

## EVALUATION OF HARD GELATIN CAPSULES AND HYDROXYPROPYL METHYLCELLULOSE CONTAINING AMPICILLIN

Graziella Gonçalves Weigert, Aniéle Posser Ineu e Patrícia Gomes\*

Curso de Farmácia, Centro Universitário Franciscano, Rua dos Andradas, 1614, 97010-032 Santa Maria - RS, Brasil

Recebido em 23/1/11; aceito em 1/7/11; publicado na web em 9/8/11

This study aims to develop and evaluate formulations containing ampicillin in capsules of gelatin and hydroxypropyl methylcellulose (HPMC). Two formulations (A and B) were developed. The final product quality was evaluated by testing for quality control and the results were in agreement with the Brazilian Pharmacopoeia. The formulations with HPMC capsules showed lower percentages of drug dissolved (99.67%, HPMC-A and 87.70%, HPMC-B) than the gelatin (100.18%, GEL-A and 101.16% GEL-B). Because of the delay of the ampicillin release observed in the dissolution profiles, it becomes necessary to evaluate the drugs that can be conditioned in the HPMC capsules.

Keywords: ampicillin; gelatin; hydroxypropyl methylcellulose.

## INTRODUCTION

Capsules are solid pharmaceutical forms usually destined to the oral use that present a good acceptance for part of the population.<sup>1-3</sup>

The involucres used for the development of capsules are usually constituted by gelatin, water, coloring and other materials including preservatives and processing aids. They are considered one of the best ways to condition drug substances because they protect them from the light, air and humidity action. The gelatin used in capsules is justifiable because it is a nontoxic substance, widely used in food, and it is readily soluble in biological fluids at room temperature.<sup>4,5</sup> Also, for being a protein, the gelatin is digested and absorbed. It is soluble in hot water and in the gastric liquid, which quickly liberates its content.<sup>3</sup>

However, main Pharmacopoeias, such as the European, Japanese and American also allow the use of other appropriate materials besides gelatin. As gelatin has a high humidity degree (13 to 16%), hard capsules have been manufactured using a material of vegetable origin, the hydroxypropyl methylcellulose (HPMC) with the main objective of producing involucres with smaller humidity tenor (3 to 8%).<sup>6</sup> The introduction of HPMC based capsules have appeared as an alternative to the conventional use of the hard gelatin capsules. Thus, the problems involving hygroscopic drugs, sensitive to the humidity and with problems in their interaction with the gelatin molecules can be circumvented.<sup>6,7</sup>

The capsules produced with HPMC involucres guarantee the hygroscopic drugs stability, such as ampicillin, a bactericidal antibiotic of wide spectrum that acts against aerobic gram-negative bacteria.<sup>8,9</sup> In the case of ampicillin, it is possible to foresee some possible degradation reactions because the ring beta-lactam is susceptible to hydrolysis (Figure 1).<sup>10,11</sup>

Hard gelatin and HPMC involucres have different compositions

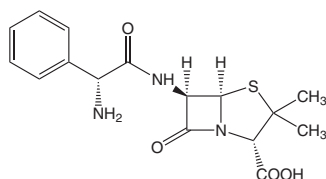


Figure 1. Chemical structure of the ampicillin

therefore it is necessary to study the development of formulations involving these involucres as well as the evaluation of the final product quality. The quality control consists in an indispensable stage of the process for medicine manufacture, regardless of its production scale. The capsules should meet the demands of weight variation, disintegration time, assay and tenor uniformity of actives described in the monograph.<sup>12,13</sup> Therefore, the aim of this study was to develop capsules starting from hard gelatin and HPMC involucres, to evaluate the quality of the final products and to compare them with each other and with the medicine reference.

## EXPERIMENTAL

## Samples and reference standard

Four formulations were developed containing 500 mg of ampicillin in hard gelatin capsules (GEL) and HPMC capsules. These capsules were purchased from market (Genix and Capsugel, Brazil), presenting humidity values of 13.8% (GEL) and 5.8% (HPMC), according to the certificate of analysis.

The formulations have been differentiated according to their content, in formulation A (ampicillin trihydrate, magnesium stearate, colloidal silicon dioxide and croscarmellose sodium) and formulation B (only ampicillin trihydrate), being denominated GEL-A, GEL-B, HPMC-A and HPMC-B. The excipients contained in the pharmaceutical dosage form were all of pharmaceutical grades and acquired from different distributors. The medicine reference Amplacilina® (Eurofarma, Brazil) was also employed in this study. Ampicillin anhydrous reference standard (98.35%) was obtained from Brazilian Pharmacopoeia (Brazil).

## Powder properties

## Granulometric analysis

The determination of the granulometric strip was done mechanically using an agitator of sieves (Bertel, Brazil). This equipment has round sieves for particle size analysis with 8.5 or 3 inches in diameter. A portion of 50 g of ampicillin for the development of the formulations was put in the sieve and submitted to sieving for 20 min. After the method application, calculations were performed to determine the granulometric strip of the ampicillin.<sup>5</sup>

\*e-mail: patriciagomes0@yahoo.com.br

### Density

The ampicillin used for the formulations development was weighed and the test was performed either to a specific number of taps or until the volume measured in the graduated glass cylinder changes by less than 2%, using tapped density or apparent volume tester (Pharma Test PT-TD1, Germany). The standard operational parameters call for a drop rate of 250 strokes per min with a drop height 3.0 mm.

### Angle of repose

The angle of repose determination was performed using the methodology proposed by Gil<sup>14</sup> employing automated powder tester (Pharma Test PTG-2, Germany). This instrument is suitable for testing powder Flow Time, the measurement of the cone angle (angle of repose) of the collected powder mound, measuring the weight, calculating the density and the volume of the powder cone as well as the EP/USP "Flowability" results which is to measure the flow time of 100 g of sample through a specified pouring nozzle. This test was performed for the powder mixture formulation for the development of hard gelatin and HPMC capsules.

## Quality control for pharmaceutical dosage form

### Determination of the mean weight

The determination of the weight of the capsules containing 500 mg of ampicillin was performed according to the Brazilian Pharmacopoeia.<sup>15</sup> To perform this test, we used 20 capsules of each manipulated formulation (A and B) and the reference product.

### Disintegration test

The disintegration test was carried out following the established methodology for Brazilian Pharmacopoeia<sup>15</sup> using the disintegration tester (Pharma Test PTZ-E, Germany). The time limit established for this test for hard gelatin, HPMC and the reference drug capsules was 45 min.<sup>15</sup>

### Assay

The assay of the examined formulations (A and B) and the reference medicine was performed through iodometric method. This trial was conducted in triplicate following the specific method described in the Brazilian Pharmacopoeia.<sup>16</sup> Standard solution was used at a concentration of 1.25 mg mL<sup>-1</sup>. The samples were diluted to concentrations similar to the pattern. The titration was performed with volumetric solution of 0.01 M sodium thiosulfate, and starch was used as indicator solution. The titration was performed until the disappearance of the blue color.

### Water determination test

The determination of water content was conducted by Karl-Fischer method using the capsules content (Reference, GEL-A, GEL-B, HPMC-A and HPMC-B).<sup>15</sup> A Karl-Fischer autotitration unit (Mettler, Brazil) was set up according to the manufacturer's instructions.

### Test and dissolution profiles

Dissolution studies were performed according to the monograph of the pharmaceutical dosage form contained in the Brazilian Pharmacopoeia.<sup>16</sup> Basket apparatus was used in the dissolution equipment (Pharma Test PTWS-3E, Germany). The dissolution medium consisted of 900 mL of distilled and degassed water, and was kept at 37 °C with speed of 100 rpm. The collection time for the dissolution test consisted of 45 min. For the dissolution profiles were used five time points: 10, 15, 20, 30, 45 and 60 min, with subsequent replacement of the dissolution medium. Six units of each formulation were subjected

to dissolution test and twelve to the dissolution profile. After testing, aliquots of 10 mL were removed from the dissolution medium, filtered and diluted in cuprum sulfate buffer to appropriate concentration (22 µg mL<sup>-1</sup>). Aliquots were transferred to the test tube with lid, heated in water bath at 75 °C for 30 min and cooled rapidly. The amount of dissolved ampicillin was determined by using a spectrophotometer UV/VIS (Shimadzu UV1650PC, Japan) and detection at a wavelength of 320 nm. To calculate the amount of ampicillin indeed dissolved in the medium, it was compared to that obtained with the ampicillin reference standard (RS) at concentration of 0.0022% (w/v) prepared under the same conditions.

### Preparation of the analytical curve

The analytical curve was prepared from a standard solution of ampicillin RS concentration of 100 µg mL<sup>-1</sup>. The linear range comprised 2-27 µg mL<sup>-1</sup> for the dissolution profiles and 16-28 µg mL<sup>-1</sup> for the dissolution test. The procedure performed in this technique is described in Test and dissolution profiles. Then, the data were treated statistically by one-way analysis of variance (ANOVA) using Graph PadPrism software (version 4.0, California, USA).

## Statistical analysis of the dissolution profiles

For the statistical analysis of data obtained with the dissolution profiles, a comparative method was used between them and the efficiency of dissolution (ED%). The ED% was calculated from the percentage curves of drug dissolved versus time. The ED% was calculated by the ratio between the area under the curve (AUC) and total area of the graph was expressed in percentage.<sup>17</sup> The graphs were obtained by Graph PadPrism software and ED% values were compared by one-way ANOVA and accomplished by Tukey post-test.

## RESULTS AND DISCUSSION

### Powder properties

#### Granulometric analysis

The granulometry found in the test for the ampicillin was superior to 600 µm. However, there are no literature values for this drug granulometry. The granulometric analysis of the ampicillin for the development of the capsules is an important parameter to be established; it represents a direct influence on the manipulation of the capsules in magisterial scale. The granulometry above 600 µm found for the ampicillin trihydrate involved a complex manipulation of the drug in magisterial scale, making the accommodation of the powders in the manual encapsulator.

#### Density

The choice of the appropriate size of the capsule for the drug to be produced is performed according to the density parameters of the powder to be encapsulated and the volume of possible capsules to be used. The high density found for the ampicillin (0.5557 g cm<sup>-3</sup>) provided the choice of capsule number 00 (0.95 mL). We opted for the addition of some excipients in the formulation A, since the amount of the present drug in the formulation is not always enough to make up the chosen capsule as well as due to other characteristics of the drug.

#### Angle of repose

After the completion of the angle of repose, we observed that the mixing of powders and the ampicillin alone have an angle greater than 40°, which characterizes a very weak flow.<sup>5</sup> This characteristic observed for the mixture of powders destined to the production of

the capsules shows that even after the addition of a small amount of excipients to the formulation A, no improvement in the flow of powders was observed. This fact associated with features of ampicillin, such as increased size, density, and hygroscopic end up making the manipulation difficult because of the resistance of the flow of powders in manual encapsulator and difficulty of accommodation in the same involucres.

### Quality control for pharmaceutical dosage form

The results of mean weight, assay, disintegration time, and water determination test for the hard gelatin capsules (A and B), HPMC capsules (A and B) and reference medicine is evidenced in Table 1. These results obtained are in agreement with the pharmacopoeia specifications<sup>15,16</sup> and they show a proper process of manipulation. In the manipulated capsules, it could be observed that the formulation containing only the drug (formulation B) presented lower values of mean weight (579.6 and 584.88 mg for GEL-B and HPMC-B, respectively), while the capsules containing excipients (formulation A) showed higher values (624.6 mg, GEL-A and 619.72 mg, HPMC-A). The reference medicine showed values of mean weight similar to the capsules with the formulation A (612.90 mg), which denotes the quality of the product elaborated in magisterial scale. The values of relative standard deviation (RSD) of mean weight were 1.79% (reference medicine), 2.60% (GEL-A), 2.10% (GEL-B), 2.61% (HPMC-A) and 2.19% (HPMC-B). The disintegration time of capsules with HPMC involucres (7 min, HPMC-A and 6 min, HPMC-B) was higher than the capsules elaborated with gelatin involucres (5 min for both formulations); this fact can be explained due to the nature of the involucres. HPMC is a cellulosic derived that is moisturized quickly, but swells and takes longer to disintegrate in body temperature; it is also more soluble in lower temperatures, such as 10 °C.<sup>18,19</sup> The gelatin is a soluble protein in hot water and in the gastric liquid, where it quickly releases its contents soluble in biological fluids at a room temperature.<sup>3</sup> Capsules of the reference medicine showed higher values for the test compared the disintegration of gelatin capsules, probably due to compression existing in the process of industrial scale production. The values obtained in this test demonstrate that the excipients added to the formulation A and the ones in the reference medicine did not aid in the disintegration process, when compared to the formulation containing only the ampicillin (formulation B). The assay values presented that ranged from 90.06 to 114.62% for HPMC-B and GEL-B, respectively. The water determination test showed lower humidity tenors for HPMC capsules than gelatin capsules. The fact is relevant because the ampicillin is susceptible to hydrolysis.<sup>10,11</sup>

**Table 1.** Results obtained in the tests of hard gelatin and HPMC capsules and reference medicine

Parameters	Reference	GEL-A	GEL-B	HPMC-A	HPMC-B
Mean weight (mg) <sup>a</sup>	612.90	624.6	579.6	619.72	584.88
Assay (%) <sup>b</sup>	95.91	107.02	114.62	91.23	90.06
Disintegration (min) <sup>c</sup>	7.0	5.0	5.0	7.0	6.0
Water determination	3.8	13.4	13.3	12.1	12.8

<sup>a</sup>n = 20, <sup>b</sup>n = 3, <sup>c</sup>n = 6

The analytical curves developed for the test and dissolution profiles presented a determination coefficient ( $R^2$ ) of 0.9969 and 0.9988, respectively. None presented linearity deviation. Regarding the dissolution test (Table 2) the hard capsules produced with gelatin

showed higher percentage values of dissolution (100.18 and 101.16% for GEL-A and GEL-B, respectively) when compared to the capsules produced with HPMC (99.67 and 87.70% for HPMC-A and HPMC-B, respectively). This fact can be explained because HPMC is a forming polymer of hydrophilic matrix used to promote a slower release of the drug conditioned in the capsules.<sup>20-23</sup> The value found for the reference medicine (90.47%) resemble the values obtained for the capsules elaborated with HPMC, although this product is made from hard gelatin capsule. This may be related to the present excipients in the reference medicine (lactose, methylcellulose, stearic acid and magnesium stearate), which are different from the excipients used in the B formulation. Furthermore, the compaction force used in the industry for the development of the reference may be delaying the drug release in dissolution medium. When the gelatin capsule is ingested, it allows the penetration of water, causing its hydration and drug release in a few min. By using a forming polymer of colloidal matrix in encapsulated excipient, the dissolution of the capsule is affected promoting a controlled release.<sup>20</sup>

**Table 2.** The dissolution tests for the hard gelatin and HPMC capsules and reference medicine

Sample	Reference (%)	GEL-A (%)	GEL-B (%)	HPMC-A (%)	HPMC-B (%)
1	88.19	99.57	111.32	100.82	92.95
2	89.44	100.57	99.45	96.07	83.82
3	89.82	96.32	102.82	97.57	82.69
4	88.45	101.82	96.07	106.32	88.45
5	89.82	100.07	100.70	100.57	89.70
6	97.07	102.70	96.57	96.70	88.57
Average	90.47	100.18	101.15	99.67	87.70
RSD	3.66	2.21	5.52	3.82	4.35

Several studies suggest the use of HPMC in formulations in order to promote modified releases of drugs. The choice of hydrophilic polymer in the matrix formulation can provide an appropriate combination of the swelling mechanisms, dissolution or erosion and determine the release kinetics in vitro.<sup>21</sup> According to previous work,<sup>22</sup> the drug incorporation in hydrophilic matrix systems is the most used method to prolong drug release dosage forms for oral use. It was observed that with increasing amounts of HPMC in the formulations it is possible to get a reduced release of the drug, which can be evidenced in the dissolution tests.<sup>23</sup> With the use of HPMC in capsules, this polymer characteristic may promote a release different from the one observed with the capsules produced with gelatin, delaying the release of the drug and consequently modifying its kinetics of release. This fact can be explained once the hydrophilic matrix, when in contact with the dissolution medium, swell and form a gelled layer that controls the subsequent entrance of water into the matrix and drug release, prolonging its release.<sup>21-23</sup> The speed of water penetration in the matrix system determines the mode of drug release. In high concentrations, the linear chains of HPMC form a tangle and result in a gelatinous layer, fairly consistent, hindering the release of the active principle. At very low concentrations, these cellulose gels have very low viscosity, allowing almost immediate release of drugs.<sup>21</sup>

The average values of ED% are shown in Table 3 and the graphical representation is shown in Figure 2.

The dissolution efficiency of the five samples (reference, GEL-A, GEL-B, HPMC-A and HPMC-B) showed significant differences among groups ( $P > 0.05$ ) in ANOVA. The Tukey test indicated the differences among the capsules in study. There was no significant

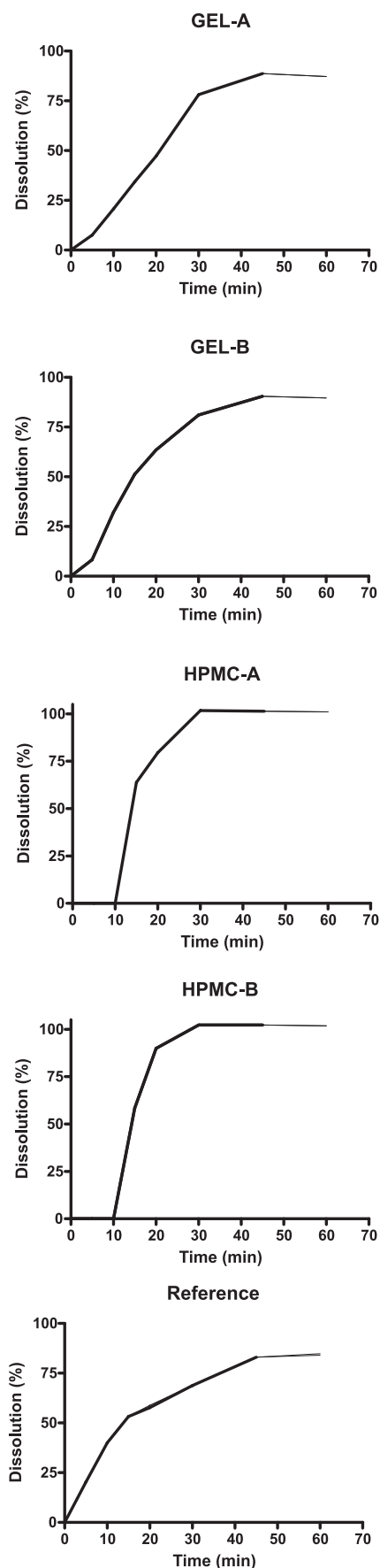


Figure 2. Graphic representation of the dissolution efficiencies of the capsules GEL-A, GEL-B, HPMC-A, HPMC-B and reference medicine

Table 3. Parameters of the dissolution efficiency for the capsules containing ampicillin trihydrate

Sample	Dissolution efficiency (%)*
Reference	62.37
GEL-A	60.97
GEL-B	66.78
HPMC-A	74.92
HPMC-B	76.18

\* n = 12

difference ( $P > 0.05$ ) in the ED% from the capsules GEL-B (66.78%), GEL-A (60.97%) and the reference medicine (62.37%) as well as between HPMC-A (74.92%) and HPMC-B (76.18%). This fact can be explained because in both comparative cases (GEL versus reference medicine and HPMC-A and HPM-B) the involucres have the same nature. In the first case, the three involucres are made of hard gelatin and in the second case they are made of HPMC. Furthermore, the  $f_2$  factor indicated the similarities between the dissolution profiles of the reference medicine and GEL-A (99.95%) and GEL-B (99.86%). This comparison cannot be performed with the reference medicine and HPMC capsules, because they are of different constitution. Although the formulations have different constituents, the excipient present in the formulation A and the reference medicine do not seem to interfere in the dissolution efficiency of the capsules in analysis. Other tested formulations showed significant differences ( $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ ), which can be explained mainly by the difference in the nature of the involucres that directly influence the dissolution efficiency.

## CONCLUSION

The results of this study suggest that differences exist between the hard gelatin and HPMC involucres used in the development of capsules containing ampicillin 500 mg, mainly in relation to disintegration, and dissolution tests. Because of the delay of the drug release in initial times of the dissolution profiles, it becomes necessary to evaluate the drugs that can be conditioned in the HPMC capsules.

In the case of drugs which need a quick release, the use of HPMC capsules must be evaluated with caution so that less damage in the therapeutic action of the medicine be observed. In the case of the ampicillin, whose plasmatic pick is reached in 2 h, a concern is not observed regarding the use of the HPMC capsule for this drug. Furthermore, by having hygroscopic characteristics, ampicillin requires greater protection against humidity and the HPMC is a capsule with low humidity tenor when compared to the gelatin, which ensures the stability of drugs with this characteristic. Stability studies are necessary to evaluate the real contribution of the hard gelatin and HPMC capsules relation to the protection of drugs conditioned in these capsules. These are important factors that must be taken into account so that the use of HPMC capsules be a viable alternative to gelatin in manipulation pharmacy and in the pharmaceutical industry for developing formulations containing ampicillin.

## REFERENCES

- Navascués, I.; Hernández, F.; *Panace* **2003**, *4*, 239.
- Gianotto, E. A. S.; Machado, H. T.; Miglioranza, B.; Fregonezi-Nery, M. M.; *Lat. Am. J. Pharm.* **2008**, *27*, 727.
- Azevedo, R. C.; Ribeiro, G. P.; Araújo, M. B.; *Rev. Bras. Cienc. Farm.* **2008**, *44*, 2628.
- Gullapalli, R. P.; *J. Pharm. Sci.* **2010**, *99*, 4107.

5. Ogura, T.; Matsuura, S.; Futuya, Y.; *Pharm. Technol. European* **1998**, *10*, 32.
6. Ku, M. S.; Li, W.; Dulin, W.; Donahue, F.; Cade, D.; Benameur, H.; Hutchison, K.; *Int. J. Pharm.* **2010**, *386*, 30.
7. Al-Tabakha, M. M.; *J. Pharm. Pharmaceut. Sci.* **2010**, *13*, 428.
8. Korolkovas, A.; França, F. F. A. C.; *Dicionário terapêutico Guanabara*, Guanabara Koogan: Rio de Janeiro, 2008.
9. Gennaro, A. R.; *A ciência e a prática farmacêutica*, 20ª ed., Guanabara Koogan: Rio de Janeiro, 2004.
10. Raj, T. J. S.; Bharati, C. H.; Ranga, K.; Rao, K. R.; *J Pharm. Biomed.* **2007**, *43*, 1470.
11. Connors, K. A.; Amidon, G. L.; Stella, V. J.; *Chemical stability of pharmaceuticals: a handbook for pharmacists*, 2<sup>nd</sup> ed., John Wiley and Sons: New York, 1986.
12. ANVISA - Agência Nacional de Vigilância Sanitária; *Farmacopéia Brasileira: Formulário Nacional*, Editora MS: Brasília, 2005.
13. Bonfilio, R.; Mendonça, T. F.; Pereira, G. R.; Araújo, M. B.; Tarley, C. R. T.; *Quim. Nova* **2010**, *33*, 377.
14. Gil, E. S.; Brandão, A. L. A.; *Excipientes: suas aplicações e controle físico-químico*, 2ª ed., Pharmabooks: São Paulo, 2007.
15. *Farmacopéia Brasileira*, 5ª ed., Atheneu: Brasília, 2010, vol.1.
16. Em ref. 15, vol. 2.
17. Khan, K. A.; *J. Pharm. Pharmacol.* **1975**, *27*, 9.
18. Fahs, A.; Brogly, M.; Bistac, S.; Schmitt, M.; *Carbohydr. Polym.* **2010**, *80*, 105.
19. Chiewele, I.; *Chem. Pharm. Bull.* **2000**, *48*, 95.
20. Ferreira, A. O.; *Guia Prático da Farmácia Magistral*, Pharmabooks: São Paulo, 2008.
21. Kamel, S.; Ali, N.; Jahangir, K.; Shah, S. M.; El-Gendy, A. A.; *Express Polymer Letters* **2008**, *11*, 758.
22. Fortunato, K. A.; Doile, M. M.; Shumücker, I. C.; Shucko, S. K.; Silva, M. A. S.; Rodrigues, P. O.; *Lat. Am. J. Pharm.* **2007**, *26*, 513.
23. Monteiro, L. M.; Souza, A. E.; Gianotto, E. A. S.; Nery, M. M. F.; Duarte, J. C.; Freitas, O.; Casagrande, R.; Baracat, M. M.; *Lat. Am. J. Pharm.* **2007**, *26*, 179.