Technological development of 40mg furosemide tablets: equivalence and bioavailability study in dogs

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Furosemide (40mg) was administered to 20 street dogs, 10 males and 10 females, in two different pharmaceutical forms: (1) compressed furosemide 40mg formulated at the Federal University of Pernambuco (UFPE-tablet), and (2) a commercial formulation with equal bioequivalence produced by the Laboratory for Pharmaceutical Technology of Pernambuco State (LAFEPE), the LAFEPE-furosemide. The study aimed to evaluate the kinetics of dissolution of the UFPE-tablet in order to analyze the behavior of bioavailability of the best formulation for veterinary use. The plasmatic concentrations of furosemide for the determination of pharmacological kinetics were analyzed by high-performance liquid chromatographic method (HPLC). The in vitro study accomplished through physicochemical analyses demonstrated that the formulas of the furosemide tablets attained the pharmaceutical requirements in agreement with USP 23 and the Brazilian Pharmacopoeia. The evaluation accomplished in dogs with UFPE-tablets given in only dose demonstrated uniformity in blood levels indicating stability in maintenance of the pharmaceutical formulation and efficiency in absorption of the active compound. These values are not significantly different in relation to the 5% confidence limit. Regarding maximum concentration (Tmax) time and global bioavailability assessed by AUC means, there were no considerable differences as well. UFPE-furosemide displayed 743.492µg/mL.h as AUC average value whereas LAFEPE-furosemide had an average of 537.284µg/mL.h.

INDEX TERMS: Furosemide, bioavailability, lyoavailability, dogs.

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niveis sanguíneos indicando estabilidade na manutenção da forma farmacêutica e eficiência na absorção do princípio ativo. Estes valores não são significativamente diferentes em relação ao estima de confiança de 5%. Em relação a concentração de máximo (Tmax) e ao tempo de biodisponibilidade global avaliados por meios de AUC, não houve nenhuma diferença considerável. O comprimido UFPE-furosemide exibiu AUC de 743.492 µg/mL h e o LAFEPE-furosemide teve uma média de 537.284 µg/mL h.

TERMOS DE INDEXAÇÃO: Furosemida, biodisponibilidade, liodisponibilidade, cães.

INTRODUCTION

Furosemide is an anthranilic acid derivative commonly used in man as a potent tubule diuretic (Pontc & Schoenwald 1990, Awad et al. 1992). It is frequently used in human edema treatment associated with congestive heart failure, hypertension and renal insufficiency. In domestic animals furosemide is used to treat various forms of edema in “azoturia”, to reduce space-filling lesions and as a prophylactic measure of epistaxis in racing horses (Roberts et al. 1978, Tobin 1978). Furosemide has a protective effect against bronchoconstriction caused by several factors including exercise (Maxson et al. 1995), and has been used illegally to ‘dilute out’ prohibited drugs ( Stevenson et al. 1990, 1994, Sweeney et al. 1990). Therefore, its use in race horses should be monitored ( Stevenson et al. 1994).

Nowadays one of the basic tasks of drug formulation is to develop an already existing dosage form in a way that makes the best drug release possible under the given circumstances, in other words, enhancing bioavailability ( Dhanaraju 1998, Sznitowska 2001, Matsuura et al. 2002). Furosemide pharmacokinetics has been studied previously in several animal species, particularly horses ( Roberts et al. 1978, Chay et al. 1983, Singh et al. 1990, Dyke et al. 1996), but also in dogs (Verbeek et al. 1981, Hirai et al. 1992, Rahman et al. 2001) and rats ( Hammarlund & Paalzow 1982).

Furosemide pharmacokinetics demonstrates 11-90% of oral absorption, 0.3-3.4% of half life, 60% of elimination in the unaltered form and 40% in the metabolized form ( Hammarlund 1984, Goodman 1996). According to Johnston (1984), furosemide is rapidly absorbed in the conventional tablet form with high diuretic peaks in dogs after one hour of administration ( Chungi 1979, Jitka Huclov 2005). In this kind of study, it is assumed that the success or failure of the pharmaceutical treatment depends largely on certain factors, which are not limited to the usual pharmacodynamics or pharmacokinetics knowledge present in pharmacology compendia used in human medicine.

This study aims to report the technological development of 40mg furosemide tablets produced at Pernambuco’s Federal University ( UFPE-tablets) in comparison with the ones developed in vitro and in vivo at Pernambuco State Pharmaceutical Laboratory (LABEPE-furosemide), using street dogs administration model to meet therapeutic needs in Veterinary Medicine practice.

MATERIALS AND METHODS

Experiment place and technological process

This study was carried out in the Laboratory for Pharmaceutical Technology of Pernambuco (LAFEPE) at Federal University of Campina Grande (UFCG) laboratories. The technological development of UFPE-tablets was based on Good Practices of Manufacture (BPF in portuguese). In the pre-formulation study the following materials were used: diluents (starch, calcium phosphate, lactose), agglutinants (arabic gum, PVP K30, carboxymethylcellulose), lubricants (magnesium stearate, powder), furosemide (active principle) and moisture (hydroholic solution). The compression process by humid via was used in tablets production.

Animals, drug administration and blood sampling

The animal selection was done with a sample of twenty dogs (street-dog), with standard weight and age. Sixteen healthy dogs with ages varying from 3-5 years and weighing between 20 and 30 kilos were selected, 10 males and 6 females. The dogs underwent clinical evaluation during a 30-day-period at the Federal University of Campina Grande (UFCG) Kennel. They were submitted to a twelve-hour fasting after the last meal of the previous day with no water restriction. A single 80mg dose (two 40mg furosemide-tablets 5 per cent w/v, Hoechst Animal Health, Bucks, UK) of the formulations under study (UFPE-tablets and LAFEPE furosemide) was administrated in the morning. The dogs were monitored during the whole study and had the same accompaniment.

Furosemide analysis

6ml of blood samples were collected in heparinized tubes in 0, 20min, 40min, 60min, 90min, 120min, and 180min intervals. The plasma, which was protected from the light until furosemide analysis, was separated by a centrifugation of 3,500 rpm for 10 minutes kept at a -20°C temperature. All the samples of the same animal were analyzed in the same day to avoid variation among analysis. Furosemide plasma concentration was determined by High Pressure Liquid Chromatography (HPLC).

Analytic methodology

The quality of the products under study was evaluated in two stages, according to the 4th edition of the Brazilian Pharmacopeia (Brasil, Ministério da Saúde 1988) and the 23rd edition of the United States Pharmacopeia (USP 1995). At first, physicochemical assay of average weight, hardness, friability and disintegration were fulfilled. Subsequently, essays on assay, content uniformity and liberation kinetics were carried out. These stages determine the in vitro study.

Plasma samples preparation

Furosemide concentration in plasma samples was determined by HPLC, HPLC acetonitrile degree and Merck methanol were used as solvents. The mobile phase was a mixture of water: acetonitrile (70:30) and the pH was adjusted with NaOH 0.2N or glacial acetic acid (1:100) to 6.5. The HPLC system chromatographic conditions consist of a solvent system; a 20 µL Rheodyne valve; an UV detector; an integrator; ODS 25µm spherisorb waters, a 1mL/min air flow and a 235 nm wave length.

Extraction of samples procedure

The following procedure was used in order to determine the furosemide in plasma samples. Firstly, 0.2mL of plasma was taken and 2.4µg/mL of fencateine in methanol was added. This material was stirred in vortex for 40 seconds and then centrifuged at 3000 rpm for 12 minutes. Secondly, the methanol layer was decanted and...
centrifuged for 5 minutes. A 1mL portion was transferred to a volumetric balloon concluding the mobile phase. A 20µL volume was injected. A threefold procedure was carried out. The standard curve (n=6, r=0.9899) was used to calculate the unknown furosemide plasma concentrations. The validation of the analytic methodology employed furosemide methanoic solutions and plasma furosemide. The feasibility of analysis reproduction was assessed through the extraction of six plasma samples with concentrations of 200, 240, 320, 360 and 400µg/mL. The following parameters were compared by means of variance analysis (ANOVA): maximum plasma concentration (C<sub>max</sub>), time for reaching maximum concentration (T<sub>max</sub>) and area under the time of maximum concentration curve (AUC).

RESULTS AND DISCUSSION

The in vitro study carried out through physicochemical essays showed that the furosemide tablet formulations met the pharmaceutical requirements according to USPC (1995) and Brazilian Pharmacopoeia, 4<sup>th</sup> edition (Table 1). The data concerning the studied dissolution formulation of Furosemida tablets reached the monography requirements - more than 65% was dissolved after 30min (Table 2). Still, the profiles showed that the products reach 65% of dissolution in vitro in 10 minutes after the beginning of the test and show a similar profile in the active principle liberation (Fig.1).

The furosemide in the dog’s plasma was carried out as a flowing stage (Table 3). The calibration line was determined with the results obtained from the standard plasma furosemide solutions and underwent statistic treatment, with (R = 0.9942) and p <0.0001. The correlation coefficient was R = 0.9899 (Fig.2).

<table>
<thead>
<tr>
<th>Parameters analyzed</th>
<th>Specification</th>
<th>UFPE-tablets</th>
<th>LAFEPE-furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (mg)</td>
<td>200 mg ± 5%</td>
<td>206.6</td>
<td>203.2</td>
</tr>
<tr>
<td>Hardness (kgf/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>&gt; 3.5 (kgf/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>8.15</td>
<td>7.11</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>&lt; 1.5 %</td>
<td>0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>Disintegration (min)</td>
<td>&lt; 30 minute</td>
<td>8 min</td>
<td>5 min</td>
</tr>
<tr>
<td>Humidity (KF)</td>
<td>&lt; 2.0 %</td>
<td>0.83</td>
<td>1.23</td>
</tr>
<tr>
<td>Assay (90-110%)</td>
<td>100 %</td>
<td>98.75%</td>
<td></td>
</tr>
<tr>
<td>Uniformity Content</td>
<td>&gt; 80%</td>
<td>103.01</td>
<td>95.41</td>
</tr>
<tr>
<td>Dissolution (%)</td>
<td>85-115%</td>
<td>98.63</td>
<td>96.68</td>
</tr>
</tbody>
</table>

Table 2. UFPE tablets and LAFEPE-furosemide dissolution data in vitro according to USP 23 methodology

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>% Dissolution UFPE-tablets</th>
<th>% Dissolution LAFEPE-furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>24.10</td>
<td>30.20</td>
</tr>
<tr>
<td>5</td>
<td>52.50</td>
<td>62.83</td>
</tr>
<tr>
<td>10</td>
<td>76.80</td>
<td>77.62</td>
</tr>
<tr>
<td>15</td>
<td>86.00</td>
<td>84.57</td>
</tr>
<tr>
<td>20</td>
<td>94.00</td>
<td>87.64</td>
</tr>
<tr>
<td>30</td>
<td>97.10</td>
<td>91.21</td>
</tr>
<tr>
<td>45</td>
<td>101.58</td>
<td>94.44</td>
</tr>
<tr>
<td>60</td>
<td>103.01</td>
<td>95.41</td>
</tr>
</tbody>
</table>

(p <0.05)

The bioavailability evaluation according to study specification entails the determination of pharmacy absorption quantity in 20, 40, 60, 90, 120 and 180 min intervals, i.e., the velocity in which this process happens (United States Pharmacopoeia 1990, Ritchel 1992) (Table 4). Since bio-equivalence is the bioavailability comparative study between two phar-
The liberation half times (t½) for UFPE and LAFEPE furosemide (40mg) is different in relation to the 5% confidence limit. Regarding maximum concentration (Cmax) time and global bioavailability assessed by AUC means, there were no considerable differences as well. UFPE-furosemide displayed 743.492μg/mL.h as AUC average value whereas LAFEPE-furosemide had an average of 537.284μg/mL.h.

CONCLUSION

The liberation half times (t½) for UFPE and LAFEPE furosemide vary between 60 and 90 minutes. The results obtained from this study indicate that following a single oral dose of two 40mg furosemide tablets, the differences shown by the data related to the test and reference products were considered statistically non-relevant within the 5% confidence level that, in turn, demonstrates that the two products are bioavailable.

The bioavailability data in plasma obtained after furosemide oral doses administration assure that it is possible to compare the bioequivalence of the products with absolute security. Based on the comparison of in vitro data and on bioavailability blood plasma levels it is possible to guarantee the bioequivalence of the products.

Hence, there is an urgent need for the government to put into practice the current policy for the Brazilian generics which will make the bioequivalence studies a compulsory item by invigilating organs hence, improving security, efficiency and quality of commercialized veterinary products.

Finally, we believe that the bioequivalence study carried out through the product development (pharmaceutical development) compared to the reference product has proved to be a superior gain over two unknown products.

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REFERENCES


