

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPLICATION NUMBER: 83607**

**BIOEQUIVALENCE REVIEW(S)**

NDA 83-607

AF 28-724

MAY 21 1975

Richlyn Laboratories, Inc.  
Attention: Mr. E.W. Rebollo  
Castor & Kensington Avenues  
Philadelphia, PA 19124

Gentlemen:

Reference is made to the bioavailability studies you submitted for Hydrochlorothiazide Tablets, 50 mg.

The studies have been reviewed by our Division of Biopharmaceutics and they have the following comments:

1. The additional information supplied in the October 30, 1974, submission viz, disintegration times and dissolution rates (99 to 100 percent of the drug in solution in 30 minutes), does meet the compendial specifications and is acceptable. However, this additional information in no way changes the conclusions reached in the previous review (by S. Dighe, dated 4/5/75).

2. In the case of 4 subjects who showed low bioavailability, for the Richlyn product, the applicant has submitted, in aforementioned letter, urinary output data to demonstrate the effectiveness of the drug. The study was conducted to demonstrate the bioequivalence of the test product with the reference product by determining the unchanged drug in the urine. One cannot use two different yardsticks in evaluating the bioavailability performance of the drug in order to explain away the deficiencies. It is neither consistent nor scientific.

RECOMMENDATION: The company should be informed that the additional information in no way changes the conclusions reached about the study in the previous review. The company should conduct a new bioequivalency study by determining the unchanged drug in urine or the electrolytes (sodium, potassium and chloride) in the urine.

/S/

✓ Marvin Seife, M.D.  
Director  
Division of Generic Drug Monographs  
Office of Drug Monographs  
Bureau of Drugs

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NDA 83-607

AF 28-724

Richlyn Laboratories, Inc.  
Attention: Mr. E. M. Rebollo  
Castor & Kensington Avenues  
Philadelphia, PA 19124

APR 15 1974

Gentlemen:

Reference is made to the bioavailability study you submitted for Hydrochlorothiazide Tablets, 25 mg. and 50 mg.

The study has been reviewed by our Division of Clinical Research and they have the following comments:

1. The data on the validation of the assay methodology contains raw data on the recovery of the drug in plasma and standard curves. The data demonstrates the specificity, linearity and sensitivity of the assay method. The lowest detectable drug level with accuracy and reproducibility is 10 mcg/ml.
2. The individual values of the drug level in urine - whether mcg/ml or mg/hr are quite erratic. The peak heights are also erratic. The areas under the curve for the test and reference drugs are 203.745 mcg/ml and 250.48 mcg/ml respectively. This indicates a mean bioavailability of 81.3% for the applicant's products in comparison to Hydrodiuril (reference product). However, examination of the individual areas under the curve reveal that in 4 out of 13 subjects (30%) the bioavailability of the Richlyn product ranges from 24 to 60% of that of the reference drug.
3. The differences detectable at a significance level of 0.05 and a power of the test of 0.80 for various sampling intervals range from 40% to 100%. The detectable differences at the same significance level and the power of the test for AUCs, peak heights and cumulative excretion are 50%. Cursory calculations at the significance level of 0.05 and the power of the test of 0.60 indicate that the detectable differences still could be in the range of 40% to 90%. These differences are much higher than the customarily acceptable value of 20% detectable differences. The data thus clearly indicates the insufficiency of the number of subjects included in the study.

RECOMMENDATIONS:

The study is unacceptable and accordingly is not approved.

/S/

Rayvin Saito, M.D.  
Director  
Generic Drug Staff  
Office of Scientific Evaluation  
Bureau of Drugs

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Hydrochlorothiazide  
25 and 50 mg Tablets  
ANDA 83-607

Richlyn Laboratories  
Philadelphia, Pennsylvania  
AF 28-724  
Submission Dated:  
October 11, 1973

REVIEW OF ADDITIONAL BIOAVAILABILITY STUDY DATA

1. In his review dated August 21, 1973 Dr. Jerome P. Skelly requested data on the validation of the assay method, revised analysis of variance, and difference detectable between the test and reference products at a significance level of 0.05 and a power of the test of 0.80. The submission contains the requested information.
2. The study was performed by for the Richlyn Laboratories. Twelve normal volunteers 21-55 years of age, weighing 135-220 pounds were employed in a 6 x 6 crossover study. Each volunteer received a single 50 mg dose in each phase of the crossover. Urine samples were collected at 0, 1, 2, 3, 4, 6, 8, 12, and 24 hours. The subjects ingested about 8 oz of water with each urine collection time to ensure an adequate urinary flow. Hydrochlorothiazide levels in urine were determined by the method of Sheppard et. al.
3. The subjects abstained from medication and alcohol 2 weeks prior to the initiation of the study and during the study day.
4. In response to the telephone request from the undersigned, the company has submitted separately (March 20, 1974) data in support of validation of the assay methodology.

COMMENTS:

1. The data on the validation of the assay methodology contains raw data on the recovery of the drug in plasma and standard curves. The data demonstrates the specificity, linearity and sensitivity of the assay method. The lowest detectable drug level with accuracy and reproducibility is 10 mcg/ml.
2. The individual values of the drug level in urine - whether mcg/ml or mg/hr are quite erratic. The peak heights and times to attain the peak heights are also erratic. The areas under the curve for the test and reference drugs are 203.745 mcg/ml and 250.48 mcg/ml respectively. This indicates a mean bio-availability of 81.3% for the applicant's products in comparison to Hydrodiuril (reference product). However, examination of the individual areas under the curve reveal that in 4 out of 13 subjects (30%) the bioavailability of the Richlyn product ranges from 24 to 60% of that of the reference drug.

3. The differences detectable at a significance level of 0.05 and a power of the test of 0.80 for various sampling intervals range from 40% to 100%. The detectable differences at the same significance level and the power of the test for AUCs, peak heights and cumulative excretion are 50%. Cursory calculations at the significance level of 0.05 and the power of the test of 0.60 indicate that the detectable differences still could be in the range of 40% to 90%. These differences are much higher than the customarily acceptable value of 20% detectable differences. The data thus clearly indicates the insufficiency of the number of subjects included in the study.

RECOMMENDATION:

The company should be informed of our comments 1 through 3.  
The study is unacceptable for approval.

/S/

Shrikant V. Dighe, Ph.D.  
Clinical Research Branch

Hydrochlorothiazide Tablets USP  
25 and 50 mg.  
ANDA 83-607

Richlyn Labs., Inc.  
AF 28-724  
Submission dated 5/14/73

REVIEW OF A BIOAVAILABILITY STUDY

1. This study was implemented pursuant to an April 23, 1973 review by DCR of the applicants protocol submitted for our evaluation on March 22, 1973.
2. The study was performed for the applicant by  
It incorporated recommendations made by DCR  
ament additional sampling times, inclusion of an SGOT in the pre-  
clinical screening and the measurement of pH on all urine samples.
3. Twelve normal male volunteers 21-55 years of age weighing  
135-220 pounds were employed in this 6 x 6 crossover study. The  
crossover interval was one week.
4. Subjects fasted for 10 hours prior to test initiation and abstained  
from coffee, tea and carbonated beverages on the test day.
5. In addition to a history and physical, a number of pre clinical  
lab tests were administered. These included WBC, Ht, Hb, urinalysis  
(including microscopic), calcium, phosphorus, glucose, BUN, LDH, uric  
acid, cholesterol, total bilirubin, serum alkaline phosphatase,  
glucose-6-phosphate dehydrogenase and an SGOT.
6. Merck Sharp & Dohme's Hydrodiuril<sup>R</sup> was used as the reference drug.
7. Applicants submission dated 3/22/73 contained formulation data in-  
dicating that the 25 mg. tablet has exactly one half of the drug  
substance and excipient content as the 50 mg. tablet. On this basis  
the results of this bioavailability study would be applicable to the  
25 mg. tablet as well.
8. Each volunteer received a single 50 mg. dose in each phase of the  
crossover. Urine samples were collected at 0, 1, 2, 3, 4, 6, 8, 12,  
and 24 hours. Eight ounces of water was ingested with each urine  
collection to ensure on adequate urinary flow. Volume and pH of  
each urine sample was determined.
9. Richlyn Labs lot 25785 and Merck Sharp & Dohme lot P1666 were  
used in the study. Composite assay and content uniformity analyses  
were 99.4% and 96.5 to 103.5% Richlyn: 100.6% and 96.2 to 107.4%  
MSD.

COMMENTS:

The applicant did not respond to a number of DCR recommendations. The following information is required before an adequate review of the data submitted can be made:

- a) The difference that can be detected between test and reference products at a significance level of 0.05 and a power of the test of 0.80.
- b) The period of time that the subjects abstained from other drugs and alcohol.
- c) The sensitivity, specificity, and linearity of the laboratory method employed must be determined by the laboratory at the level of drug expected in the clinical specimens. All standard curves, recovery data, etc. must be submitted.
- d) The analysis of variance employed did not contain reference to all the variables desired. It should include subject, period or time effects, drug, and sequence effects. A similar analysis will be required for cumulative urine excretion.

RECOMMENDATION:

The applicant should submit the data requested in "a" thru "d".

/S/

Jerome P. Skelly, Ph.D. *U*  
Acting Supervisor  
Division of Clinical Research

Hydrochlorthiazide 25 & 50 mg Tablets  
ANDA 83-607

Richlyn Labs., Inc.  
(AF 28-724)  
Submission dated 3/22/73

#### REVIEW OF A BIOAVAILABILITY PROTOCOL

1. This submission contains a protocol for a bioavailability study to be conducted for the applicant by
2. The study will employ 12 normal male volunteers 21-55 years of age, weighing 135-220 pounds. The applicant should be informed that the number of subjects employed should be sufficient to determine a reasonable difference between test and reference drugs at a significance level of 0.05 and a power of the test of 0.80. This difference should be reported. Also, the subject weights should be within 10% of those contained in the Metropolitan Life Insurance Company's Statistical Bulletin '40', Nov.-Dec., 1959.
3. The protocol indicates that the subjects will abstain from other drugs for at least one week prior to the study. They should, however, abstain from other drugs for two weeks and from alcohol for 48 hours prior to dose administration.
4. They will abstain from coffee, tea, and carbonated beverages on the test day.
5. Subjects will fast for 10 hours prior to dosage and for two hours post dosage.
6. A medical history will be taken and a physical examination given to each volunteer to rule out subjects with a history of ulcers, blood dyscrasia, allergy, renal and/or hepatic diseases, and of hypersensitivity to diuretics. Written informed consent will be obtained.
7. Laboratory studies will include WBC, hematocrit, hemoglobin, urinalysis (including microscopic) calcium, phosphorus, glucose, BUN, LDH, uric acid, cholesterol, total bilirubin, serum alkaline phosphatase, and glucose-6-phosphate dehydrogenase. An SGOT should be included.
8. The reference drug will be Merck, Sharp, and Dohme's Hydrodiuril 50 mg tablets. The test drug will be the applicant's 50 mg tablet. The results will be applicable to the applicant's 25 mg tablet insofar as the latter has exactly 1/2 of the drug substance and excipient content as the 50 mg tablet.
9. The volunteers will receive a 50 mg oral dose with 100 ml water.
10. Urine collection will occur at 0, 2, 4, 6, 8, 12, 16 and 24 hours. It should be better fractionated, however, collection at 0, 1, 2, 3, 4, 6, 8, 12, and 24 hours is recommended.
11. Eight ounces of water should be ingested at each urine collection to ensure an adequate urinary flow.

12. The urine collected will be measured for volume and 100 mg will be retained for lab analysis. This is satisfactory if the drug content can be quantitated by the analytical methodology employed. In addition, however, pH should be determined.

13. The crossover interval was not given but should have been. It should not occur until after 10 halflives of the drug. For hydrochlorthiazide we recommend an interval of one week.

14. Hydrochlorthiazide levels in urine will be determined by the method of Sheppard, et al. The applicant should be informed that the sensitivity, specificity, and linearity of the method must be determined by the laboratory at the level of the drug expected in the clinical specimen. All standard curves, recovery data, etc. should be submitted with the final report.

15. The test and reference product should be assayed for potency and content uniformity.

RECOMMENDATIONS; Acceptance of the protocol provided that points 2, 3, and 7, and 10 through 15 are satisfactorily incorporated.

Jerome P. Skelly, Ph.D.  
Acting Supervisor  
Division of Clinical Research

Hydrochlorothiazide  
50 mg Tablets  
ANDA 83-607

Richlyn Laboratories, Inc.  
Submission Dated:  
February 11, 1977

### REVIEW OF A BIOAVAILABILITY STUDY

#### SUMMARY:

This was a randomized, two way crossover study comparing the bioavailability of hydrochlorothiazide 50 mg tablets of Richlyn Laboratories, Inc. with Hydrodiuril 50 mg tablets of Merk, Sharpe, and Dohme as reference standard. The study was performed by \_\_\_\_\_ under the direction of \_\_\_\_\_. Chemical analysis of hydrochlorothiazide in urine was carried out at the \_\_\_\_\_ supervision of \_\_\_\_\_. Twenty four male subjects originally entered the study. Statistical analysis was carried out on 20 subjects (see comment # 1). Urinary excretion of hydrochlorothiazide was measured over 24 hours following a single 50 mg oral dose. The average cumulative amount excreted in 24 hours was 21.6 and 22.2 mg respectively for the reference and test dosage form. Analysis of variance indicated there was no statistically significant differences between the test and reference drugs in rates of excretion or cumulative amount excreted at different time intervals.

#### RESULTS:

1. The lot numbers of the 50 mg hydrochlorothiazide tablets tested were 30234 - Richlyn Labs. and T3001 - Merk, Sharpe, and Dohme.
2. The study consisted of 24 male subjects, ages ranging from 13 to 37 years and weight from 120 to 200 lbs. All subjects were considered in good health on the basis of medical history, physical exam SMA-12 chemistries, CBC with differential and urinalysis including microscopic examination. No subject deviated more than 10% from their ideal weight.
3. Subjects refrained from all medication including OTC preparations for one week prior to and during the study. The use of Xanthine-containing beverages or alcoholic beverages was prohibited from 12 hours prior to the study. A 50 mg tablet was administered with 8 oz of water after a 12 hour fast. Food was withheld for an additional 4 hours. Urine was voided at the start of the study. (0 hour collection) and collections were made at the following intervals: 0-1, 1-2, 2-3, 3-4, 4-6, 6-8, 8-12, and 12-24 hours. Eight ounces of water were ingested at 1, 2, 4 and 6 hours to maintain proper urine flow. Samples were frozen until analysis. One week separated each phase of the study.
4. The chemical analysis of hydrochlorothiazide was performed by the method of Shepherd, H., Mowles, T.F., and Plummer, A.J., : J. Am. Pharm. Assoc. 49:722 (1960). Urine is extracted with ethylacetate, diazotized and coupled with Bratton - Marshall reagent. The absorbance of the resulting color is measured spectrophotometrically at 510 nm. Daily standard curves were run and were reproducible. Recovery data was variable (see comment # 2).

5. The bioavailability study showed the following:

COLLECTION TIMES (HOURS)	RATE OF EXCRETION (MG/HR)	
	MSD	RLI
0 - 1	0.115 ± 0.298	0.198 ± 0.604
1 - 2	2.863 ± 2.328	2.785 ± 2.673
2 - 3	4.634 ± 2.166	5.072 ± 3.859
3 - 4	2.619 ± 2.038	3.792 ± 3.992
4 - 6	1.660 ± 1.064	1.872 ± 1.242
6 - 8	1.726 ± 1.092	1.323 ± 0.703
8 - 12	0.507 ± 0.318	0.541 ± 0.367
12 - 24	0.198 ± 0.175	0.187 ± 0.133

No statistically significant differences were observed at any time period.

TIME (HOUR)	CUMULATIVE AMOUNT EXCRETED	
	MSD	RLI
1	0.115 ± 0.298	0.198 ± 0.604
2	2.973 ± 2.469	2.983 ± 2.907
3	7.655 ± 4.052	7.903 ± 5.953
4	10.274 ± 4.870	11.696 ± 9.122
6	13.591 ± 5.569	15.424 ± 9.430
8	17.039 ± 6.070	18.064 ± 9.637
12	19.063 ± 6.261	20.055 ± 9.872
24	21.156 ± 7.236	22.206 ± 10.621

No statistically significant differences were observed at any time period.

6. Dissolution studies were performed in a rotating basket apparatus run at 150 rpm in dilute HCl. The results are shown below. The same lots were tested as in the excretion study.

TABLET	PERCENT DISSOLVED IN 30 MINUTES	
	MSD	RLI
1	100.0	92.5
2	102.8	92.5
3	104.2	93.4
4	98.8	93.7
5	96.3	87.3
6	99.7	95.7

COMMENTS:

1. Although 24 subjects entered the study the statistical analysis was carried out on 20 subjects. One subject dropped out. The data of the two subjects was eliminated because of missing values. One subject received the test drug twice.
2. The standards of hydrochlorothiazide were made up in urine and carried through the extraction procedure. A standard curve was run each day. Because of good reproducibility a composite standard curve from the

9 daily standard curves was made and used for all calculations. Recovery at low concentrations, ie below 15 mcg in a 2 ml aliquot, dropped off. Therefore samples with concentrations of drug in this lower range are probably overestimated.

3. The cumulative amount recovered for both the test and reference dosage form are somewhat below the values this reviewer has observed in other studies.

4. A large intersubject variability in the excretion of hydrochlorothiazide was seen. This is a usual observation with hydrochlorothiazide.

RECOMMENDATIONS:

The study has shown that the Richlyn Laboratories, Inc., 50 mg hydrochlorothiazide is bioequivalent to 50 mg Hydrodiuril, MSD and therefore approvable.

The Division of Biopharmaceutics approves lot #30234 as being bioequivalent to MSD lot #T3001 and from the biopharmaceutics viewpoint the ANDA is approvable.

(gr)  
Robert M. Blum, Ph.D., Expert,  
Division of Biopharmaceutics

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NDA 63-607  
AP 28-724

Richlyn Laboratories, Inc.  
Attention: Mr. Edward Rebolio  
Castor & Kensington Avenues  
Philadelphia, Pennsylvania 19124

APR 25 1973

Gentlemen:

Reference is made to your protocol for bioavailability studies for Hydrochlorothiazide Tablets, 25 and 50 mg.

This protocol has been reviewed by our Division of Clinical Research and they have the following comments:

1. The study will employ 12 normal male volunteers 21-55 years of age, weighing 135-200 pounds. The applicant should be informed that the number of subjects employed should be sufficient to determine a reasonable difference between test and reference drugs at a significance level of 0.05 and a power of the test of 0.80. This difference should be reported. Also, the subject weights should be within 10% of those contained in the Metropolitan Life Insurance Company's Statistical Bulletin (1961, Nov.-Dec., 1960).
2. The protocol indicates that the subjects will abstain from other drugs for at least one week prior to the study. They should, however, abstain from other drugs for one week and from alcohol for 48 hours prior to dose administration.
3. Laboratory studies will include: CBC, hematocrit, hemoglobin, urinalysis (including microscopy), calcium, cholesterol, glucose, BUN, LFT, uric acid, cholesterol, total bilirubin, serum alkaline phosphatase, and glucose-6-phosphate dehydrogenase. An ECG should be measured.
4. Urine collection will occur at 0, 2, 4, 6, 8, 12, 16 and 24 hours. It should be better fractionated, however. Collection at 0, 1, 2, 3, 4, 6, 8, 12, and 24 hours is recommended.

5. Eight ounces of water should be ingested at each urine collection to insure an adequate urinary flow.
6. The urine collected will be measured for volume and 100 mg will be retained for lab analysis. This is satisfactory if the drug content can be quantitated by the analytical methodology employed. In addition, however, pH should be determined.
7. The crossover interval was not given but should have been. It should not occur until after 10 half-lives of the drug. For hydrochlorothiazide we recommend an interval of one week.
8. Hydrochlorothiazide levels in urine will be determined by the method of Shepard, et al. The applicant should be informed that the sensitivity, specificity, and linearity of the method must be determined by the laboratory at the level of the drug expected in the clinical specimen. All standard curves, recovery data, etc. should be submitted with the final report.
9. The test and reference product should be assayed for potency and content uniformity.

RECOMMENDATION:

The protocol is acceptable provided that the above 9 points are satisfactorily incorporated into the protocol.

Sincerely yours,

Marvin Seife, M.D.  
Director  
Division of Actions Implementation  
Drug Efficacy Study Implementation  
Project Office  
Bureau of Drugs

Hydrochlorothiazide  
50 mg Tablets  
ANDA 83-607

Richlyn Laboratories  
AF #28-724  
Submission Dated: *JF*  
June 28, 1976

REVIEW OF A BIOAVAILABILITY PROTOCOL

This protocol was submitted to FDA in June 1976. I looked it over and found it to be satisfactory, as I called Mr. Rebollo, Vice President to Richlyn Laboratories and told him that I was sorry for the delay in replying to the protocol and that it was satisfactory. He thanked me and said this was good news because the study was underway.

*12/23/76*

The protocol was written by  
assays will be done by  
to review the protocol further.

ne  
There is no need

RECOMMENDATION:

Approval of the protocol is recommended.

*ISI*

Harold R. Murdock, Ph.D.  
Biopharmaceutics Review Branch

*Noted  
as above  
ISI  
12/23/76*