartlett and Cochran test for homocedasticity. The variables were compared by parametric analysis of variance <sup>4</sup> or, when they did not present normal distribution, by the nonparametric Kruskal-Wallis test <sup>5</sup>.

AUC and  $C_{max}$  data were submitted to the normality and homoscedasticity tests and were normalized after submitting to logarithm transformation :  $\log(x+0)$  type.

Means presenting significant differences ( $P < 0.05$ ) were analyzed by the Tuckey test <sup>5</sup>, and the *in vitro-in vivo* correlation was obtained by the Pearson correlation coefficient. Significance was determined by the Student t-test.

## **RESULTS AND DISCUSSION**

At first, the percentual activity of the samples was determined and the obtained results were: 109,60% for laboratory A; 92,23% for laboratory B; and 102,3% for laboratory C.

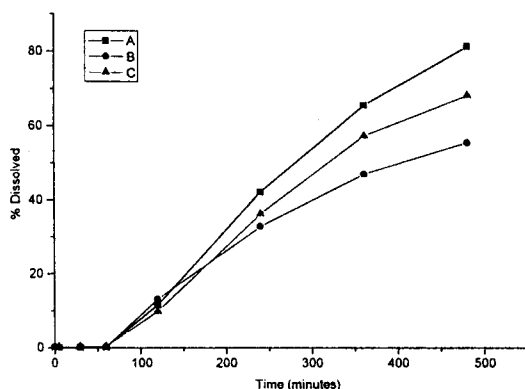
Lotus 123 software was used to determine the area under the dissolution curve for the tablets from the three laboratories according to the Khan <sup>7</sup> method, who determined dissolution efficiency (DE%) using the following equation:

$$DE\% = \frac{(\text{Area under the curve of \% dissolved up to time } t) \times 100}{100\% \times \text{sampling time } t}$$

Differences in the release of the active substance *in vitro* can be determined by calculating dissolution efficiency as proposed by Khan <sup>7</sup> (Table 1).

| Laboratory           | DE% in water       | DE% in 0.1 N HCl   | DE% in pH 7.5 phosphate buffer |
|----------------------|--------------------|--------------------|--------------------------------|
|                      | Means $\pm$ SD     | Means $\pm$ SD     | Means $\pm$ SD                 |
| <b>Lab. A (n=11)</b> | 0.0190 $\pm$ 0.024 | 0.0540 $\pm$ 0.020 | 48.236 $\pm$ 6.37              |
| <b>Lab. B (n=16)</b> | 0.0280 $\pm$ 0.012 | 0.1720 $\pm$ 0.134 | 28.9011 $\pm$ 11.17            |
| <b>Lab. C (n=14)</b> | 0.1930 $\pm$ 0.097 | 0.1260 $\pm$ 0.053 | 40.706 $\pm$ 6.17              |

**Table 1.** Mean  $\pm$  SD values of Dissolving Efficiency (DE%) in the dissolving fluids of coated erythromycin stearate tablets from laboratories A, B and C. The times were: Water- 5 min, 0.1N HCl- 60 min, pH 7.5 phosphate buffer- 480 min.



**Figure 1.** Dissolution patterns of coated erythromycin stearate tablets from laboratories A (■), B (●) and C (▲) in water, 0.1 N hydrochloric acid, and sodium phosphate buffer, pH 7.5. The results are reported as means.

The present study on the dissolving rate of coated erythromycin stearate tablets was carried out in a flow cell apparatus, which had the advantage of maintaining a constant and non-turbulent flow and of using a system that permitted constant fluid renewal <sup>8</sup>. The results (Fig. 1) showed a similar dissolution pattern for the samples from the three laboratories, despite the different amount of active substance released during the experiment. Formulation A released 81.1% of the drug, while formulation B released 55.33%, with higher percent dissolution in water and 0.1 N hydrochloric acid compared to the other formulations. Formulation C released 68.11% of the active substance. The ab-

sence of full erythromycin release led us to measure the residue present in the flow cell, in which the remaining percentage was detected.

This method presents some advantages such as facilitating comparison of a large number of formulations and theoretically permitting comparison with *in vivo* data as long as these are evaluated in the same manner as *in vitro* data <sup>9</sup>.

When submitted to the normality and homocedasticity tests, DE% data in water and hydrochloric acid were found not to be homogeneous and not to present normal distribution. DE% values using 0.1 N hydrochloric acid as dissolution fluid were evaluated by a nonparametric test (Kruskal-Wallis) which detected significant differences among tablets from laboratory A and tablets from the other two laboratories in which did not differ from each other. Tablets from laboratory A released less active substance into 0.1 N hydrochloric acid, suggesting that the employed coating, called cellulose acetophthalate, was efficient as a coating material.

According to Prista <sup>10</sup>, this is one of the most resistant coatings and its dissolution occurs by enzymatic hydrolysis in the intestine, regardless of the pH of the medium. Gennaro <sup>8</sup> disagrees with this statement since the enzymatic decomposition of a film is very slow with the coating dissolving at pH higher than 6.0.

DE% data for sodium phosphate buffer, pH 7.5, were considered normal and the parametric analysis of variance revealed significant differences for the three laboratories. According to Tuckey test, the result of laboratory A was statistically different ( $p < 0,05$ ) from laboratory B, but not from laboratory C. The tablets from laboratory B released the active substance at a significantly slower rate. This may be explained by the use of Eudragit E<sup>R</sup>, a material that is appropriate for dissolution in acid medium but it is insoluble in sodium phosphate buffer, impairing disaggregation of the nucleus and release of the active substance into this medium.

Serum erythromycin concentration was determined by the microbiological method of agar diffusion for all samples, using the adjusted standard curve:

$$y = -3.1036 + 0.1418x$$

$$r = 0.9969.$$

The study of *in vivo* availability of the active substance consisted in the administration of a single oral dose of the tablets to rabbits. The choice of rabbits as a biological model was due to the easy handling of this animal <sup>11</sup>, which has been frequently used in research <sup>12,13</sup>.

The microbiological method used in the present study is still extensively used in studies of bioavailability despite the recent technological advances and the development of easier, more rapid and sophisticated methods for quantification <sup>14,15,16</sup>. The method proved to be adequate in terms of sensitivity and reproducibility of the results. According to Stubbs *et al.* <sup>17</sup>, serum samples to be tested by this method can be stored at temperatures of 4 °C or -15 °C for 12 weeks.

The serum concentrations observed presented a wide dispersal among individuals which may be caused by biological variation, manufacturing technology, type of coating used, and the wide variability inter- and intra-individual in absorption reported in previous studies on erythromycin <sup>18-20</sup>. The pharmaceutical form itself (coated tablet) represents an additional barrier in the release of the active substance and the differences in release time directly affect the variability of the results. Due to the protective coating against acid pH, the release of larger amounts of the active substance in the intestine abruptly increases its concentration in blood, characterizing a normal curve. Fig. 2 presents the mean serum concentration curves for the three laboratories.

The pharmacokinetic parameters are presented on Table 2: area under the serum concentration curve (AUC), which is the most important parameter and represents the amount of active substance absorbed, maximum concentration peak ( $C_{max}$ ), which indicates the intensity of action, time of maximum concentration peak ( $t_{max}$ ), which indicates the rate of absorption of the active substance, elimination constant ( $K_e$ ), mean life ( $t_{1/2}$ ) and mean residence time (MRT).

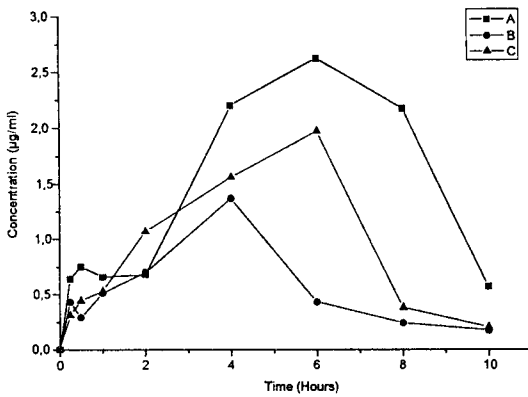
Parametric analysis of variance (ANOVA), utilized for the comparison of data of normal distribution data, is one of the methods for the determination of bioequivalence since it permits the comparison among subjects and among treatments and it evaluates the order of administration of the treatment and the residue <sup>2</sup>.

$AUC_{0-10}$  and  $C_{max}$  were submitted to the Lilliefors test for normality and to

|                               | LAB A<br>Means ± SD | LAB B<br>Means ± SD | LAB C<br>Means ± SD |
|-------------------------------|---------------------|---------------------|---------------------|
| Ke (h <sup>-1</sup> )         | 0,326 ± 0,169       | 0,292 ± 0,171       | 0,415 ± 0,301       |
| t <sub>1/2</sub> (h)          | 2,663 ± 1,308       | 3,398 ± 2,360       | 2,402 ± 1,373       |
| AUC <sub>0-∞</sub> ( µg.h/ml) | 20,042 ± 10,857     | 6,792 ± 3,265       | 10,908 ± 6,782      |
| AUC <sub>0-10</sub> (µg.h/ml) | 17,075 ± 9,275      | 5,917 ± 3,044       | 10,267 ± 6,387      |
| C <sub>max</sub> (µg/ml)      | 4,855 ± 2,890       | 1,648 ± 1,026       | 3,200 ± 6,782       |
| t <sub>max</sub> (h)          | 4,438 ± 2,500       | 3,125 ± 2,173       | 3,458 ± 1,751       |
| MRT (h)                       | 6,549 ± 2,380       | 6,167 ± 2,841       | 5,141 ± 0,963       |

The results are reported as means ± SD of the pharmacokinetic parameters obtained with 12 rabbits.

**Table 2.** Mean ± SD pharmacokinetic parameters obtained after oral administration of coated 250 mg erythromycin stearate tablets for laboratories A, B, and C.



**Figure 2.** Serum erythromycin concentration curves obtained after oral administration of coated 250 mg erythromycin stearate tablets for laboratories A (■), B (●) and C (▲) in 12 rabbits. The results are reported as means.

the Bartlett and Cochran test for homocedasticity and then transformed into logarithmic expressions. After normalization, data were compared by the parametric analysis of variance ( $p < 0.05$ ), which showed significant differences in AUC<sub>0-10</sub> and C<sub>max</sub> among the three laboratories. The Tuckey test focused these differences, with laboratory A differing statistically from B but not from C.

The t<sub>max</sub> data were found to be normal when tested for normality and homocedasticity. Comparison of the means by non-parametric analysis of variance showed no significant differences among laboratories ( $p < 0.05$ ).

The half life of erythromycin in humans ranges from 1.4 to 1.6 h, but in rabbits it increased to 2.7 h, probably due to a lower absorption and elimination rate tested on this method. However, the half life of erythromycin is known to be 1.6 h and an week intermission is believed to be sufficient to evaluate the bioavailability of this active substance<sup>14,19,22</sup>.

After the administration of an oral bolus dose of drug, a large number of drug molecules was distributed throughout the body. The mean residence time (MRT) describes the average time for all drug molecules to reside in the body<sup>23,24</sup>.

The parameters Ke, t<sub>(1/2)</sub> and MRT did not present differences statistically significant among them.

The experimental design employed did not permit the detection of the effect of time of administration, which evaluated some environmental exchange, fatigue or the residual effect of another medication.

Bioequivalence can also be evaluated by determining the confidence interval<sup>25,26</sup>. According to FDA (USA = Food and Drug Administration), the maximum acceptable limit of difference in bioavailability is 20%. Products are considered to be

bioequivalent when no significant differences in  $ASC_{0-10}$ ,  $C_{max}$  or  $t_{max}$  are detected among them.

On the basis of the present results, we conclude that the coated erythromycin stearate tablets from laboratories A and C showed similar bioavailability in rabbits while the results from laboratory B were statistically different from laboratories A and C with respect to the amount of active substance absorbed.

### ***In vitro-in vivo correlation***

The percentage of absorbed erythromycin was calculated by the equation of Wagner & Nelson <sup>27</sup>.

The *in vitro-in vivo* correlation was obtained by comparing the mean dissolved fraction to the absorbed fraction at times of 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 h. The significance of the *r* value present in Table 3 was tested by the Student *t*-test.

The *in vivo-in vitro* correlation is defined as the relationship among biological properties, or between some parameters derived from a pharmaceutical form and from a physicochemical characteristic of this same formulation <sup>28</sup>. This correlation is determined in order to predict the *in vivo* behavior of a pharmaceutical formulation, so that the *in vitro* test may be applied to each lot of a product of the same formulation <sup>29,30</sup>. Stawchansky *et al.* <sup>31</sup> demonstrated a significant *in vivo-in vitro* correlation when they tested 5 formulations of coated 250 mg erythromycin stearate tablets, confirming the feasibility by using the *in vitro* test under experimental conditions as a quality control for the *in vivo* behavior.

We determined the correlation of the absorbed-dissolved fraction for the three laboratories using the Pearson correlation coefficient (*r*), as well as the equation of the regression line. The significance of the *r* values was determined by the Student *t*-test, which showed on Table 3 a significant correlation for all three laboratories, although the correlation was stronger for laboratory A. The *in vitro-in vivo* correlation was lower but still significant for laboratory B, being this result mainly due to the dissolved fraction, which was significantly lower for this laboratory.

The investigation of the bioavailability of an active substance in animals, although it does not reflect the results obtained in humans, may be considered as a method for predicting its behavior and for verifying its quality. The importance of these studies in the development and orientation of pharmaceutical research is incontestable, with *in vitro* and *in vivo* tests being indispensable to evaluate and compare the therapeutic efficiency of the formulations.

| Laboratory | <i>r</i> | $T_{calc}$          |
|------------|----------|---------------------|
| A          | 0.9969   | 25.321 <sup>a</sup> |
| B          | 0.8883   | 3.869 <sup>a</sup>  |
| C          | 0.9593   | 6.386 <sup>a</sup>  |

<sup>a</sup>*p* < 0.05

**Table 3.** Correlation coefficient (*r*) and *t*<sub>calc</sub> values for laboratories A, B, and C.

## REFERENCES

1. Washington II, J. A.(1985) Eritromicina Revisão. Perspectiva microbiológica e clínica após 30 anos de utilização terapêutica. *Separata de Mayo Clin. Proc.* **60**: 189-203
2. Sande, N. A. & Mandell, G.L.(1987) Drogas Antimicrobianas. In: Gilman, A.G.; Goodman, L.S.; Rall, T.W.; Murad, F. (eds). Goodman e Gilman. *As Bases Farmacológicas da Terapêutica*. 7ª ed. Rio de Janeiro: Guanabara-Koogan, cap. 52, pp. 767-85
3. Posti, J. & M. Salonen (1983) *Int. J. Pharm.* **17**: 225-35
4. Yakatan,G.J., W.J. Poynor, R.G. Harris, A. Martin, R.G. Leonard, A.H. Briggs & J.T. Do Luisio (1979) *J. Pharmacokin. Biopharm.* **7**: 355-69
5. Steel, R.G.D. & J.H. Torrie (1980) *Principles and Procedures of Statistics*. 2nd ed. New York, McGraw-Hill
6. Pabst, G. & H. Jaeger (1990) *Eur. J. Clin. Pharmacol.* **38**: 5-10
7. Khan, K.A.(1975) *J. Pharm. Pharmacol.* **27**: 48-9
8. Gennaro, A.R. (1985)(Ed.) *Remington's Pharmaceutical Sciences*. 17th ed. Mack, Easton
9. Vaughan, D.P. & G.T.Tucker (1976) *J. Pharm. Pharmacol.* **28**: 932-3
10. Prista, L.N., Correia Alves. & R.M.R. Morgado (1981) *Técnica Farmacêutica e Farmácia Galênica*. 3ª ed. Lisboa: Fundação Calouste Gulbenkian, V. 1
11. Maeda, T., H. Takenaka, Y. Yamahira & T. Noguchi (1977) *J. Pharm. Sci.* **66**: 69-73
12. Holland, D.R. & J.F Quay (1976) *J. Pharm. Sci.* **65**: 417-9
13. Hermann, T.W., F.K. Gtowka & E.R. Garret (1993) *J. Pharm. Sci.* **82**: 1102-11
14. Malmborg, A.S.(1980) *Curr. Ther. Res.* **27**: 733-40
15. Houin, G, J.P. Tillement, F. Lhoste, N. Rapin, C.J. Soussy & J. Duval(1980) *J. Clin. Med. Res.* **8**: 9-14
16. Prescott, J.E., D.J. Hoover & J.R. Dohoo (1983) *J.Vet. Pharmacol. Therap.* **6**: 67-73
17. Stutbs, C., J.M. Haigh & I. Kanfer (1987 ) *J. Liq. Chrom.* **10**: 2547-57
18. Bell, S.M. (1971) *Med. J. Aust.* **2**: 1280-3
19. Saarni, H., J. Kanto & H. Allonen (1979) *Ann. Clin. Res.* **11**: 196-8
20. Yakatan, G.J.,W.J. Poynor, S.A. Breeding, C.E. Lankford, S.V. Righe, A.N. Martin & J.T. Doluisio (1980) *J. Clin.Pharmacol.* **20**: 625-38
21. Vila Jato, J.L. & J.J.Torres (1989) *Rev. Asoc. Esp. Farm. Hosp.* **13**: 63-71
22. Griffith, R.S. & H.R.Black (1964) *Am. J. Med. Sci.* **274**: 69-74
23. Shareel, H. & Andrew B.C. Yu (1992) *Applied Biopharmaceutics and Pharmacokinetics*. 3rd ed. Norwalk- Connecticut. Appleton & Lange
24. Gai H., M.N. & A. Arancibia O. (1992) *Acta Farm Bonaerense* **11**: 7-12
25. Westlake, W.J. (1972) *J. Pharm. Sci.* **61**: 1340-1
26. Schuirmann, D.J. (1987) *J. Pharmacokin. Biopharm.* **15**: 657-80
27. Wagner, J.G.,E. Nelson (1964) *J. Pharm. Sci.* **53**: 1392-403
28. Lieberman, H.A. & Lachman, L.(1990) (Ed.) *Pharmaceutical Dosage Forms: Tablets*. New York: Marcel Dekker, v. 2
29. Smolen, V. F. & W.A. Weigand (1976) *J. Pharm. Sci.* **65**: 1718-23
30. Cartwright, A.C. (1984) *Pharm. Int.* **5**: 196-8
31. Stavchansky, S., J.T. Doluisio, A. Martin, C. Martin, B. Cabàna, S. Dighe & A. Loper (1980) *J. Pharm. Sci.* **69**: 1309-10