

Food and Drug Administration
Rockville MD 20857

1982 APR 15 AM 10:22

APR 15 1982

Edward J. Hiross, Ph.D.
Director
Drug Regulatory Affairs
Sterling Drug Inc.
90 Park Avenue
New York, NY 10016

Re: Docket No. 77N-0094
Comment No. C00110

Dear Dr. Hiross:

Reference is made to your company's protocol (number GSA 81-25) for a bioavailability study on enteric-coated aspirin, which was submitted to FDA by cover letter dated January 25, 1982 and is identified as C00110 under docket number 77N-0094.

The Bureau of Drugs has reviewed your company's protocol and has found it to be unsatisfactory. The Bureau offers the following comments for your consideration:

1. The specific objective(s) of the study are unclear and need to be clearly stated. For example, is the objective to demonstrate bioequivalence of your firm's product and Bayer aspirin, or of your firm's product and a particular brand of enteric-coated aspirin?
2. The rationale for the doses chosen should be stated. In addition, the doses of the drugs are unclear and not specified (e.g., "two (or one) tablets as one dose").
3. The four treatments to be used should be explicitly described by brand name. In addition, the drug treatments are not consistently listed. The abstract describes the four treatments as: (A.) "two enteric coated, 325 mg.," (B.) "two enteric coated, 325 mg., tablets," (C.) "one enteric coated, 650 mg., tablet," and (D.) "three, 325 mg., aspirin tablets." The aspirin tablets in (D.) are presumably uncoated Bayer tablets. Is this correct? In the informed consent form for the patient, the four treatments are described as: (1.) "2 x 325 mg enteric coated aspirin tablets," (2.) "2 x 325 mg enteric coated aspirin tablets," (3.) "1 x 650 mg enteric coated aspirin tablets," and (4.) "2 x 325 mg enteric coated aspirin tablets."
4. The protocol states that the study will be a crossover study involving four treatments, but a complete description of the experimental design is not given. A possible experimental design is the following:

77N-0094

ANS //

	<u>Week</u> <u>1</u>	<u>Week</u> <u>2</u>	<u>Week</u> <u>3</u>	<u>Week</u> <u>4</u>
Sequence 1	A	B	C	D
Sequence 2	B	D	A	C
Sequence 3	C	A	D	B
Sequence 4	D	C	B	A

This is a four-treatment, four-period crossover design balanced for the presence of residual effects of the treatments. The four treatments would be randomly assigned to the four treatment labels: A, B, C, and D. Each subject participating in the study should be randomly assigned to one of the four treatment administration sequences, such that an equal number of subjects is assigned to each sequence. Thus, the number of subjects should be a multiple of 4.

In using this design, it is assumed that the clinical facilities are adequate to permit treatment administration to be carried out on each subject on the same day. If the subjects are dosed on different days in each study week, then the design will have to be altered. In addition, the protocol provides a seven day washout period between treatment administrations. Your firm should verify that this period is long enough to prevent direct carryover of drug from one study week to the next.

The above design is still usable if your firm decides to sample blood as well as urine.

5. The measurement of total salicylate from urine is unacceptable for the evaluation of the bioequivalence of enteric-coated aspirin to nonenteric-coated aspirin. The plasma concentrations of the active therapeutic moieties, aspirin and salicylic acid, should be determined. The rate and extent of absorption of both therapeutic moieties should be evaluated. A possible assay method that your firm may wish to consider is enclosed.

6. Your firm should provide assay validation data, i.e., assay sensitivity, specificity, linearity, and reproducibility data. In addition, representative chromatograms should be provided for control, spiked, and collected plasma samples for different concentrations.

7. Precautions should be taken to prevent aspirin degradation during urine and blood sampling, handling, and storage. These precautions should be described in the protocol.

8. The proposed urine collection times do not allow for continuous collection. For example, collection times are proposed for the 0-1 and 2-3 hour interval, but not for the 1-2 hour interval.

9. If an estimate of intrasubject variability is available from previous studies of these dosage forms, the necessary number of subjects may be determined. (Variability estimates for 0-3 hour salicylate excretion and for total cumulative salicylate excretion may possibly be obtained from the data in the paper by Bicket et al., included with your firm's protocol.)

10. The rationale for the selection of treatment groups should be stated. No rationale has been given for the sample size of 12 subjects in the protocol. In addition, there is no discussion of the proposed statistical analysis.

11. Your firm has not discussed the possibility of a potential lag time in absorption following the administration of the enteric-coated product. In addition, your firm has not considered what effect the ingestion of food 4 hours post-drug administration will have on the absorption of the drug.

12. The Bureau suggests that, before conducting a full-scale study, your firm conduct a pilot study in a few volunteers to determine the most accurate blood sampling times for defining the time to each maximum plasma concentration (Tmax), maximum plasma concentration (Cmax), and extent of absorption (AUC) for both aspirin and salicylic acid in each product.

We suggest that you consider the above comments in revising your protocol. After you have had an opportunity to review these comments, we will be happy to meet with you to discuss your proposed study. You may call Mr. Melvin Lessing at (301) 443-4960 to arrange a mutually convenient time. If a meeting is held, it would be helpful if you submit your revised protocol sufficiently in advance to allow us to review it prior

to the meeting. In addition, we would be interested in receiving a curriculum vitae for each of your investigators.

We hope this information is helpful.

Sincerely yours,

A handwritten signature in black ink, appearing to read "W. E. Gilbertson". The signature is written in a cursive style with a large, sweeping flourish at the end.

William E. Gilbertson, Pharm. D.
Director
Division of OTC Drug Evaluation
Bureau of Drugs

Enclosure

Introduction

Several HPLC assay methods for the simultaneously quantitation of aspirin and salicylic acid in biological fluids have been published (1-3). In the method of Peng et al (1), no consideration was given to loss of salicylic acid by sublimation or loss of aspirin by hydrolysis. In addition, the precision and reproducibility work was only carried out at high drug and metabolite concentrations (aspirin - 50 µg/ml; salicylic acid - 100 µg/ml). Amick and Mason (2) used a u.v. detector (280 nm) to monitor the aspirin and salicylic acid. Because of the low molar absorptivity of these compounds at 280 nm and the resulting weak chromatographic peaks, the choice of this detection wavelength is questionable. Although an aspirin sensitivity of 0.2 µg/ml was claimed, validation was only carried out in the 1-7 µg/ml range. The most recent method of Lo and Bye (3) utilized a u.v. detector set at 234 nm. Precision data was given in the report at the 1 µg/ml level for aspirin.

In this study, elements of the previously published methods were evaluated. A method based primarily on that of Lo and Bye was developed and validated (3).

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Apparatus:

A modular HPLC system consisting of a Waters Model M6000A constant flow pump, a Waters Model U6K valve-type injector, a Schoeffel Model SF770 variable wavelength ultraviolet detector (235 nm) and a Kipp and Zonen strip chart recorder (0.5 cm/min) was used in these studies. It was equipped with a Waters reverse-phase µBondapak C-18 column. The mobile phase was a solution of methanol:0.085% phosphoric acid (45:55). A flow rate of 1 ml/min (1500 psig) was established.

Reagents and Materials:

Aspirin, salicylic acid, gentisic acid, salicylic acid and 4-methoxyphenylacetic acid were obtained from a commercial source (Aldrich Chemical Company). Solvents were HPLC grade (Burdick and Jackson Labs.). Standard solutions of each substance were prepared in methanol. (Stock solution - 10 mg/ml; working solution - 0.1 mg/ml). Concentrated phosphoric acid (Fisher Scientific) was diluted to 5% with deionized water (Millipore's Milli Q System).

Assay:

To 0.2 ml plasma in a 20 x 150 mm disposable glass culture tube, 10 µl of solution of 4-methoxyphenylacetic acid (internal standard), 1 ml of 5% H₂SO₄ and 10 ml of CHCl₃ were added. The tubes were vortexed for 30 seconds and centrifuged for five minutes. After aspiration of the top

aqueous phase, the organic phase was transferred by pipet to a second 50 x 150 mm disposable culture tube. The tubes were placed into an ice water bath and the organic phase evaporated to dryness under a gentle stream of nitrogen. The residue was then taken up in 0.5 ml of mobile phase. (Thorough rinsing of the entire walls of the test tube was absolutely necessary to recover all of the salicylic acid.) A 50 μ l aliquot of the solution was injected onto the HPLC column.

Recovery:

Plasma aliquots (0.2 ml) were spiked with known serial quantities of aspirin and salicylic acid. After the samples were treated as described, the peak heights were compared to the peak heights obtained from injection of known quantities of the compounds. Recovery was also estimated by determining the ratio of slopes of the standard plots.

Precision and Accuracy:

Normal human plasma aliquots were spiked with known amounts of aspirin and salicylic acid to give several concentrations. The samples were "blinded" and then assayed as described.

Specificity:

Direct injection of standard solutions of two other known metabolites (i.e., gentisic acid and salicyluric acid) were made using the chromatographic conditions described. In addition, several control human plasma samples were carried through the method as described.

Stability Studies:

To 5 ml control plasma containing 50 μ l of a 50% aqueous solution of NaF (w/v) and 50 μ l of an aqueous solution of heparin (1000 I.U./ml) were added known amounts of aspirin and salicylic acid. Ten aliquots of each spiked plasma sample were taken and immediately frozen. Subsequently, duplicate samples were assayed at 0, 1, 2, 3 and 4 weeks.

Results and Discussion

Studies were carried out to optimize the conditions for the assay of aspirin and salicylic acid. Previously published extraction procedures were evaluated. In two publications (1,2), a benzene-ethyl acetate extraction solvent system was employed. Excellent recovery was reported using this system. However, during our evaluation solvent impurities, as well as residual solvent in the samples, resulted in extraneous interfering chromatographic peaks. In addition, the extended evaporation time that would be necessary to assure the complete evaporation of benzene, raises the potential for loss of salicylic acid by sublimation. Thus this solvent system was not considered acceptable.

Another investigator (3) used chloroform for extraction after protein precipitation with acetonitrile. In our hands, excellent recovery was achieved, however, the emulsions that formed were very difficult to break. Therefore we examined the possibility of using chloroform alone as the extraction solvent. The distribution ratios for aspirin and salicylic acid between chloroform and plasma were determined. From these studies it was established that an absolute recovery of better than 90% was possible using 10 ml of chloroform and 0.2 ml plasma which had been acidified with 1 ml of 5% H_3PO_4 .

The loss of salicylic acid by sublimation during the evaporation process has been reported (1-4). To overcome this problem, it has been suggested that the evaporation process be carried out with the test tubes immersed in an ice bath. However, another investigator (5) found that the test tubes must be removed from the evaporation system within 30-60 seconds after the sample had reached dryness. After examination of this problem, we have found that the sublimed salicylic acid can be recovered. In our study, salicylic acid was added to a group of test tubes. The samples were placed in an ice bath and nitrogen was passed into the test tubes (20 x 150 mm) for up to one hour. After thorough rinsing of the walls of the test tubes, all of the added salicylic acid was recovered (Table I). It should be noted that a larger test tube is needed to facilitate the complete recovery of salicylic acid.

An internal standard has been used in the published methodologies. The internal standards used were: phthalic acid (1,5), p-toluic acid (2) and 3,4-dimethylbenzoic acid (3). Phthalic acid elutes too early for it to be a useful internal standard. The other two substances elute with retention times around twice that of the salicylic acid. In our work we found that 4-methoxyphenylacetic acid eluted between aspirin and salicylic acid. Therefore 4-methoxyphenylacetic acid was used in our subsequent studies.

The method was evaluated for specificity. First, it was determined that no endogenous substances interfered with the peak for 4-methoxyphenylacetic acid. Only very small peaks (less than 2 mm at 0.01 AUFS), having retention times corresponding to aspirin and salicylic acid, were occasionally found in the chromatograms of control plasma samples. Second, standard solutions of two other metabolites were prepared and their retention times determined. Gentisic acid and salicylic acid with retention times of 5.2 and 6.0 minutes respectively, would not interfere in our analysis of clinical samples.

Chromatograms obtained in the analysis of control human plasma and plasma spiked with varying amounts of aspirin and salicylic acid are shown in Figure 1. The retention times for aspirin, 4-methoxyphenylacetic acid and salicylic acid were 6.8, 8.4, and 10.8 minutes, respectively.

A standard linear plasma calibration curve was obtained in the 0.4 to 10 $\mu g/ml$ range for aspirin and in the 4 to 40 $\mu g/ml$ range for salicylic acid (Figure 2). Present sensitivity studies showed that at least 0.4 $\mu g/ml$ aspirin and 4 $\mu g/ml$ salicylic acid could be quantitated. Further studies at lower concentrations will be carried out. The absolute recovery of

aspirin and salicylic acid added to control plasma was studied by comparison of peak heights and the ratio of slopes obtained for the plasma extraction standard curves and the direct standard curves. The absolute recoveries for aspirin and salicylic acid were over 90%. The recovery for 4-methoxyphenylacetic acid was over 75% (Table 2).

To study method precision and accuracy, known amounts were added to control plasma. These samples were assayed in quadruplicate as described. The coefficients of variation (cv) for the assay of aspirin and salicylic acid over the ranges of 0.4-8 μ g/ml for the aspirin and 4-40 μ g/ml for salicylic acid are reported in Table 3.

The stability study of aspirin and salicylic acid in plasma has been initiated. Results of the study will be made available in three weeks.

Conclusions

A relatively simple, precise and reliable analytical method has been developed. The method is sufficiently sensitive so that single dose (650 mg) pharmacokinetic studies can be carried out. Great care, however, is required after the evaporation step to insure that all of the salicylic acid has been dissolved in the mobile phase prior to injection of the sample onto the chromatographic column.

References

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mean recovery (C.V.) 101 \pm 12%

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94%

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	<u>Salicylic Acid</u>	
4.0	3.5	6.2
15.0	16.8	2.9
25.0	23.9	9.4
35.0	35.3	6.3

CHART NO. KZ NR-9

S = 0.01

0 10 20 30 40 50 60 70 80 90

Std
ASA = 5
SA = 20
S = 0.01

Std
ASA = 2.5
SA = 10
S = 0.02

100 90 80 70 60 50 40 30 20 10

Std
ASA = 5 ug
SA = 5 ug

S = 0.01

Blank plasma injection

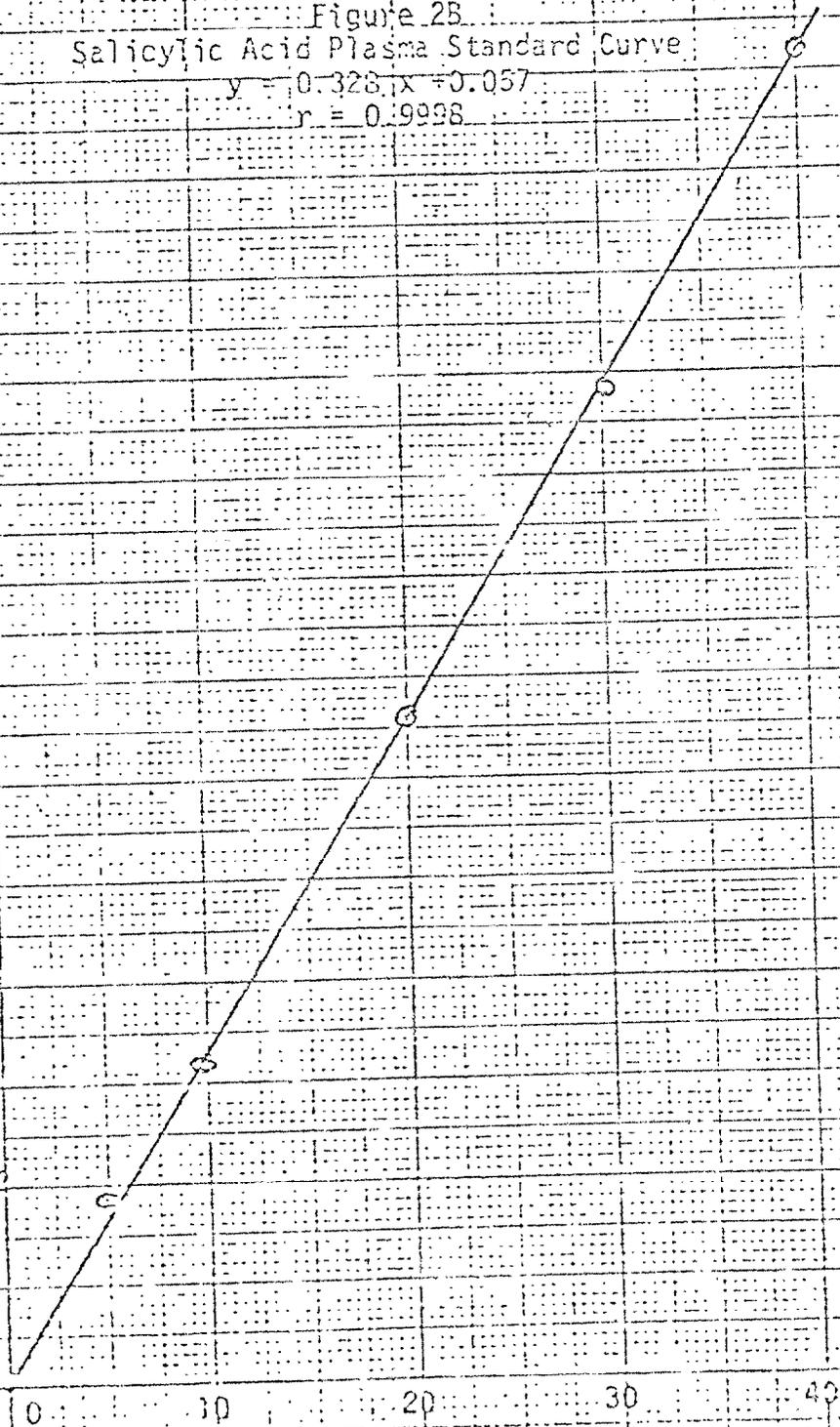
FIGURE 1

CHROMATOGRAMS FROM ASSAY OF ASPIRIN AND SALICYLIC ACID IN PLASMA

PEAK HEIGHT RATIO

Figure 2B
Salicylic Acid Plasma Standard Curve

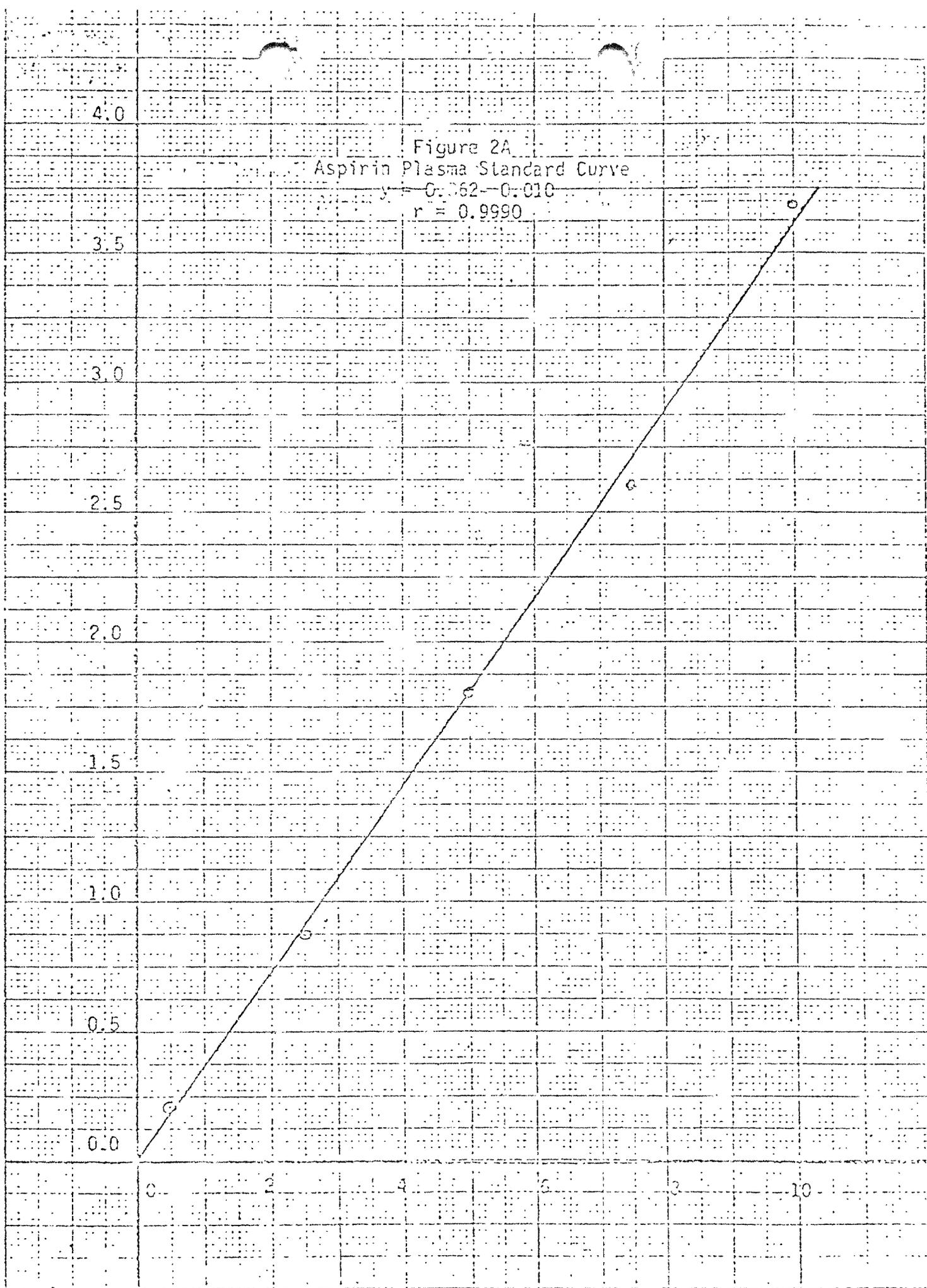
$$y = 0.328x + 0.057$$
$$r = 0.9998$$



SALICYLIC ACID (µg/ml)

20 X 20 (11) P-9101

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CHART NO. 17 XR-9

S=0.1

0 20 30 40 50 60 70 80 90

↑ STD
ASA = 5
SA = 20
S = 0.1

↑ STD
ASA = 2.5
SA = 10
S = 0.2

100 90 80 70 60 50 40 30 20 10

↑ STD
ASA = 5.4
SA = 5.4

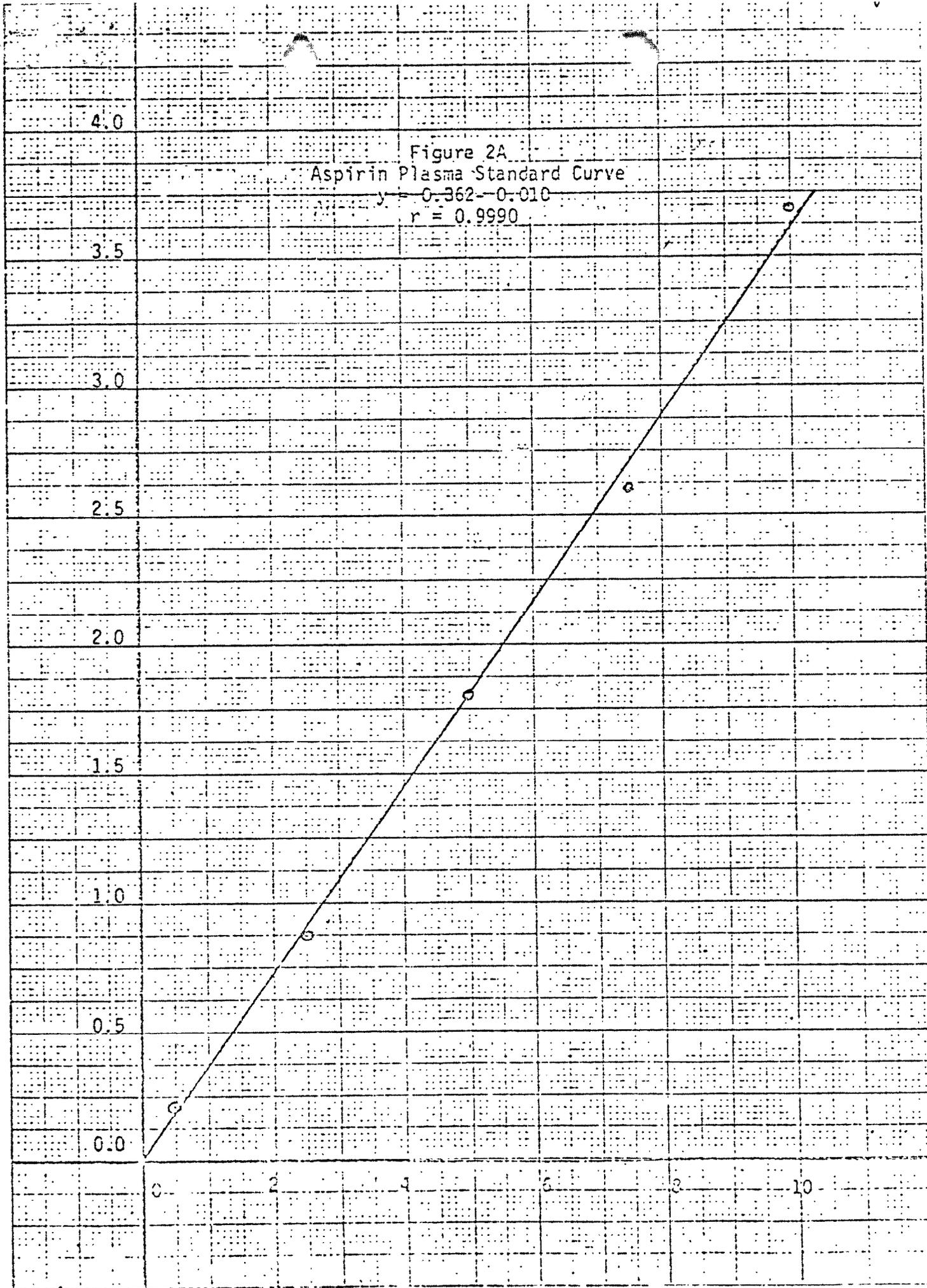
S=0.1

Blank plasma injection

FIGURE 1
CHROMATOGRAMS FROM ASSAY
OF ASPIRIN AND SALICYLIC
ACID IN PLASMA

30 X 20 PER INCH

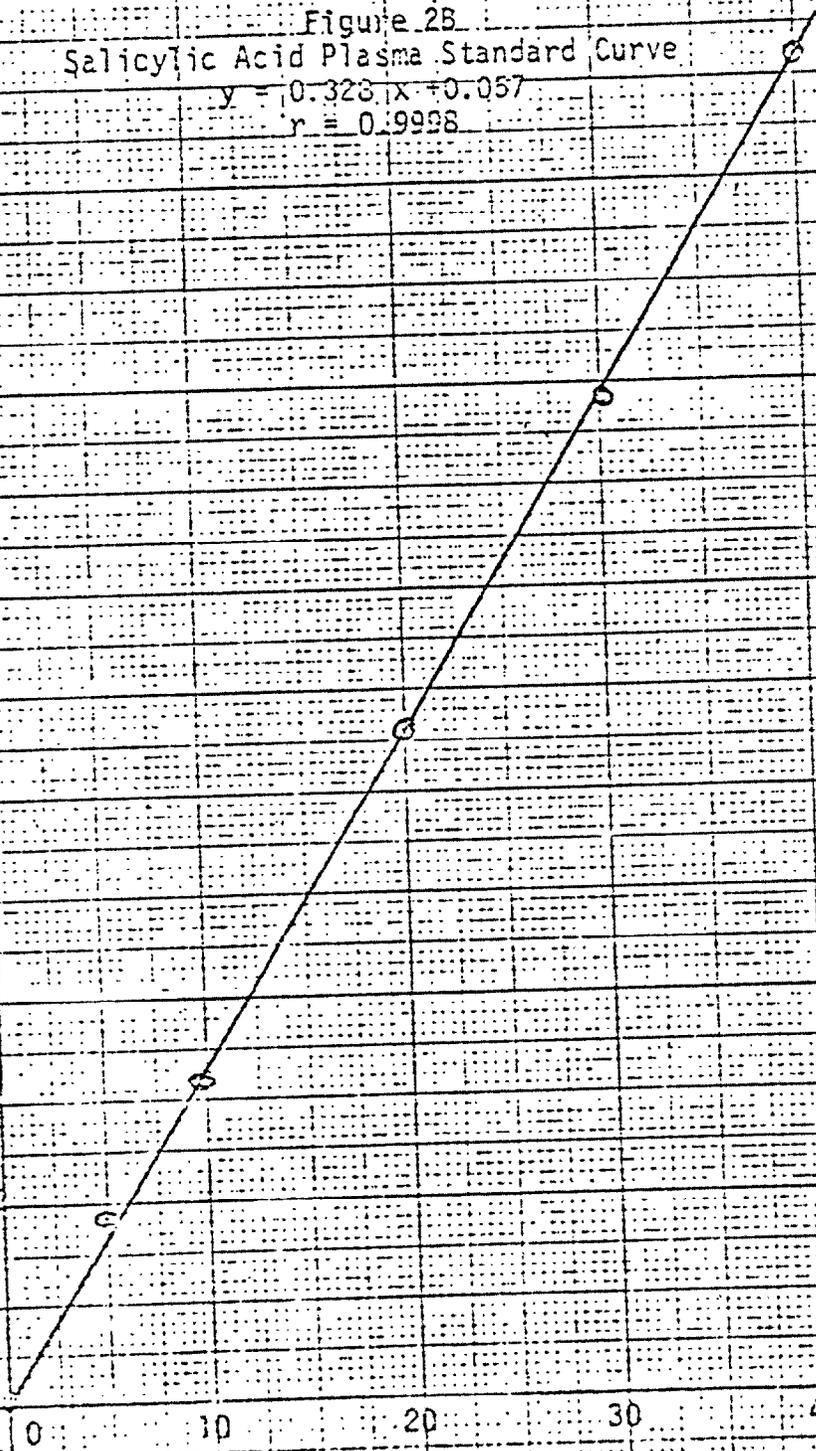
PEAK HEIGHT RATIO



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Figure 2B
Salicylic Acid Plasma Standard Curve

$$y = 0.328x + 0.057$$
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SALICYLIC ACID (µg/ml)

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Dockets Management Branch (HFA-305)

DATE: APR 15 1982

FROM : Director
Division of OTC Drug Evaluation (HFD-510)

SUBJECT: Material for Docket No. 77N-0094

- The attached correspondence should be placed on public display under the above referenced Docket No.
- This correspondence should be cross-referenced to Comment C00110.



William E. Gilbertson, Pharm. D.

Attachment