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Dissolution medium—a critical parameter to identify bioavailability problems of furosemide tablets

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Summary

The pH–solubility profiles of furosemide bulk drug and the dissolution profiles of two brands of furosemide tablets as a function of pH have been determined. The dissolution rate increased as the pH of the medium increased. Even though dissolution rate differences were noted around the pK_a (3.60) of furosemide, such differences were negligible in the phosphate buffer region. One of these brands was alleged to cause therapeutic failures. The brand that dissolved poorly at pH 4.6 also exhibited inferior bioavailability. The data suggests the need for careful selection of pH and buffers composing dissolution media to make in vitro results meaningful and consistent with in vivo data.

Introduction

Furosemide (I) is an anthranilic acid derivative used to treat edematous states of hepatic, cardiac and renal origin (Stason et al., 1966; Kirkendall et al., 1968). The patent for this widely used diuretic has expired resulting in the generic manufacture of products. At present there are no in vitro dissolution specifications for furosemide tablets in the U.S.P. Furthermore the U.S. Food and Drug Administration has recalled an unapproved brand of furosemide tablets because of its alleged therapeutic failure. It is very well known that pharmaceutic excipients as well as the process factors such as method of granulation, addition of lubricant and subsequent blending and blending times etc. contribute to differences observed in the dissolu-

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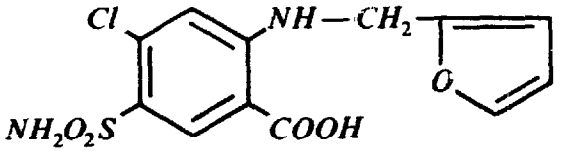
tion characteristics. Such in vitro dissolution differences are also often associated with differences in in vivo performance. It is not very often that dissolution media



are chosen on a rational basis. Furthermore, it was alleged that the brand of furosemide tablet which was recalled by F.D.A. had similar dissolution characteristics as the reference product (i.e. innovator). Rubinstein and Price (1977) while studying the effect of 5 h disintegrants and processing on the bioavailability of furosemide tablets have shown that bioavailability differences can be detected by sensitive and specific dissolution tests. The present investigation was undertaken to identify in vitro dissolution differences, if any, that existed between FDA recalled and the innovator products which might have contributed to the alleged therapeutic failures. An attempt will be made to rationalize the in vivo results with the observed dissolution rates.

Materials and methods

Buffer solutions used as dissolution media were prepared according to standard procedures. The following buffers were used: pH 1.6 (HCl/KCl) (United States Pharmacopeia, 1980a), pH 2.6 (citrate/phosphate), pH 3.8 and 4.6 (acetate), pH 5.6, 6.6 and 7.4 (phosphate) (Documenta Geigy, 1973).

Solubility determinations were carried out by equilibrating an excess of furosemide powder in the appropriate buffer at 37.0 ± 0.2°C. The concentration of furosemide in the unknown solution was determined by measuring the absorbance at 277 nm and comparison with solutions of known concentrations or from a standard curve.

Dissolution procedure

A 6 unit dissolution equipment [1] conforming to the specification of United States Pharmacopeia XX Apparatus 2 (United States Pharmacopeia, 1980b) was used. The dissolution medium consisted of 900 ml of the appropriate buffer which was brought to 37 ± 0.2°C by immersing the dissolution vessel [2] containing the buffer into a water bath made of plexiglass. The water bath temperature was maintained by a constant temperature water pump [3]. The dissolution medium was stirred at 50 rpm. The dissolution medium was filtered through a millipore membrane and pumped through the spectrophotometer [4] flow-through cell at a rate of approximately 5–7

[1] Hollow Spindle Stirrer, Model 72S115, 1978 Model, Hanson Research, P.O. Box 35, Northridge, CA 91324, U.S.A.

[2] Dissolution Glass Vessel supplied by Kimble Glass Company, Part No. 33730.

[3] Circulating System-200, GCA/Precision Scientific, West Cortland St., Chicago, IL 60647, U.S.A.

[4] Beckman Model 25 Spectrophotometer with Flow-Through Cells, Beckman Instruments, Fullerton, CA 92634, U.S.A.

ml/min. The dissolution medium was returned to the dissolution vessel. The spectrophotometric absorbances determined at 277 nm that were recorded were compared with the absorbance of a furosemide U.S.P. reference standard solution prepared by dissolving 10 mg of furosemide powder in 12 ml of ethanol and diluting to 250 ml with the appropriate buffer [5]. The absorbances measured at 277 nm were utilized to calculate the concentration of furosemide dissolved in the dissolution medium.

Furosemide 40 mg tablets manufactured by the innovator, Brand A [6], and one batch of the recalled product Brand B [7], were employed in this study. Bulk active drug (furosemide powder) was also obtained from the above two manufacturers and dissolution profiles were carried out by filling 40 mg of the furosemide powder into '0' size gelatin capsule. The capsules were restrained in wire spiral as described in U.S.P. XX (1980b).

Results and Discussion

Solubility studies

Solubility–pH profiles of furosemide of $37.0 \pm 0.2°C$ are shown in Fig. 1. The curve is consistent with the pK_a of furosemide which is reported to be 3.6 (8). To study the influence of buffer components on the solubility of furosemide at pH 7.6, Tris buffer (Documenta Geigy, 1973) was used in contrast to the phosphate buffer. Furosemide solubility was markedly suppressed in this medium which may be attributed to interaction between furosemide and Tris(hydroxymethyl)aminomethane.

Dissolution studies

Dissolution profiles in pH 7.4 phosphate buffer at $37.0 \pm 0.2°C$ of Brands A and B are shown in Fig. 2. Each data point represents an average of 6 tablets. No significant differences in the dissolution profiles between the two brands were observed and the data shows that the products are indistinguishable at the 30- and 60-min intervals. This close similarity between the two products would lead one to believe that there may not be any differences in the bioavailability of the two products. However, the F.D.A. received complaints on several batches of Brand B. Brand B furosemide tablet was not submitted to F.D.A. for approval and thus is an F.D.A. unapproved product. Since bioavailability studies are time consuming, it was felt that a thorough and comparative investigation of the dissolution profiles of Brand B and the innovators product Brand A would give clues to in vitro differences, if any, that exist between the two products. Furthermore, investigation of the bulk active drugs that the two firms employed could also shed light on the differences between the two brands.

The results of the dissolution profiles of Brands A and B in different buffers at

[5] The concentration of alcohol in the final solution shall not be greater than 5%.

[6] Hoechst-Roussel Pharmaceuticals, Somerville, NJ 08876, U.S.A.; Batch 602098.

[7] Pharmadyne Laboratories, Hackensack, NJ 07601, U.S.A.; Batch 8137.

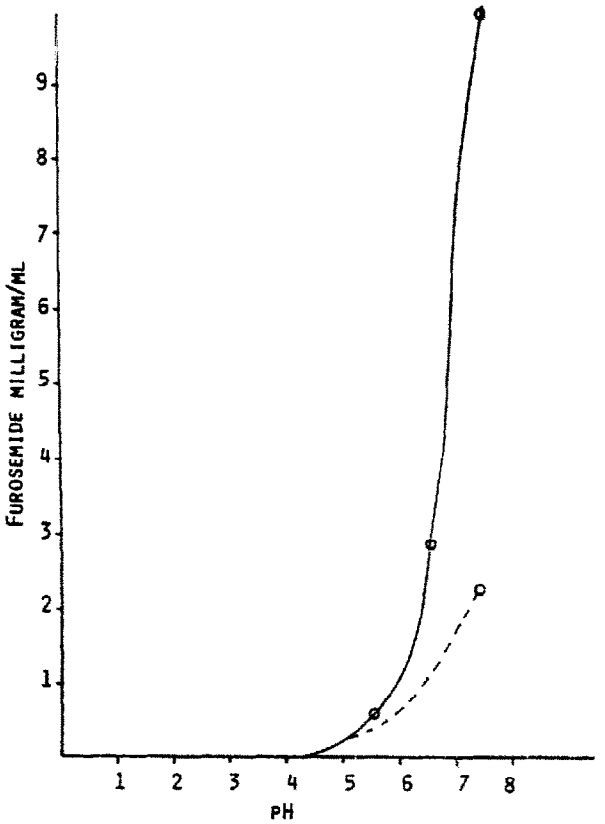


Fig. 1. Solubility–pH profiles of furosemide bulk powder in various buffers at 37±0.2°C. Dashed line denotes Tris buffer solution.

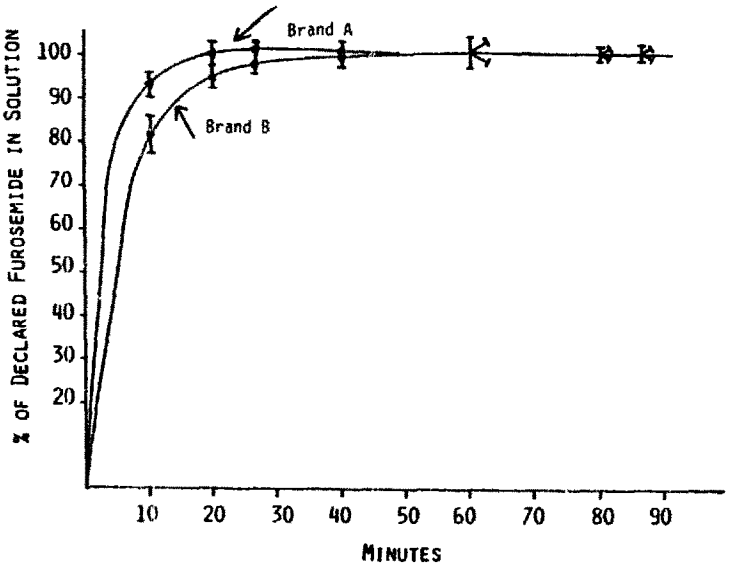


Fig. 2. Dissolution profiles of furosemide 40 mg tablets in pH 7.4 phosphate buffer at 37±0.2°C. Brand A and Brand B.

37.0 ± 0.2°C are shown in Fig. 3. It is quite evident that as the pH of the medium is increased away from the pK_a, the differences in dissolution decreases and at pH 7.4, they virtually disappear. Thus if one were to rely only on a two time point (i.e. 30 and 60 min) determination in pH 7.4 buffer, it could be wrongly concluded that Brands A and B are similar in quality, in spite of the therapeutic failures with Brand B.

Rubinstein (1980), Rubinstein and Price (1977) have reported significant changes in dissolution rates of furosemide tablets which depended on the nature of the disintegrant as well as a wet or dry granulating process that was employed. Rubinstein and Rughani (1978) have also shown that tablet binders can also influence the dissolution with consequent alteration in bioavailability of furosemide tablets. In the study on the effect of disintegrants (Rubinstein, 1980; Rubinstein and Price, 1977), they have reported a correlation coefficient of 0.79 between $t_{50\%}$ and bioavailability when all formulations were considered and the dissolution test was carried out in pH 5.0 buffer using the basket method described in the British Pharmacopeia (1977). As described above, our studies employed the U.S.P. XIX rotating paddle method, which is different from the B.P. method.

Chungi et al. (1979) have demonstrated that undissociated furosemide (acidic moiety) is substantially better absorbed in the rat gut than the dissociated furosemide molecule. This finding is substantiated by the well known Brodie theory (1957) that to facilitate drug passage through biomembrane, the molecule should be undissociated. Even though Chungi's work points out that pH 3.6 buffer would be more predictive of in vivo absorption, it is contrary to Rubinstein's findings who found that at pH 3.5 the correlation coefficient is 0.071 for tested tablets.

One of the major objectives of a dissolution test is that it identifies poorly bioavailable products from therapeutically acceptable products. Furthermore, ex-

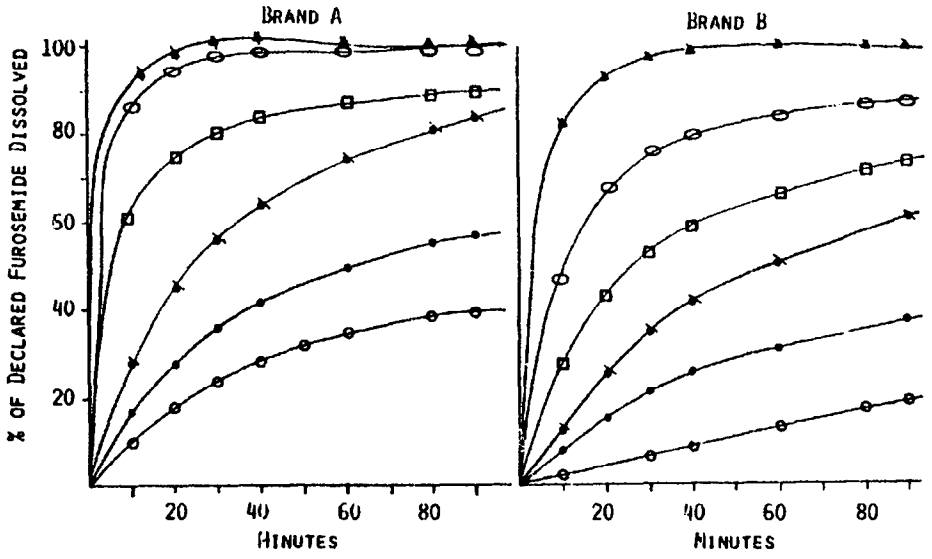


Fig. 3. Dissolution profiles of furosemide 40 mg tablets in buffers of different pH values at 37.0 ± 0.2°C. pH 2.6, ○; 3.8, ◕; 4.6, ●; 5.6, □; 6.6, ⊖; 7.4, ▲.

perimental conditions giving rise to very low dissolution results (such as 25% or lower) of products with acceptable bioavailability are misleading. Therefore, if possible, such dissolution test conditions should be avoided. A buffer at pH 4.6 would satisfy the above criteria for the two brands of furosemide tablets, i.e. exhibits maximum differences between formulations A and B. Therefore all other tests were carried out in pH 4.6 acetate buffer.

Dissolution differences of dosage forms have sometimes been traced to bulk active drug. This is often true when synthetic procedures and/or solvents of crystallization etc. are different. Therefore, dissolution in pH 4.6 buffer of several batches of the bulk active drug (furosemide) of the two firms were tested in no. 0 size capsules and results are summarized in Fig. 4. One batch of the active drug from firm B was quite different, whereas other batches were identical to those of firm A. Although Brand A bulk active drug dissolved considerably less than batch 1 of Brand B, its (Brand A) formulation dissolved significantly more in acidic buffers. Thus dissolution rate differences between Brands A and B therefore can be attributed to formulation and process factors and not to bulk active drug.

Dissolution rate differences are often traced to formulations. A systematic search has indicated that furosemide is incompatible, among other chemicals, with calcium gluconate and tetracycline (Merck Index, 1976). Calcium salts such as dicalcium orthophosphate, calcium sulfate and calcium carbonate are often used as diluents in tablets (Marshall, 1979). Furthermore, tablet matrix consisting of calcium carbonate

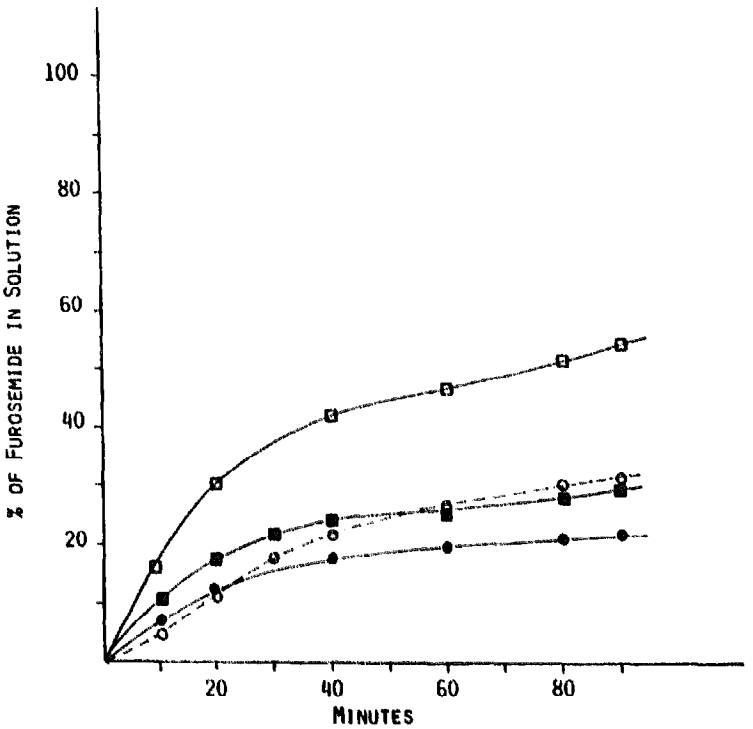


Fig. 4. Dissolution profiles of furosemide 'Active Bulk Powder' of Brands A and B in pH 4.6 acetate buffer at 37±0.2°C. Brand A ● Brand B: Batch 1, □; Batch 2, ■; Batch 3, ○.

or dicalcium phosphate dissolve more readily under acidic conditions. Whether dissolution rates of Brand A and B were affected by increasing concentration of acid in the dissolution medium was investigated. The results are summarized in Fig. 5. A considerable suppression in dissolution of Brand B occurred whereas Brand A was unaffected. This influence of the medium on the dissolution rate of the tablet can be attributed to formulation differences and perhaps to some degree to process factors.

Effect of storage on dissolution

Influence of storage on dissolution profiles of Brands A and B at the time of collection and after storage at room temperature for 3 months are summarized in Fig. 6. Dissolution rate of Brand B had dropped dramatically within 3 months at room temperature. This could be due either to increase in hardness of the tablets or an interaction between excipients and furosemide. Product A being a different formulation was unaffected upon storage.

Bioequivalency results

Bioequivalency study was conducted in a panel of 12 healthy volunteers in a 2-way cross-over fashion on Brands A and B. The batch numbers of brands A and B that were used in the bioequivalency study were different from those used in the dissolution study. Details of this bioequivalency study shall be presented elsewhere (Martin et al., 1980). The dissolution results in pH 4.6 acetate buffer of the batches used in the bioequivalency study are shown in Fig. 7. The results are comparable to

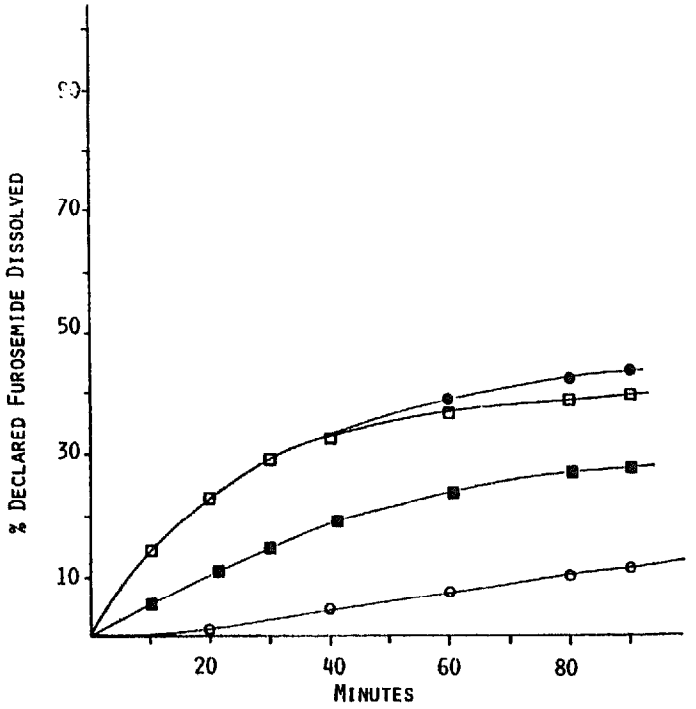


Fig. 5. Influence of concentrations of hydrochloric acid on dissolution rates of furosemide 40 mg tablets at 37.0±0.2°C. Brand A: ●, 0.1 N HCl; □, 1.0 N HCl; Brand B: ■, 0.1 N HCl; ⊙, 1.0 N HCl.

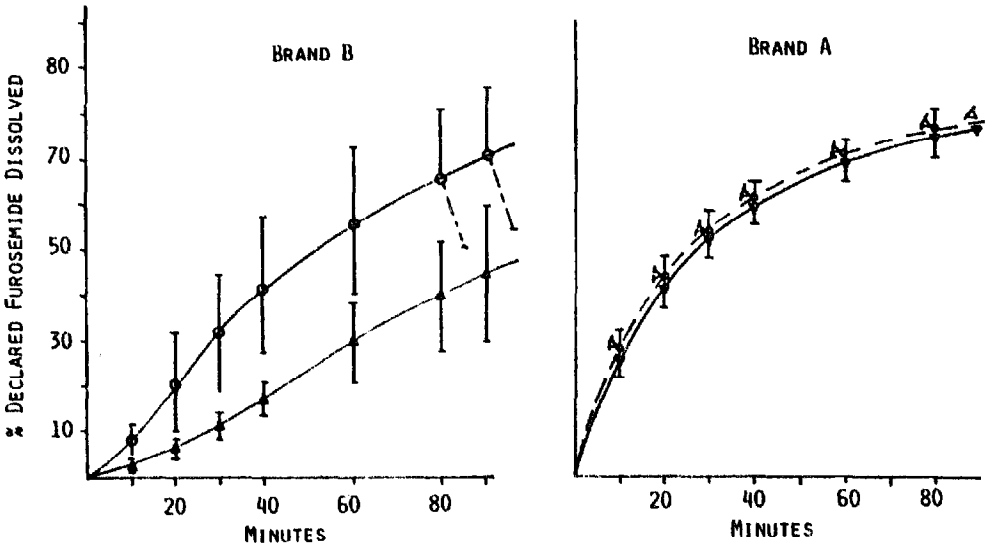


Fig. 6. Effect of aging on the dissolution profiles of furosemide 40 mg tablets of Brands A and B in pH 4.6 acetate buffer at 37.0±0.2°C. ○, time of collection; △, three months later.

the other batches that have been tested in vitro thus confirming batch-to-batch uniformity. The bioavailability results confirmed the observed dissolution differences between the two brands. The mean pharmacokinetic parameters and the individual urine and plasma levels of furosemide are summarized in Table 1. Summary (Table 2) of the more rigorous analysis of the individual subject data have

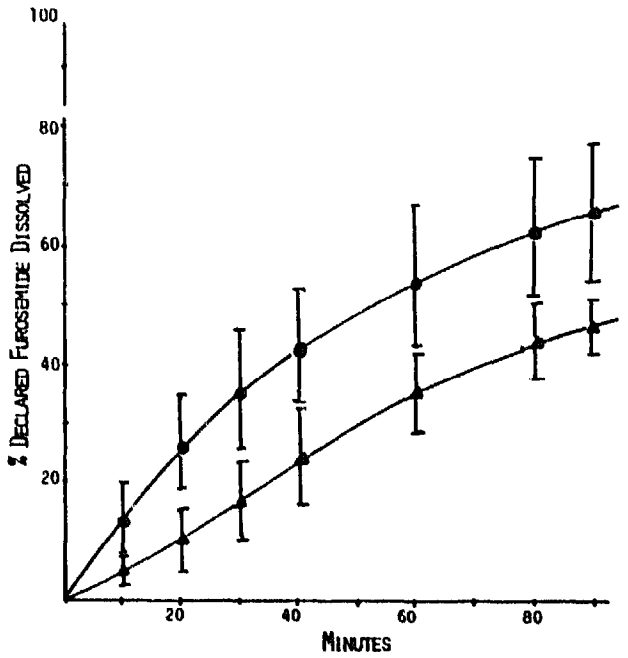


Fig. 7. Dissolution profiles in pH 4.6 acetate buffer of furosemide 40 mg tablets of Brands A (●) (Batch 600229) and Brand B (▲) (Batch 8137-07) used in bioavailability study.

TABLE 1

COMPARISON OF MEAN BIOAVAILABILITY AFTER ADMINISTRATION OF FUROSEMIDE 40 mg TABLETS OF BRANDS A AND B ***

	Brand A	Brand B
C_{max} (ng/ml) **	1658 ±585	947 ±518
t_{max} (h) n.s.	1.1± 0.4	1.6± 1.2
$AUC_{0-10\,h}$ **	2977 ±981	2006 ±772
$AUC_{0-\infty\,h}$ **	3066 ±959	2098 ±756
Urinary excretion (mg) *	13.6± 3.2	10.8± 3.1

n.s. = not significant.

* Significant at the 5% level.

** Significant at the 1% level.

*** Brand A, Batch 60029, and Brand B, Batch 8137-07, were used.

shown that Brand B is substantially different to Brand A. Thus 75% of the subjects in the study failed to achieve 80%, and 50% of the subjects failed to achieve 75% of the AUC_{0-10h} in comparison to Brand A used as the reference product. Furthermore, when Brand B was administered 75% of subjects failed to achieve 75% of the peak concentration when compared to Brand A.

Dissolution studies on furosemide tablets with two divergent formulations and process conditions have shown that pH of the medium is very critical in order to be able to associate dissolution with bioavailability. Although Rubinstein and Price (1977) have correlated dissolution with bioavailability, the formulations that they employed differed only in the amount and type of disintegrant used. The present study consisted of furosemide tablets with widely divergent formulations and processing conditions because they were produced by two different manufacturers. Since a sufficient number of divergent formulations produced under different processing conditions have not been tested at present, it is difficult to conclude that a single buffer and the test conditions used in this study are ideal and applicable to all formulations. Nevertheless, we have associated dissolution results with the bioavailability of these two brands of furosemide tablets. It is safe to conclude from

TABLE 2

NUMBER OF SUBJECTS FAILING TO MEET BIOEQUIVALENCE DECISION RULES

To achieve (%) AUC_{0-10h} of reference	No. of Subjects failing	To achieve (%) of C_{max} of reference	No. of subjects failing	To achieve (%) of urinary excretion of Reference	No. of subjects failing
75	6/11	75	9/12	75	7/12
80	9/12	80	9/12	80	8/12

our work as well as that of Rubinstein that a buffer with pH between 4.0 and 5.0 is most suited to differentiate good and bad furosemide formulations.

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