

## COMPARATIVE BIOAVAILABILITY OF FUROSEMIDE FROM SOLUTION AND 40 mg TABLETS WITH DIFFERENT DISSOLUTION CHARACTERISTICS FOLLOWING ORAL ADMINISTRATION IN NORMAL MEN

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### ABSTRACT

Furosemide tablets, with markedly different dissolution characteristics, and solution were orally administered to 21 healthy adult males to determine the effect of *in vitro* dissolution rate on *in vivo* bioavailability profiles. Furosemide 40 mg was given as Tablet A (fast dissolution characteristics), Tablet B (slow dissolution characteristics), and an aqueous solution. Both batches of tablets had identical formulae and were produced by a common process. The dissolution rate of the slower Tablet B was probably retarded by extension of the wet granulation time. Blood was collected for 12 h postdose and urine for 24 h. Peak plasma furosemide concentrations after the solution were significantly greater than after the tablets; there was no significant difference between the tablets. The time to peak occurred significantly earlier with the solution, with no significant difference between the tablets. Relative bioavailabilities of Tablet A and B were 89 per cent and 101 per cent, respectively, as determined by AUC, and 79 per cent and 84 per cent, respectively, as determined by urine recovery. These differences are not statistically significant. These results indicate that dissolution rate profiles of furosemide tablets may not be predictive of *in vivo* bioavailability.

KEY WORDS Furosemide Bioavailability Dissolution

### INTRODUCTION

It is well known that formulation factors can significantly affect the *in vivo* performance of a dosage form. Formulation techniques which influence dissolution rate may significantly affect the onset, rate, and extent of drug

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absorption. Ideally *in vitro* dissolution characteristics would be helpful in predicting *in vivo* absorption, but this may often not be the case. Significant *in vivo-in vitro* correlations are needed before dissolution rate characteristics can be used as one of many quality control tests to assure a product will perform consistently *in vivo*.<sup>1</sup>

Furosemide is an anthranilic acid derivative which is poorly soluble in water but rapidly absorbed from tablet form.<sup>2</sup> Thus, the bioavailability of this drug may be influenced by the dissolution rate. The present study was conducted to compare the bioavailability in normal healthy men of furosemide from a 40 mg tablet with rapid dissolution characteristics, a 40 mg tablet with slow dissolution characteristics, and an oral solution containing 40 mg of drug.

## EXPERIMENTAL

### *Subject selection*

Twenty-one healthy males, 19–35 years of age (mean 27), weighing between 67 and 85 kg (mean 74), who were in good physical condition as determined by physical examination, volunteered to participate in the study. Informed consent was obtained from each subject. The protocol was approved by the University of Texas at Austin Institutional Review Board.

### *Study Design*

An open Latin-square was used to study 21 subjects divided into three groups of seven. Subjects were randomly assigned to each group. A single dose of furosemide 40 mg was administered on three different days as an oral aqueous solution (10 mg ml<sup>-1</sup>), a tablet with fast dissolution characteristics (Tablet A), and a tablet with slow dissolution characteristics (Tablet B). Using the standard USP assay procedure,<sup>3</sup> analysis of furosemide content in the two tablets showed Tablet A to contain 41.6 mg/tablet and Tablet B 40.3 mg/tablet. All clinical supplies were provided by Hoechst-Roussel Pharmaceuticals Inc. as Lasix<sup>TM</sup>; Tablet A: Lot no. RP 3943; Tablet B: experimental Lot no. RP 3942; and Lasix<sup>TM</sup> solution: Lot no. X619513. The per cent dissolution of Tablets A and B were measured using USP Method 2 (paddles) with apparatus meeting the specifications of the Apparatus Suitability Test. Dissolution was determined at 50 rev min<sup>-1</sup> in pH 5.8, 0.05 M phosphate buffer for 30 min. Dissolution testing was also repeated under the same conditions but using a 0.05 M acetate buffer at pH 4.6.

All subjects abstained from medications, smoking, and alcohol for 1 week prior to, and throughout, the study. Seven-day washout periods separated the study days. Subjects fasted for 12 h before each drug administration and 3 h thereafter. Furosemide tablets were administered with 180 ml of water. Four millilitres of furosemide solution was diluted in 90 ml of water and administered with an additional 90 ml of water. Following drug administration, blood

samples (10 ml) were collected from a forearm vein using a plastic syringe with immediate transfer to heparinized tubes. Blood was collected immediately before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 h after drug administration.

Plasma was separated and frozen at  $-20^{\circ}\text{C}$  until assayed. During each study day, urine was collected immediately before drug administration and for the following periods: 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–8, 8–12, and 12–24 h. Urine volumes were recorded and an aliquot was frozen at  $-20^{\circ}\text{C}$  until assayed.

#### *Analytical methodology*

Furosemide was supplied by Hoechst-Roussel Pharmaceuticals Inc. (Somerville, New Jersey) and hydroflumethiazide by Bristol Laboratories (Syracuse, New York). Ether (ACS, absolute) and glass distilled methanol (Omnisolv<sup>TM</sup>) were obtained from MCB (Cincinnati, Ohio). All other chemicals used were reagent grade or better.

#### *Plasma assay*

To 1 ml of plasma was added 10  $\mu\text{l}$  of a stock solution of hydroflumethiazide (prepared in HPLC grade methanol; final concentration of internal standard hydroflumethiazide was approximately  $20\text{ }\mu\text{g ml}^{-1}$ ) and, in the case of standard curve samples, 100  $\mu\text{l}$  of stock solution of furosemide (prepared in HPLC grade methanol) to yield final furosemide concentrations of  $0.05\text{--}2.5\text{ }\mu\text{g ml}^{-1}$ . The sample was acidified with 100  $\mu\text{l}$  of 6 M HCl and immediately extracted with 5 ml of ether. Following a brief centrifugation to separate phases, 4 of 5 ml of the organic phase was transferred to another tube, evaporated under a gentle stream of nitrogen, and reconstituted in 0.25 ml of 0.02 M glycine buffer, pH 11. A portion (100  $\mu\text{l}$ ) of the glycine buffer phase was chromatographed using an IBM HPLC system including a 4.6 mm ID  $\times$  15 cm, 5 micron ODS column (Altex) and fluorescence detection (Gilson Spectra/Glo filter fluorometer, excitation filter 330–400 nm, emission filter 460–600 nm). The mobile phase was methanol–water–acetic acid (40:57:3) used at a flow rate of  $0.8\text{ ml min}^{-1}$ . Furosemide typically eluted with a retention time of approximately 9.8 min, while the internal standard hydroflumethiazide typically eluted with a retention time of approximately 2.7 min.

Absolute recovery of furosemide extracted from plasma was 82–89% over the concentration range  $0.05\text{--}2.6\text{ }\mu\text{g ml}^{-1}$ . The sensitivity limit for furosemide was estimated to be  $0.05\text{ }\mu\text{g ml}^{-1}$  for a signal/noise ratio greater than or equal to 5. For a group of 34 standard curves, the mean regression line obtained was  $y = 0.2013x + 0.0010$  ( $r = 0.9985 \pm 0.0024$ ). Analysis of plasma samples spiked with furosemide indicated an accuracy of 99.8 per cent, with a precision of approximately 11 per cent for samples at or above the practical lower sensitivity limit of the assay. The precision is equivalent to the standard deviation of the mean of the daily accuracies of the assay results where:

$$\text{accuracy} = \frac{\text{content measured}}{\text{actual content}} \times 100 \text{ per cent}$$

### *Urine assay*

Sample extraction and chromatography of urine samples were similar to the method described above, with the following modifications: chromatograms of urine samples containing the internal standard hydroflumethiazide showed variable and irreproducible interference under the hydroflumethiazide peak from endogenous urine components. Thus, the internal standard was omitted in the urine assay and the method of external standardization was employed to determine final furosemide concentrations.<sup>4</sup> Also, a back extraction step was added to the extraction procedure. Four of the 5 ml ether phase obtained from the extraction of acidified sample (cf. above) was transferred to another tube and back extracted with 1 ml of 0.02 M glycine buffer, pH 11. The glycine buffer phase (100  $\mu$ l) was then chromatographed as described above with the exception that the flow rate used was 1.45 ml min<sup>-1</sup>. Furosemide typically eluted with a retention time of 5.5 min.

Absolute recovery of furosemide extracted from urine was 71–87 per cent for samples prepared in the concentration range 0.52–52.0  $\mu$ g ml<sup>-1</sup>. The sensitivity limit for furosemide was estimated to be 0.05  $\mu$ g ml<sup>-1</sup> for a signal/noise ratio greater than or equal to 5. For a group of 23 standard curves, the mean regression line obtained for the concentration range of 0.5 to 50  $\mu$ g ml was  $y = 47712x + 4866$  ( $r = 0.9995 \pm 0.0007$ ). Analysis of urine sample spiked with furosemide indicated an accuracy of 99.3 per cent, with a precision of approximately 11 per cent for samples at or above the practical lower sensitivity limit of the assay.

### *Dissolution data*

Five sets of six tablets each were used to determine a mean dissolution value of  $82.93 \pm 4.9$  per cent (S.D.) for Tablet A. Nine sets of six tablets each were used to determine a mean dissolution value of  $49.07 \pm 15.03$  per cent (S.D.) for Tablet B. The USP standard for furosemide tablet dissolution requires at least 65 per cent dissolution after 30 min. Although the two tablets are produced from a common formula, the dissolution of Tablet B is probably retarded by extension of the wet granulation time. The disintegration time is similarly affected, being 43 s for Tablet A and 79 s for Tablet B. At pH 4.6, dissolution was retarded for both tablets as compared with pH 5.8. However, the differences between tablets did not disappear, dissolution being  $41.14 \pm 4.05$  per cent for Tablet A and  $16.92 \pm 8.47$  per cent for Tablet B.

### *Data analysis*

Area under the plasma concentration–time curve (AUC) was calculated for 0–12 h using the trapezoidal rule. The AUC was corrected to infinity (AUC<sub>∞</sub>) with the terminal slope correction factor,  $Cp^n/B$ , where  $Cp^n$  is the last measured

concentration-time point, and  $B$  is the negative slope of the terminal log-linear phase of the semilog plot of concentration versus time. The maximum plasma concentration achieved ( $C_{p_{\max}}$ ) and time to maximum plasma concentration ( $t_{\max}$ ) were observed from the measured plasma concentrations.

Renal clearance ( $Cl_R$ ) was calculated from the equation:

$$Cl_R = \frac{X_{u\ 0-24\ h}}{AUC_{0-\infty}} \quad (1)$$

where  $X_{u\ 0-24\ h}$  is the total amount of unchanged drug eliminated in the urine in 24 h.

The relative bioavailability ( $F$ ) was determined from plasma and urine data from:

$$F_{\text{plasma}} = \frac{AUC_{0-\infty}^{\text{tab}}}{AUC_{0-\infty}^{\text{soln}}} \quad (2)$$

$$F_{\text{urine}} = \frac{X_{u\ 0-24\ h}^{\text{tab}}}{X_{u\ 0-24\ h}^{\text{soln}}} \quad (3)$$

The software package MINITAB, release 81.1, was used for statistical analysis, and analysis of variance with the least significant difference test was utilized for *a posteriori* comparison and *t*-test for paired data. An alpha level of 0.05 was accepted as evidence of statistical significance.

## RESULTS AND DISCUSSION

Twenty of the 21 recruited subjects completed the study. One subject dropped from the study for reasons unrelated to the administered drug. Data collected from this subject were not included in the data analysis.

Mean plasma furosemide concentrations resulting from the administration of the three dosage formulations are depicted graphically in Figure 1. Pharmacokinetic parameters are shown in Table 1. The  $C_{p_{\max}}$  from solution was significantly greater than those associated with tablets. There was no significant difference in  $C_{p_{\max}}$  resulting from the two tablets.

Mean  $t_{\max}$  from solution was 0.69 h, which was significantly less than  $t_{\max}$  values of 1.39 and 1.48 h associated with Tablet A and Tablet B, respectively. There was no significant difference in  $t_{\max}$  from the tablets.

The  $AUC_{0-\infty}$  from the three treatments were not significantly different. Relative bioavailabilities of Tablets A and B were 89 per cent and 101 per cent respectively, also not significantly different.

Cumulative urine recovery of furosemide is illustrated in Figure 2. Significantly more furosemide was recovered from the solution than from the

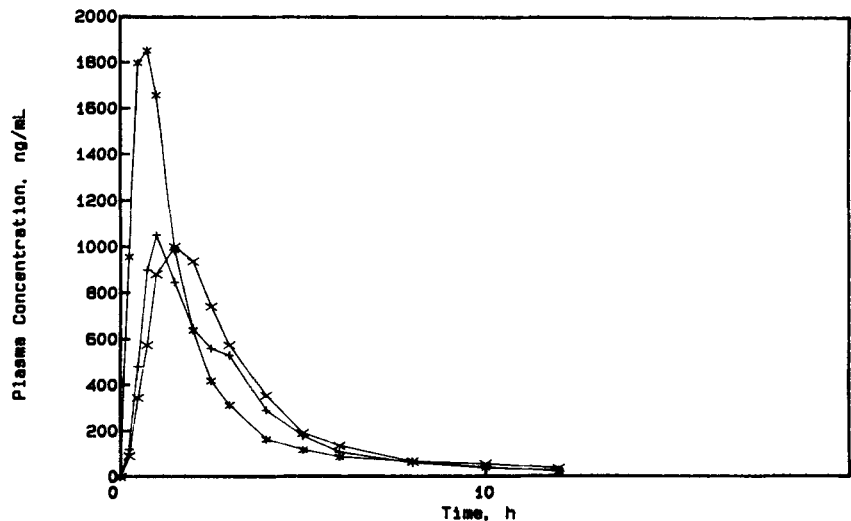


Figure 1. Mean furosemide plasma concentrations following oral administration of furosemide 40 mg as Tablet A (+), Tablet B (x), and Solution (\*) to 20 healthy adult males

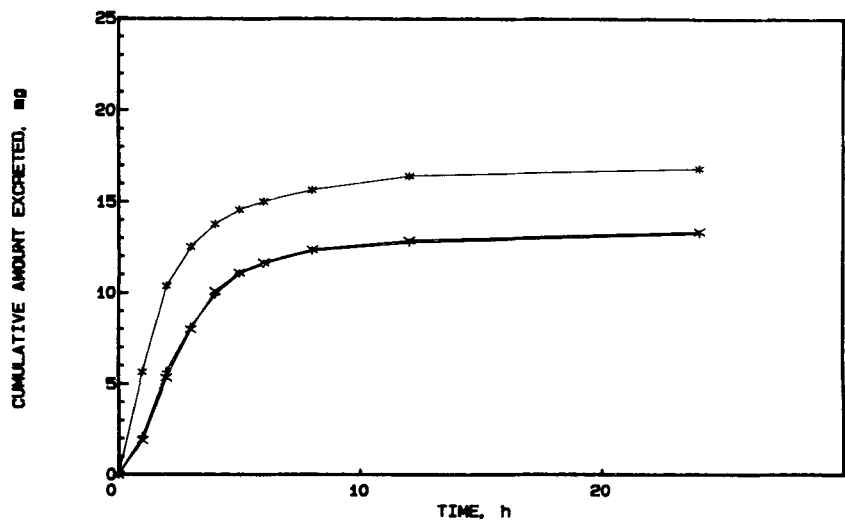


Figure 2. Mean cumulative amounts of furosemide in urine following oral administration of furosemide 40 mg as Tablet A (+), Tablet B (x) and Solution (\*) to 20 healthy adult males

Table 1. Pharmacokinetic parameters (mean  $\pm$  S.D.) in 20 normal males following administration of furosemide 40 mg

	Tablet A	Tablet B	Solution
$C_{p_{\max}}$ (ng ml <sup>-1</sup> )	1502.1 $\pm$ 797.6	1581.1 $\pm$ 872.6	2053.5 $\pm$ 558.6
$t_{\max}$ (h)	1.39 $\pm$ 0.84	1.48 $\pm$ 0.85	0.69 $\pm$ 0.21
AUC <sub>0-<math>\infty</math></sub> ( $\mu$ $\cdot$ min $\cdot$ ml <sup>-1</sup> )	192.0 $\pm$ 67.0	208.2 $\pm$ 100.5	232.1 $\pm$ 88.8
$X_u$ 0-24 (mg)	12.42 $\pm$ 3.96	12.52 $\pm$ 3.43	16.18 $\pm$ 3.82
( $n = 18$ )			
Cl <sub>R</sub> (ml min <sup>-1</sup> )	70.45 $\pm$ 20.2	69.23 $\pm$ 28.5	79.81 $\pm$ 25.5
( $n = 18$ )			
$F_{\text{plasma}}$	0.89 $\pm$ 0.32	1.01 $\pm$ 0.61	
$F_{\text{urine}}$	0.79 $\pm$ 0.26	0.84 $\pm$ 0.28	
( $n = 18$ )			

tablets. There was no significant difference in recovery from the two tablets. Renal clearance of furosemide from the three dosage formulations was not significantly different. Relative bioavailabilities of Tablets A and B were 79 per cent and 84 per cent, respectively, as determined by renal recovery of furosemide. These are not significantly different.

Thus, the bioavailabilities of Tablets A and B were not significantly different, as determined by both plasma and urine data. It should be noted, however, that because of the large standard deviations in the data that the statistical power was less than 0.8. It would have taken a population size of at least 26 to detect a 20 per cent difference in AUC between the two tablets.

The correlation of *in vitro* dissolution rate data with *in vivo* bioavailability profiles of furosemide would give assurance of *in vivo* drug performance with relatively simple *in vitro* testing. The use of dissolution data, in addition to other drug-dependent characteristics, to predict *in vivo* drug behaviour would obviously be very valuable in quality control of dosage formulations. However, drug absorption is not entirely influenced by drug dissolution, making dissolution only one of many properties of a drug product which may correlate with bioavailability. Therefore, dissolution rate alone may not be a good predictor of bioavailability profiles for some drugs.

Rubinstein<sup>5</sup> has reported the only previously published evaluation of effect of dissolution rate on furosemide bioavailability. The bioavailability of two unidentified proprietary furosemide products, one (Formulation 1) with short disintegration time and a long dissolution half-life (0.65 and 86.2 min, respectively), and another (Formulation 2) with a relatively long disintegration time and fast dissolution half-life (1.14 and 16.6 min, respectively), was determined in five normal male volunteers. The total cumulative amounts (mean  $\pm$  S.D.) of furosemide in the urine were 22.76  $\pm$  4.34 and 15.47  $\pm$  2.89 mg for Formulation 1 and 2, respectively. The author concluded that the dissolution rate did not correlate with furosemide bioavailability because Formulation 1 with slower dissolution rate was more bioavailable than Formulation 2. These

study results would indicate that furosemide bioavailability is not improved by the manufacture of tablets with fast dissolution rates. However they must be considered as preliminary data because of the small sample size. Unfortunately, he also does not explain the methodology used in the *in vitro* testing.

Based on data generated in the present study and Rubenstein's preliminary data, dissolution rate may not be a good predictor of furosemide's bioavailability profile. In the present study, despite administering two tablets with distinctly different dissolution rate profiles, relative bioavailability determined by both plasma and urine data of the two tablets was not significantly different. Thus, the dissolution rate profiles associated with furosemide Tablets A and B were not predictive of *in vivo* drug performance. However, it is recognized that the large standard deviations in data and the small sample size could have resulted in a statistically significant difference not being seen where one actually existed. Even though dissolution was examined in two media with different pH, it would be helpful to examine furosemide dissolution in a variety of different conditions.

The lack of a direct association between the *in vitro* test and clinical data may be due to drug properties other than dissolution rate which are more critical in determining furosemide absorption. *In vitro* dissolution rates could be useful in predicting bioavailability only when absorption rate is dissolution rate limited. If dissolution rate is not the rate limiting step in determining absorption, then other factors affecting absorption may become more important. Additional studies need to be performed examining the ability of *in vitro* data to predict *in vivo* results with different drugs under a variety of experimental conditions and utilizing larger sample sizes for the *in vivo* experiments.

#### ACKNOWLEDGEMENT

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#### REFERENCES

1. S. A. Kaplan, in *Dissolution Technology*, L. J. Lesson and J. T. Carstensen (Eds), American Pharmaceutical Association, Washington, 1974, pp. 163-86.
2. E. S. Waller, S. F. Hamilton, J. W. Massarella, M. A. Sharanevych, R. V. Smith, G. J. Yakatan and J. T. Doluisio, *J. Pharm. Sci.*, **71**, 1105 (1982).
3. *U.S. Pharmacopeia*, 21st rev. U.S. Pharmacopeial Convention, Rockville, Maryland, 1984, pp. 453-454.
4. R. V. Smith and J. T. Stewart, *Textbook of Biopharmaceutic Analysis—A Description of Methods for the Determination of Drugs in Biologic Fluids*, Lea and Febiger, Philadelphia, 1981, p. 107.
5. M. H. Rubinstein, *Drug Dev. Ind. Pharm.*, **6**, 105 (1980).