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**Preformulation Studies of Selected  
Pretreatment and Therapeutic Compounds**

**Annual Report**

**John L. Lach  
Douglas R. Flanagan  
Lloyd E. Matheson, Jr.**

**July, 1982**

**Supported by**

**U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701**

**Contract No. DAMD17-79-C-9136**

**College of Pharmacy  
University of Iowa  
Iowa City, Iowa 52242**

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Preformulation Studies of Selected  
 Pretreatment and Therapeutic Compounds

Annual Report



John L. Lach  
 Douglas R. Flanagan  
 Lloyd E. Matheson, Jr.

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Summary

This annual report represents preformulation and formulation studies conducted in the third year of this contract on the following drugs:

WR6026·2HCl

WR171,669·HCl

WR142,490·HCl

WR180,409·H<sub>3</sub>PO<sub>4</sub>

This work consists of stability studies and liposome development work on WR6026·2HCl; development of a high pressure liquid chromatographic assay for WR180,409·H<sub>3</sub>PO<sub>4</sub> in blood; production of placebo tablets of WR142,490·HCl and placebo capsules of WR171,669·HCl; and production and quality control reports on WR6026·2HCl placebo, one mg and five mg capsules.

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Quarterly Report Number 8  
(August 1, 1981 to September 30, 1981)

Liposome Development Studies  
on WR6026·2HCl

John L. Lach, Principal Investigator  
Douglas R. Flanagan, Assistant Principal Investigator  
Lloyd E. Matheson, Jr., Assistant Principal Investigator

October, 1981

Supported by  
U.S. Army Medical Research and  
Development Command  
Fort Detrick  
Frederick, Maryland 21701

Contract No. DAMD 17-79-C-9136

College of Pharmacy  
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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Summary

This quarterly report contains further studies on the development of a liposomal drug delivery system for WR6026·2HCl.

These studies include further investigations on improving entrapment efficiency and reproducibility, release of entrapped WR6026·2HCl from negative and neutral liposomes and scale-up of liposome batch size. ↗

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### Introduction

Liposome development studies have centered on further methods to optimize entrapment efficiency, investigating the leakage characteristics of washed liposomes, and scaling up the batch size of liposomes produced. All work has employed WR6026·2HCl as the drug entrapped with UV spectral assay for measuring entrapment.

### Experimental

The methods for preparing liposomes have been presented in our previous reports (See Annual Report dated July 1981) as well as methods for assaying entrapment. It was considered worthwhile to briefly review them for the sake of completeness.

Liposomes are prepared by depositing dipalmitoylphosphatidyl choline (DPPC) and cholesterol on the wall of a round bottom flask from a chloroform solution with a vacuum rotary evaporator with gentle heating (<40°C). The usual molar ratio of DPPC and cholesterol is 4:3 and other additives like dicalcylphosphate (DCP) and  $\alpha$ -tocopherol (Vit. E) are often added in much lower molar ratios (i.e. less than 1 part to 7 parts lipid). The chloroform is completely removed from the lipid layer in a vacuum desiccator. The lipid is then swollen into liposomes by gently shaking the lipid-coated flask in a water bath at 40°C with a swelling solution of WR6026·2HCl at a particular concentration. Swelling may require from less than an hour to overnight to remove all of the lipid from the flask wall. Other swelling techniques to speed the process employing glass beads and ultrasonification have met with varying success.

Once the liposomes are swollen, the entire dispersion can be assayed for drug content (entrapped and unentrapped) by removing an aliquot (usually 0.1 ml) and dissolving the entire contents in isopropanol and then reading the absorbance for this isopropanol solution adjusted to be acidic with HCl at 289 nm ( $\epsilon = 20,251$ ) on a Pye-Unicam 8100 spectrophotometer. This total dispersion assay allows correction for volume changes due to lipid incorporation and/or evaporation during the swelling process.

To measure entrapment, an aliquot of the dispersion (0.1 - 1.0 ml) is centrifuged and washed three times with normal saline or appropriate dilutions of normal saline with re-centrifugation after each washing. The decanted swelling solution and washings are pooled, adjusted to pH2 with HCl and then its absorbance is measured at 262 nm ( $\epsilon = 17,548$ ). The assay of the pooled washing solutions and decanted swelling solution gives a measure of entrapped drug by taking the difference from the total dispersion assay. This is not as accurate an assessment of entrapment as direct assay of the washed liposomes.

Direct assay of the washed liposomes is obtained by dissolving them in isopropanol and adding a drop of concentrated HCl with UV analysis at 289 nm as with the total dispersion assay. The value so obtained for entrapment is used in our report for the entrapment efficiency assessment.

Further Entrapment Studies

As a continuation of entrapment efficiency studies discussed previously (see Annual Report dated July 1981) we investigated using a 1 mg/ml concentration of WR6026 for swelling. At 5 mg/ml and 10 mg/ml, it was shown that up to 20% could be entrapped in neutral liposomes by increasing the lipid concentration (i.e., decreasing swelling solution volume). At 1 mg/ml the lipid swells and is dispersed in approximately 10 minutes with gentle shaking and no ultrasonification. Direct assay of the washed liposomes gives an average entrapment of 20.3% (19.6% and 22.0% for two samples).

The study at 5 mg/ml was repeated with WR6026 added to the lipid solution rather than in the swelling solution. For the neutral liposomes produced by swelling the lipid/drug layer with distilled water an average entrapment of 20.35% was obtained (two samples give 21.9% and 19.9%).

To determine the reproducibility of entrapment when the swelling volume is reduced by two-fold for a given amount of lipid, a total of nine samples were prepared of negatively charged (DCP-containing) liposomes swollen with a isotonic WR6026 solution. Given below are the concentrations of entrapped WR6026

| <u>Sample #</u> | <u>Entrapped Conc (mg/ml)</u> |
|-----------------|-------------------------------|
| 1               | 12.74                         |
| 2               | 9.73                          |
| 3               | 10.39                         |
| 4               | 12.08                         |
| 5               | 10.91                         |
| 6               | 12.38                         |
| 7               | 11.99                         |
| 8               | 12.33                         |
| 9               | 17.65                         |

The results average around 12% entrapment with only one sample (#9) falling outside a reasonable range around this average.

Further confirmation that decreasing the swelling volume would lead to a higher entrapped fraction of the swelling solution was obtained by preparing identical neutral lipid-coated flasks and swelling one with 1.5 ml and the other with 3.0 ml of 104 mg/ml WR6026. Direct assay of the washed liposomes gave 1.86 mg/ml entrapped (1.93 mg/ml and 1.79 mg/ml) for the 3 ml swelling volume while the 1.5 ml swelling volume gave 3.36 mg/ml entrapped (3.19 mg/ml and 3.54 mg/ml). This study adds further confirmation to the proposal that more concentrated lipid dispersions will entrap a higher fraction of the swelling solution. We are now determining the practical lower limit for the swelling solution volume before the viscosity becomes too high to disperse the lipid.

Since the lipid thickness on the flask wall appears to be an important factor to efficient entrapment, we decided to increase its area of coverage by the use of fine glass beads. The beads were either 0.25-.3 mm or 0.1-.11 mm in diameter. It was hoped that incorporating 4 or 8 gm of these beads into the lipid solution would increase the area over which the lipid was deposited upon evaporation and thus improve reproducibility and/or degree of entrapment upon swelling.

In practice the major problem with this method was that the lipid caused the beads to clump together rather than

staying dispersed individual beads. Upon swelling the beads dispersed once the lipid was removed. Entrapment was as high as 2.3% when 4 gm of 0.25-.3 mm beads were used and as low as 0.92% when 8 gm of 0.1-.11 mm beads were used. We are exploring methods to individually spray coat these glass beads with lipid to explore this method further because theoretically it should provide the surface area for deposition required for preparation of large batches without requiring flask size that are inconvenient for shaking or rotating on the flash evaporator.

#### Liposome Leakage Studies

Since the liposome entrapped drug will require shipping and periods of storage before being used it was deemed necessary to determine how fast entrapped drug would be released from washed liposomes. To this end an aliquot of washed neutral and negative liposomes was dispersed in 1.5 ml of normal saline and sealed in a dialysis bag (molecular weight cut-off = 50,000). The dialysis bag was then shaken in 40 ml of normal saline at room temperature. The normal saline was replaced at various time intervals and assayed for WR6026 content. After 25 hours the drug content remaining inside the liposomes was determined by dissolving the contents of the dialysis bag in isopropanol and assaying by the UV method for drug content. Figure 1 and Table I show the cumulative percent released for the negative and neutral liposomes.

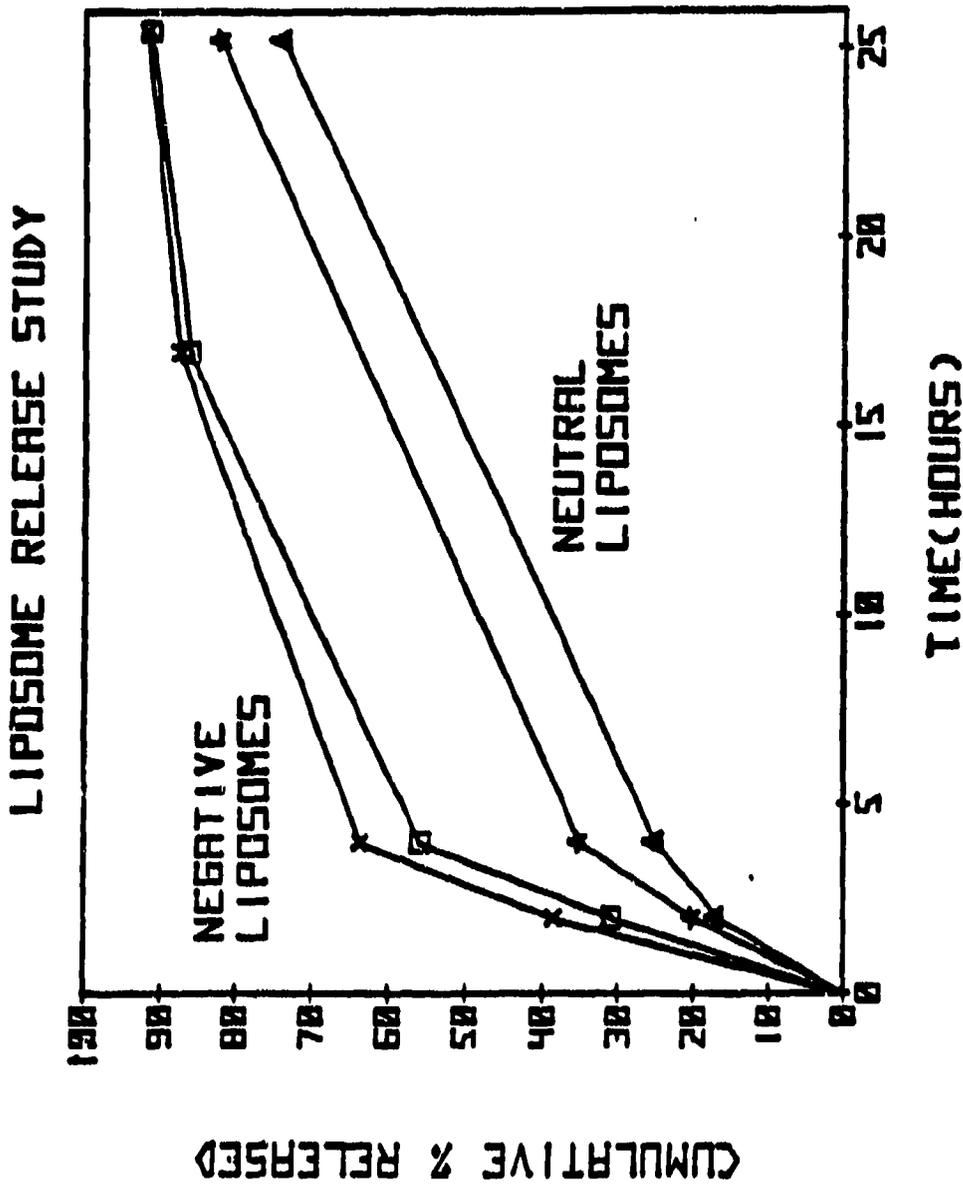


Figure 1. Release of WR6026-ZnCl from Liposomes. Key: x, ■ - Negative Liposomes; ▲, Δ - Neutral Liposomes



Table I  
Percent Release of WR6026  
from Washed Liposomes

|                       | <u>Sample No.</u> | <u>2 hours</u> | <u>4 hours</u> | <u>17 hours</u> | <u>25.5 hours</u> |
|-----------------------|-------------------|----------------|----------------|-----------------|-------------------|
| Negative<br>Liposomes | 1                 | 30.9           | 55.9           | 86.3            | 91.4              |
|                       | 2                 | 38.6           | 63.7           | 87.7            | 91.9              |
| Neutral<br>Liposomes  | 1                 | 16.9           | 25.0           | -               | 74.0              |
|                       | 2                 | 20.0           | 35.1           | -               | 82.2              |

Initially, it appears that the negative liposomes release drug faster than neutral liposomes. This is probably due to the fact that the negative liposomes tend to be smaller and stay better dispersed than the neutral liposomes. Thus the apparent permeability differences between the two are not due to intrinsic differences in wall permeability but rather to other physical characteristics which affect the total release characteristics of the liposomes. It is readily apparent though that both types of liposomes are quite leaky and thus cannot be kept in the washed state for any significant period of time before a substantial fraction of the entrapped contents diffuses out.

It was then considered necessary to determine the stability of centrifuged liposomes maintained as a plug in contact with swelling solution incorporated in the plug. Neutral liposomes were prepared with a 104 mg/ml WR6026 swelling solution, centrifuged and the excess swelling solution decanted. The liposome plug was then stored under various conditions, and assayed at periodic intervals for

drug content remaining entrapped. Table II gives the results of storage of refrigerator, freezer and 40°C.

Table II  
Entrapment Stability of Unwashed Liposomes  
Stored at Various Temperatures

| <u>Time (Days)</u> | <u>Temperature</u>       |                       |             |
|--------------------|--------------------------|-----------------------|-------------|
|                    | <u>4°C(Refrigerator)</u> | <u>-10°C(Freezer)</u> | <u>40°C</u> |
| 0                  | 4.30 mg/ml               | 4.30 mg/ml            | 4.30 mg/ml  |
| 3                  | 3.49 mg/ml               | 1.56 mg/ml            | 3.77 mg/ml  |
| 10                 | 3.49 mg/ml               | -                     | -           |
| 13                 | 3.69 mg/ml               | -                     | -           |
| 20                 | 3.73 mg/ml               | -                     | -           |
| 26                 | 3.52 mg/ml               | -                     | -           |

Table III gives the results of a repeat of these entrapment stability studies at freezer, 40°C and room temperature (20-25°C) conditions.

Table III  
Further Entrapment Stability of Unwashed  
Liposomes at Various Temperatures

| <u>Time (Days)</u>                  | <u>-10°C(Freezer)</u> | <u>40°C<sup>a</sup></u> | <u>R.T.(20-25°C)<sup>a</sup></u> |
|-------------------------------------|-----------------------|-------------------------|----------------------------------|
| 0                                   | 4.51 mg/ml            | 4.51 mg/ml              | 4.05 mg/ml                       |
| 0<br>(freeze & thaw<br>immediately) | 4.41 mg/ml            | -                       | -                                |
| 4                                   | -                     | -                       | 4.17 mg/ml                       |
| 5                                   | 1.92 mg/ml            | 4.67 mg/ml              | -                                |
| 8                                   | 2.00 mg/ml            | 4.32 mg/ml              | -                                |
| 10                                  | -                     | -                       | 4.55 mg/ml                       |
| 28                                  | -                     | -                       | 5.07 mg/ml                       |

<sup>a</sup>Liposomes dry out, become solid and are difficult to redisperse.

These studies demonstrate that freezing has a deleterious effect on liposome entrapment while refrigerator, room temperature and 40°C have a less deleterious effect on entrapment. It appears that entrapment may even go up with long term storage but this is probably an artifact of the liposome plug drying out and becoming more concentrated with time. To redisperse the dried liposome plug one is in effect rehydrating the lipid which can give variable entrapment unless one is careful in redispersing such a desiccated system.

It is possible that the liposomes could be transported as centrifuged plugs with the swelling solution removed and then redispersed in an injection vehicle before administration. Before this can be done with confidence the amount of unentrapped drug entrained between the liposomes must be determined to learn what fraction of administered drug would be outside the liposomes. Washing solutions were assayed separately to estimate untrapped drug within the liposome plug. It was found that 14.25 mg (average of three samples) was removed in the first washing which amounts to approximately 0.14 ml of the 104 mg/ml swelling solution remaining within the liposome plug. Second and third washings gave 0.33 mg and 0.25 mg removed, respectively. The amount of drug remaining within the plug of unwashed liposomes is over 3-fold higher than the entrapped level and is probably not acceptable for normal clinical administration since the entrapped drug is significantly outweighed by untrapped drug. The only

situation in which with could be used would be in cases in which the entrapped drug is far superior clinically to unentrapped, in which case the unentrapped drug would be substantially less active.

Since the levels of unentrapped drug left in the unwashed liposome plug were unacceptably high, the leakage of drug from a liposome plug that was washed once was investigated. In this case neutral liposomes were employed and the decanted supernate contained 84.4 mg/ml of WR6026 from an original 104 mg/ml swelling solution. The first washing removed 12.5 mg/ml additionally and the liposome plug was left at room temperature (20-25°C). Direct assay of the liposomes initially gave 3.88 mg/ml entrapped, after 2 days, 2.73 mg/ml was entrapped, after 7 days, 2.07 mg/ml was entrapped and after 18 days, 1.76 mg/ml was entrapped. Thus the leakage into the fluid between the liposomes is quite substantial once the swelling solution has been replaced by normal saline. It appears that the liposomes may have to be transported in contact with their swelling solution and washed once before administration to test animals or subjects to remove unentrapped drug.

Further work on reducing the unentrapped drug in liposome plugs is centering on evaluation of special centrifuge tubes which contain a long narrow tip at the bottom. These tips hold around 0.5 ml and may permit more complete separation of swelling solution once the liposome plug has been centrifuged into the tip. We will report on these studies in our next report.

Previously we reported on attempts to force WR6026·HCl into liposomes by raising the pH to change its degree of protonation to determine if the monoprotinated or free base forms could be pushed into the liposomes (see Annual Report dated July 1981). This did not prove to be a very successful method for loading the liposomes. Since we have observed a fairly fast leakage out of WR6026·2HCl, we felt that would be worthwhile to use doubly protonated WR6026 and try to get it to penetrate empty liposomes. This reverse diffusion method employed neutral empty liposomes prepared by swelling in normal saline. The liposomes were centrifuged, the normal saline decanted off and replaced with 2.5 ml of 116 mg/ml WR6026·2HCl (Note - the higher concentration was used to counteract the dilution by normal saline in and around the liposomes in the centrifuged plug). The liposomes were redispersed and shaken for 20 hours at 40°C. Direct assay of the washed liposomes gave 3.53% entrapped WR6026 from the 290 mg contained in the 2.5 ml of 116 mg/ml concentration. This reverse diffusion method into empty liposomes may offer some advantages for large scale preparation since large batches of blank liposomes can be quickly prepared because the lipid hydrates rapidly in normal saline. Reverse diffusion will also be explored further as a means to enhance entrapment by reducing the drug solution volume used for loading the liposomes so that a higher fraction could be loaded into the liposomes.

### Scale-Up of Liposome Batch Size

Since most liposome test batches were less than 10 ml in size it was considered valuable to gain experience with the preparation of larger batch sizes in anticipation of having to prepare large quantities of liposome entrapped drug for animal or clinical trials.

Table IV contains the results of these scale-up studies for various batch sizes each prepared in a 3 liter round bottom flask instead of a 100 ml round bottom flask.

Table IV  
Scale-Up of Liposome Batches and The  
Resulting Entrapment Efficiency

| <u>Batch Size<br/>(ml)</u> | <u>Swelling Time<br/>(hours)</u> | <u>Entrapped Conc<br/>(mg/ml)<sup>a</sup></u> | <u>%<br/>Entrapped</u> |
|----------------------------|----------------------------------|---|------------------------|
| 255                        | 4                                | 2.08 (2.04, 2.04,<br>2.17, 2.08)              | 2.10                   |
| 105                        | 8                                | 4.27 (4.68, 3.87)                             | 4.14                   |
| 55                         | 6 5                              | 5.44 (5.69, 5.18)                             | 5.35                   |
| 25                         | 21.5                             | 5.09 (5.14, 4.58,<br>5.25, 5.39)              | 4.72                   |
| 15                         | 17.5                             | 3.39 (4.00, 3.70,<br>4.08)                    | 3.77                   |

<sup>a</sup>Values in parentheses are actual individual assay values.

After 6 days in the refrigerator, the percentage entrapments were 2.16% (255 ml batch), 3.50% (105 ml batch) and 4.72% (55 ml batch). The entrapment reductions observed are the same as seen previously with maintaining the liposome plugs in the refrigerator in contact with their swelling solution. Also the 55 ml batch size appears to give the highest entrapment in the 3 liter flask. Reductions in

entrapment with larger batches are observed because the lipid layer is thicker and does not hydrate as efficiently. In going from a 100 ml round bottom flask to a 3 liter the area for deposition of lipid increases 10-fold. Thus, if the usual batches in a 100 ml flask were 3-10 ml, an equivalent scale-up would be a 30-100 ml batch size in a 3 liter flask. This is only an approximate calculation but suffices to demonstrate that the 105 ml and 255 ml batch sizes probably would require a 5 liter round bottom flask to obtain an equivalent surface area. Above a 3 liter size is impractical for swelling because of the requirement of continuous shaking. The 25 ml and 15 ml batch sizes are too small to be considered practical but were prepared to determine whether spreading the lipid out over a large flask area would give even higher percent entrapment than obtained with the 55 ml batch. They did not, most likely because it was quite difficult to move the small volume of swelling solution evenly over the flask surface to obtain efficient swelling of all the lipid. Thus, it appears that 55 ml is a practical batch size to work with and if larger batches are needed, multiple 55 ml batches can be prepared and pooled.

The preparation of a 55 ml batch size was repeated a number of times to determine the reproducibility of entrapment. Table V contains the entrapment efficiency for each 55 ml liposome batch.

Table V  
Entrapment Reproducibility for 55 ml  
Liposome Batches

| <u>Batch No.</u> | <u>Entrapped Conc. (mg/ml)</u> | <u>% Entrapped</u> |
|------------------|--------------------------------|--------------------|
| 1                | 5.44 (5.69, 5.18)              | 5.35               |
| 2                | 4.07 (4.12, 3.97, 4.06, 4.14)  | 3.84               |
| 3                | 3.19 (3.27, 3.14, 3.09, 3.25)  | 3.00               |
| 4                | 3.58 (3.57, 3.56, 3.61, 3.59)  | 3.46               |
| 5                | 3.06 (3.05, 3.11, 3.13, 2.96)  | 2.93               |
| 6                | 2.53 (2.83, 2.23)              | 2.48               |
| 7                | 1.86 (1.82, 1.89)              | 1.82               |
| 2a*              | 4.51 (4.53, 4.51, 4.50)        | 4.26               |
| 4a*              | 4.30 (4.14, 4.14, 4.60)        | 4.16               |
| 2a-4a*           | 4.05 (4.03, 4.04, 4.07)        | 3.86               |

\*Reassay of batches and mixtures of batches for use in other studies.

It appears that there is significant variation in entrapment with this 55 ml batch. We have not been able to obtain the high entrapment of batch 1 again and batches 6 and 7 are low because apparently the round bottom flask had a slight residue of cleaning detergent remaining. The round bottom flasks must be carefully cleaned, rinsed and dried or else entrapment is reduced because the deposition of lipid is uneven and it then does not hydrate properly. We are looking into other possible causes of the observed variability to learn whether there are uncontrolled variables which we need to consider in more detail.

Based upon our present experience we feel that a 4% entrapment can be reasonably expected with this scaled up size of 55 ml which would give 220 mg of entrapped drug for each batch. This should be sufficient for screening studies in small animals since the dose reported by Alving (1) in his work did not exceed 1 mg/kg. For larger animals or human trials a more efficient means of preparing large batches will be required.

To this end we are investigating the use of an ultrasonic sprayer for preparing liposomes continuously. Behrens (2) has shown that atomizing the lipid solution into a warm solution of the compound to be entrapped produced a liposomal system with entrapment that equaled or exceeded the flask deposition method. In this method the lipid solution droplet governs the amount of lipid per liposome and ultimately its size. The warm drug solution should evaporate the lipid solvent or it may evaporate during atomization. Behrens (2) worked with only a crude insufflator to atomize his lipid solution to explore this method.

We have obtained an ultrasonic spray nozzle that can generate fine droplet sizes from 0.1-10 microns. It is highly efficient and reproducible in its spray characteristics and requires only a 30 psi source of compressed air or nitrogen to generate the fine mist. The compressed gas serves as a sonic generator inside the nozzle into which liquid is pushed which in turn is broken up into a fine aerosol mist. We will be evaluating this system for the

atomization of the lipid solution as a fine mist which can then be directed into a solution of WR6026·2HCl where the solvent will evaporate leaving the lipid to entrap drug. We will report on the results of our feasibility study with this method in our next report.

#### Electron Microscopy

We have been working with the University of Iowa Electron Microscopy Service to obtain negative stain electron micrographs of our liposomal preparations. We have run into a number of problems because the typical negative staining agents (ammonium molybdate, phosphotungstic acid, etc.) react or interact with WR6026 to alter, distort, aggregate or destroy the liposomes. We have also learned (private communication from Dr. C.A. Hunt, University of California at San Francisco) that the success rate for obtaining acceptable electron micrographs by negative staining of liposomes is around 20-25%. Many electron microscopic grids need to be prepared and examined to find a few good ones. We are continuing this work and hope to have acceptable electron micrographs in the near future. We are also planning to use the freeze fracture technique to obtain replicas of frozen liposome dispersions but we must wait for the equipment for this procedure to be installed in our Electron Microscopy Lab.

### Conclusions

These liposomes containing WR6026·2HCl have been shown to be quite leaky which will require special shipping and/or handling consideration to deliver liposomes to animals or human subjects with known drug content. Further work will be conducted on exploring means by which leakage can be retarded or eliminated by the addition of additives (i.e. hydrophilic gums, albumin, etc.) to the washed liposomes. The use of WR6026 in liposomes may ultimately be limited by the leakage characteristics of the liposomes which will limit the time and conditions under which they can be stored or shipped.

Large batches of liposomes can be prepared but the batch size is governed by the flask that can be practically used with the common rotary evaporators. Other methods (i.e., using an ultrasonic sprayer) need to be explored to eliminate this restriction of flask size governing batch size and entrapment efficiency.

### References

1. C.R. Alving, E.A. Steck, W.L. Chapman, B. Waits, L.D. Hendricks, G.M. Swartz, Jr. and W.L. Hanson, *Life Sci.*, 26, 2231 (1980).
2. B.C. Behrens, Ph.D. Thesis, University of Wisconsin, Madison, 1980, pp. 134-163.

AD \_\_\_\_\_

Quarterly Report Number 9  
(October 1, 1981 to December 31, 1981)

- I. Placebo Capsule Production for WR171,669·HCl  
(Halofantrine Hydrochloride)
- II. Placebo Tablet Production for WR142,490·HCl  
(Mefloquine Hydrochloride)

John L. Lach, Principal Investigator  
Douglas R. Flanagan, Assistant Principal Investigator  
Lloyd E. Matheson, Jr., Assistant Principal Investigator

January, 1982

Supported by  
U.S. Army Medical Research and  
Development Command  
Fort Detrick  
Frederick, Maryland 21701

Contract No. DAMD 17-79-C-9136

College of Pharmacy  
University of Iowa  
Iowa City, Iowa 52242  
(319/353-4520)

Distribution limited to U.S. Government agencies only for contract or performance evaluation; January, 1982. Other requests for this document must be referred to the Commander, U.S. Army Medical Research and Development Command (ATTN: SGRD-AJ) Fort Detrick, Frederick, Maryland 21701.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

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### Introduction

Placebo capsules of WR171,669·HCl (Halofantrine Hydrochloride) were produced to be indistinguishable in appearance and formulation characteristics from those produced according to the procedure developed by Lafayette Pharmacal, Inc. (1) and previously employed to produce active capsules (2). Starch was employed to replace the active drug and the wetting agent (Pluronic L-101) was deemed unnecessary.

Placebo tablets of WR142,490·HCl (Mefloquine Hydrochloride) were produced to be similar in appearance and formulation characteristics to those produced by Lafayette Pharmacal, Inc. (3). Lactose was employed to replace the active drug and a number of the components which were deemed unnecessary (i.e., Pluronic L-101, Keltose and a portion of the Avicel PH-101) were omitted.

### Formulation

Since the goal of placebo production is to reproduce the active formulation in appearance and general performance characteristics a number of economies can be effected which do not materially affect the final product.

In the placebo capsule for WR171,669·HCl, it was deemed unnecessarily difficult to match the bulk density of the drug with an excipient with the same bulk density to obtain capsules of identical weight. It was thought unlikely that the active capsules could be distinguished from placebo capsules if they were nominally within 50 mg of each other.

Also, the wetting agent, Pluronic L-101, was considered superfluous since no hydrophobic drug was incorporated in the formulation.

In the placebo tablets for WR149,490·HCl, the first wet granulation step used by Lafayette (3) was deemed unnecessary since it is primarily involved with producing drug granules of optimum tableting characteristics. Without active drug there is no difficulty in producing good tablets by compression. Also, there was no need for the wetting agent, Pluronic L-101, Keltose and the Avicel PH-101 in this first granulation step. Thus, Lactose (USP) was employed to constitute the entire portion of the formulation (421.2 mg) identified as WR-142490 granulation. For the product mixture, the same components and levels were used as reported by Lafayette (3).

#### Production

Sufficient quantities of Sta-Rx 1500 Starch were employed to produce 1560 capsules (1440 capsules packaged and shipped). These capsules weighed 406.2 mg (theoretical - 390 mg) while the active capsules weighed 344.5 mg (theoretical - 347.5 mg). This weight difference was considered insignificant to be detected by clinicians or subjects.

The Sta-Rx 1500 Starch was filled into the same Parke-Davis #0 white opaque capsules (Stock #046-021-999-999, Lot #TD0493-2) used for the production of active capsules (2). A Parke-Davis capsule filling machine was used to fill the capsules.

Capsules were removed for the following tests: weight variation and disintegration.

The finished capsules were packaged into 11 dram amber glass vials with standard closures (Owens-Illinois, PW-4011) using a Drug-O-Matic automatic filling machine. The packaged capsule vials were labeled with individual labels numbered 1 through 72 with 20 capsules in each vial and a shrink seal added to each. The packaged capsules were shipped by Federal Express to Walter Reed Army Institute of Research. Further details of the manufacturing procedure can be found in Appendix I on page 31.

For the WR142,490-HCl placebo tablets, sufficient quantities of Lactose (USP), Avicel PH-10L, Sta-Rx 1500 Starch, Magnesium Stearate (NF) and Talc (NF) were employed to produce 1225 tablets (1200 tablets packaged and shipped).

With the elimination of the wet granulation, all components except the lubricants could be dry blended in a V-blender. Magnesium stearate and talc were then successively blended into the powdered mix. The powdered mix was then compressed on a Manesty single punch tablet machine with a standard concave (7/16") punch and die set.

Tablets were removed during the tabetting process for monitoring weight variation, hardness and thickness. The final tablets were vacuumed, inspected for defects, samples taken for disintegration testing and packaged.

The tablets were packaged into 11 dram amber glass vials with standard closures (Owens-Illinois, PW-4011) using a Drug-O-Matic automatic filling machine. The packaged tablet vials were labeled with individual labels numbered 1 through 60

with 20 tablets in each vial and a shrink seal added to each. The packaged capsules were shipped by Federal Express to Walter Reed Army Institute of Research. Further details of the manufacturing procedure can be found in Appendix II on page 39.

#### Quality Control Results

No in-process or final assay of contents were required for the capsules or tablets since they contained no active components.

For the WR171,669 placebo capsules the weight variation (based on 20 capsules) was less than 5% around an average weight of 406.2 mg. Even though this is 50 mg greater than the active capsule weight, it is doubtful that this difference can be detected. A disintegration test in 900 ml of water at 37°C gave an average (6 determinations) disintegration time of 4 minutes which is the same as the active capsules.

For the WR142,490 placebo tablets, the weight variation (based on 20 tablets) was less than 2.5% around an average weight of 563.9 mg. Disintegration times in 900 ml of water (37°C) were around 35 seconds for six tablets. Each tablet had an average thickness of 5.9 mm with a hardness of 4.6 kg. These tablets were produced to show the same characteristics as the active tablets produced by Lafayette which showed poor integrity with a high tendency to chip and split. These placebo tablets should have the same general characteristics.

Conclusions

Both the WR171,669 placebo capsules and WR142,490 placebo tablets should serve as satisfactory placebos for clinical trials and be indistinguishable to clinicians and subjects from those containing active drug.

References

1. Arthur L. Kunz, Lafayette Pharmacal, Inc., Report No. 74-4-206, Supplemental No. 1-206-51, May 1974.
2. John L. Lach, Interim Report No. 2, April, 1981, Contract No. DAMD 17-79-C-9136, University of Iowa, College of Pharmacy, Iowa City, Iowa.
3. Robert A. Sharp, Lafayette Pharmacal, Inc., Report No. 80-4-406, Supplemental No. 1-406-65, January, 1980.

APPENDIX I

Manufacturing Formula and Quality Control Tests on WR171,669-HCl  
(Halofantrine Hydrochloride) Placebo Capsules



Product Placebo Capsules for WR 171,669 HClList No. WRA 2Batch Size 1500Control No. WRA-2-12291

Action or Special Instructions

507

| BATCH CONTAINS | INGREDIENTS AND DIRECTIONS  | RAW MAT'L<br>CONTROL NO. | INITIAL | AMOUNT<br>PER BATCH |
|----------------|---|--------------------------|---------|---------------------|
| 150 mg.        | 1. Weigh Corn Starch.   | Staley A.E.              | KS MV   |                     |
|                | 2. Fill #0 White Opaque Capsules<br>on Parke-Davis capsule machine.<br>Weigh 10 capsules from each ring<br>(the weight should be 3.9 gm). | lot: 678781              | KS MV   |                     |
|                | 3. Clean capsules with salt.  | Parke-Davis              | KS MV   |                     |
|                | 4. Yield: 1560 Capsules   | lot: 720491-2            | KS MV   |                     |
|                | # of capsules packaged: 1440  |                          | KS MV   |                     |
|                | Retain sample: 120 Capsules   |                          | KS MV   |                     |
|                | # (30 Capsules for 11 drawn bottles)  |                          |         |                     |



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1847

**WEIGHT VARIATION OF FINISHED CAPSULES**

Lot # WRA-2-12291

| <u>No.</u> | <u>Mg/Capsule</u> | <u>No.</u> | <u>Mg/Capsule</u> |
|------------|-------------------|------------|-------------------|
| 1          | 400               | 11         | 417               |
| 2          | 426               | 12         | 414               |
| 3          | 395               | 13         | 412               |
| 4          | 426               | 14         | 390               |
| 5          | 400               | 15         | 404               |
| 6          | 400               | 16         | 409               |
| 7          | 420               | 17         | 398               |
| 8          | 410               | 18         | 404               |
| 9          | 392               | 19         | 400               |
| 10         | 407               | 20         | 400               |

Average fill: 406.2 mg/Capsule

Deviation from low (390) = 4.0%

Deviation from high (426) = 4.8%

Balance was tared with empty capsule.

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1847

**DISINTEGRATION TEST**

Item: Placebo Capsules for WR 171669 AD

Lot No.: WRA-2-12291

Apparatus: USP XX, p. 958

Temperature: 37° C.

Medium: 900 ml Distilled Water

Test: 4 minutes (average of six determinations)

1720

D. R. Berg  
File

Mrs. Mary Hansen  
University of Iowa  
College of Pharmacy  
Iowa City, Iowa 52244

## STA-Rx Starch USP

G-73781

6/6/79

NO. 1720

DATE RECEIVED

.012

|                           |      |
|---------------------------|------|
| Moisture, %               | 11.5 |
| Iron - PPM                | 0    |
| Ash, %                    | .06  |
| pH                        | 6.2  |
| Color                     | 2.7  |
| Flavor                    | OK   |
| Odor                      | OK   |
| Screen Analysis:          |      |
| On #80 (X)                | .92  |
| On #325 (X)               | .93  |
| Sulphur Dioxide, PPM      | 21.0 |
| Oxidizing Substance       | OK   |
| FM                        | 2.0  |
| Standard Plate Count      | 40.0 |
| Mold/Gram                 | 0    |
| Yeast/Gram                | 0    |
| Salmonella                | Neg. |
| E. coli                   | Neg. |
| Pseudomonas aeruginosa    | Neg. |
| Coag. Pos. Staph. Species | Neg. |

*S. Martin*  
S. Martin

**Product: STA-Rx 1500 starch**

**Manufacturer: Staley Manufacturing Co.  
Lot G-73781**

**Identification Tests:**

a) Heat with hot water: Translucent and jelly

b) Iodine Test: Positive

**Result: Meets USP requirements**

**Control No. PG-117-059**

*Stc.*

## APPENDIX II

Manufacturing Formula and Quality Control Tests on WR142,490-HCL  
(Mefloquine Hydrochloride) Placebo Tablets



Product Placebo Tablets for WR 142,490 HClList No. WRA 3Batch Size 1500Control No. WRA-3-12291

Caution or Special Instructions

1507

| EACH CONTAINS | INGREDIENTS AND DIRECTIONS   | RAW MAT'L CONTROL NO.          | INITIAL | AMOUNT PER BATCH |
|---------------|--|--------------------------------|---------|------------------|
| 421.2 mg      | 1. Weigh Lactose USP Anhydrous.  | Sheffield                      | BZ m    | 632 gm           |
| 60.0 mg       | 2. Weigh Avicel PH 101.  | Lot # INC 19<br>FMC            | BZ m    | 90 gm            |
| 55.0 mg       | 3. Weigh Sta-Rx 1500.  | Lot # 1114<br>Colson           | BZ m    | 82.5 gm          |
|               | 4. Blend Lactose, Avicel and Sta-Rx in a V-blender for 10 minutes.<br>Mixing started at 10 <sup>00</sup> AM; stopped at 10 <sup>15</sup> AM  | 12/29/01                       | BZ m    |                  |
| 5.5 mg        | 5. Weigh Magnesium Stearate and transfer it to the V-blender (4).  | Mullinckraht<br>Lot KMKH       | BZ m    | 8.25 gm          |
|               | 6. Blend it for 5 minutes.<br>Time: Mixing started: 10 <sup>20</sup> AM; mixing stopped 10 <sup>25</sup> AM  | 12/29/01                       | BZ m    |                  |
| 11.0 mg       | 7. Weigh Talc and transfer it to the same V-blender (4).   | Thompson Minerals<br>Lot 94478 | BZ m    | 16.5 gm          |
|               | 8. Blend it for an additional 5 minutes.<br>Time: Mixing started: 10 <sup>30</sup> AM; mixing stopped 10 <sup>35</sup> AM  | 12/29/01                       | BZ m    |                  |
|               | 9. Compress on single punch tablet machine (Manisty) using 7/16 inch S.C. (standard concave) punch and die set. Monitor tablet weight (5.52 gm for 10 tablets), hardness (> 4 kg) and thickness. |                                | BZ m    |                  |
|               | 10. Vacuum tablets, inspect and package.   |                                | BZ m    |                  |
|               | 11. Package 20 tablets in 11 dram amber glass vials using Drug-O-Matic automatic filling machine.  |                                | BZ m    |                  |
|               | 12. Yield: 1225 tablets  |                                | BZ m    |                  |
|               | # of tablets packaged: 1200  |                                |         |                  |
|               | Retain sample: 25 tablets  |                                | BZ m    |                  |
|               | * (20 tablets per 11 dram bottles)   |                                |         |                  |

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1947

**WEIGHT VARIATION OF FINISHED TABLETS**

Lot No: WRA-3-12291

| <u>No.</u> | <u>Mg/Tablet</u> | <u>No.</u> | <u>Mg/Tablet</u> |
|------------|------------------|------------|------------------|
| 1          | 566              | 11         | 561              |
| 2          | 572              | 12         | 560              |
| 3          | 559              | 13         | 557              |
| 4          | 568              | 14         | 561              |
| 5          | 571              | 15         | 557              |
| 6          | 565              | 16         | 562              |
| 7          | 568              | 17         | 560              |
| 8          | 556              | 18         | 568              |
| 9          | 577              | 19         | 564              |
| 10         | 562              | 20         | 565              |

Average fill: 563.9 mg/tablet

Deviation from low (556)=1.4%

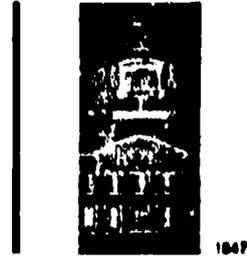
Deviation from high (577) = 2.3%

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**DISINTEGRATION TEST**

Item: Placebo Tablets for WR 142,490 HCl

Lot No.: WRA-3-12291

Apparatus: USP XX, p. 958

Temperature: 37°C

Medium: 900 ml Distilled Water

Test: 15 seconds (average of six tablets)

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1847

**IN-PROCESS CONTROL FOR PLACEBO TABLETS**

Lot # WRA-3-12291

| <u>Hardness</u><br>( $> 4$ kg) | <u>Thickness</u><br>( $5.9 \pm 0.5$ mm.) |
|--------------------------------|--|
| 5.0                            | 5.90                                     |
| 4.8                            | 5.92                                     |
| 4.0                            | 5.88                                     |
| 5.2                            | 5.90                                     |
| 6.0                            | 5.90                                     |
| 4.6                            | 5.92                                     |
| 4.0                            | 5.90                                     |
| 4.4                            | 5.90                                     |
| 4.4                            | 5.88                                     |
| 4.0                            | 5.90                                     |

Average - 4.6 kg

Average - 5.90 mm



FMC CORPORATION  
 Food & Pharmaceutical Products Division  
 1301 Oglatown Road  
 Newark, Delaware 19711

PRODUCT QUALITY CONTROL REPORT

PRODUCT: AVICEL PH-101  
 Microcrystalline Cellulose, N.F.

LOT NO: 1114  
 DATE : 4/9/81

Identification

Conforms to NF XV

Loss on Drying, %

3.3-4.4

Heavy Metals, ppm

<10

Residue on Ignition, ppm

41

Water Soluble Substances, mg/5g

5.2

Particle Size, WT. % + 60 mesh

<0.1

WT. % + 200 mesh

11-29

pH

6.4

Assay, % cellulose

98.6

Search Test

negative

Retained on a screen having 37 um openings, wt. %

> 5

Identification

passes

*R. B. Wortz*  
 R. B. Wortz  
 Quality Control Manager

1981  
 PURCHASING DEPT.



COLORCON, INC. 2117 N. GALE STREET, INDIANAPOLIS, INDIANA 46218  
(317) 545-6211

STA-RX 1500 STARCH PECTACOL

BATCH NO: 905029

DATE OF REPORT 4/16/80

ANALYTICAL DATA:

|                      |        |
|----------------------|--------|
| Loss on drying       | 10.6%  |
| Residue on ignition  | 0.12%  |
| Iron                 | <10ppm |
| pH                   | 5.6    |
| Oxidizing Substances | NEG    |
| Sulfur Dioxide       | OK.    |

Microbial Limits:

|                             |     |
|-----------------------------|-----|
| Standard Plate Count, per g | <10 |
| Mold, per g                 | <10 |
| Yeast, per g                | <10 |
| Salmonella                  | NEG |
| E. Coli                     | NEG |
| Pseudomonas Aeruginosa      | NEG |
| Coagulase Positive          | NEG |
| Staphylococcus Species      | NEG |

Screen Analysis:

|                               |      |
|-------------------------------|------|
| On U.S. No: 8, %              | 0.0  |
| On U.S. No: 40, %             | 0.01 |
| Through U.S. No: 100, %       | 93   |
| Cold Water Solubles, % d.s.b. | 11.7 |

APPROVED FOR SHIPMENT BY

*G.A. Hunter*

COLORCON, INC.

# Mallinckrodt, Inc.

48

Page 9 of 10 pages

PARIS BY-PASS

PO. BOX 11

PARIS, KENTUCKY 40361

(606) 987-7000

ITEM - MAGNESIUM STEARATE NF

CODE 2256

LOT KHKH

## TESTS

Identification

Loss on drying

Lead (Pb)

Assay (MgO)

Sieve test US Standard #325 Mesh

## RESULTS

Passes Test

3.72%

0.0001%

7.72%

99.8%

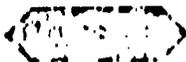
It is hereby certified that the above is a true copy of the actual analysis of the subject item.



Ted Dubowski  
Manager Quality Control  
Mallinckrodt, Inc.  
Paris, Kentucky

8/31/81

bc



SCIENCE PRODUCTS COMPANY

Product: Magnesium Stearate USP

Manufacturer: Mallinckrodt, Inc.

Lot # KHKH

IDENTIFICATION TESTS:

Result: Meets USP Requirements  
(USP XX, p 1235)

Control No.: Z-051-048

AD \_\_\_\_\_

Quarterly Report No. 10  
Preformulation Studies on Antimalarials:  
Stability of WR6026·2HCl and Blood Assay  
for WR180,409·H<sub>3</sub>PO<sub>4</sub>

Quarterly Progress Report  
January 1, 1982 to March 31, 1982

John L. Lach, Principal Investigator  
Douglas R. Flana, Assistant Principal Investigator  
Lloyd E. Matheson, Assistant Principal Investigator

May, 1982

Supported by  
U.S. Army Medical Research and  
Development Command  
Fort Detrick  
Frederick, Maryland 21701

Contract No. DAMD 17-79-C-9136

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Iowa City, Iowa 52242  
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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Summary

This quarterly report contains results from ongoing stability studies on WR6026·2HCl under various conditions in aqueous media and the results of the development of a quantitative blood assay for WR180,409·H<sub>3</sub>PO<sub>4</sub> using a high pressure liquid chromatographic procedure.

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Rayonet Mini-Photochemical Reactor Stability Studies

Stability studies on WR6026·2HCl have been continuing with the recent purchase of a Rayonet Mini-Photochemical reactor since first being reported in the Annual Report dated July 1981. The advantages of this equipment over the previously used photochemical system are two-fold. First, the wavelength of the UV light is more easily controlled and secondly the temperature in the system does not rise above 35°C. The only drawback of this reactor is its relatively small capacity.

The buffer compositions were described in Annual Report No. 2 on page 42. The conditions for the high pressure liquid chromatographic assay were the same as those mentioned on page 48 of the second annual report except that a 20 µl loop was used in the Rheodyne injector. Six mg of WR6026·2HCl dissolved in 200 ml of the appropriate buffer system with and without 1% W/V ascorbic acid was used as the starting solution. Stability conditions are shown in Table I. Six ml of the starting solution was placed into

Table I: Stability Conditions for Rayonet Mini-Photochemical Reactor Stability Studies

| <u>Condition</u> | <u>Run</u> |   |   |   |
|------------------|------------|---|---|---|
|                  | 1          | 2 | 3 | 4 |
| 1% Ascorbic Acid |            | X |   | X |
| pH2 Buffer       |            | X | X | X |
| pH6 Buffer       | X          |   |   |   |
| Clear Glass      | X          | X |   |   |
| Amber Glass      |            |   | X | X |
| Air Headspace    | X          | X | X | X |

several six ml clear or amber glass vials, which were sealed with rubber stoppers. Approximately 300  $\mu$ l of solution was withdrawn with a needle for each sample alternately from two vials. This was done to check whether location of the sample within the photochemical reactor affected the results. Standard curves were prepared weekly to check the performance of the chromatographic system. The percent of WR6026 $\cdot$ 2HCl remaining over time is given in Table II and shown in Figure 1 for the conditions described in Table I.

Table II: Percent of WR6026 $\cdot$ 2HCl Remaining as a Function of Time.

| <u>Time (Hrs)</u> | <u>Run</u> |          |          |          |
|-------------------|------------|----------|----------|----------|
|                   | <u>1</u>   | <u>2</u> | <u>3</u> | <u>4</u> |
| 0                 | 100        | 100      | 100      | 100      |
| 5.5               | -          | 98       | -        | -        |
| 24                | 81         | 76       | 85       | 84       |
| 48                | 12         | 44       | 83       | 63       |
| 72                | -          | 26       | 79       | 53       |
| 96                | 9          | 6        | 80       | 39       |

Conditions: All runs had an air headspace.

- Run 1: pH6 buffer, no ascorbic acid, clear glass
- Run 2: pH2 buffer, ascorbic acid, clear glass
- Run 3: pH2 buffer, no ascorbic acid, amber glass
- Run 4: pH2 buffer, ascorbic acid, amber glass

Again it is obvious in comparing Run 2 with Run 4 that the amber glass helps to protect the drug. It also appears when comparing Runs 3 and 4 that the antioxidant ascorbic acid is decreasing the stability of the drug. The reason for this

is not yet known.

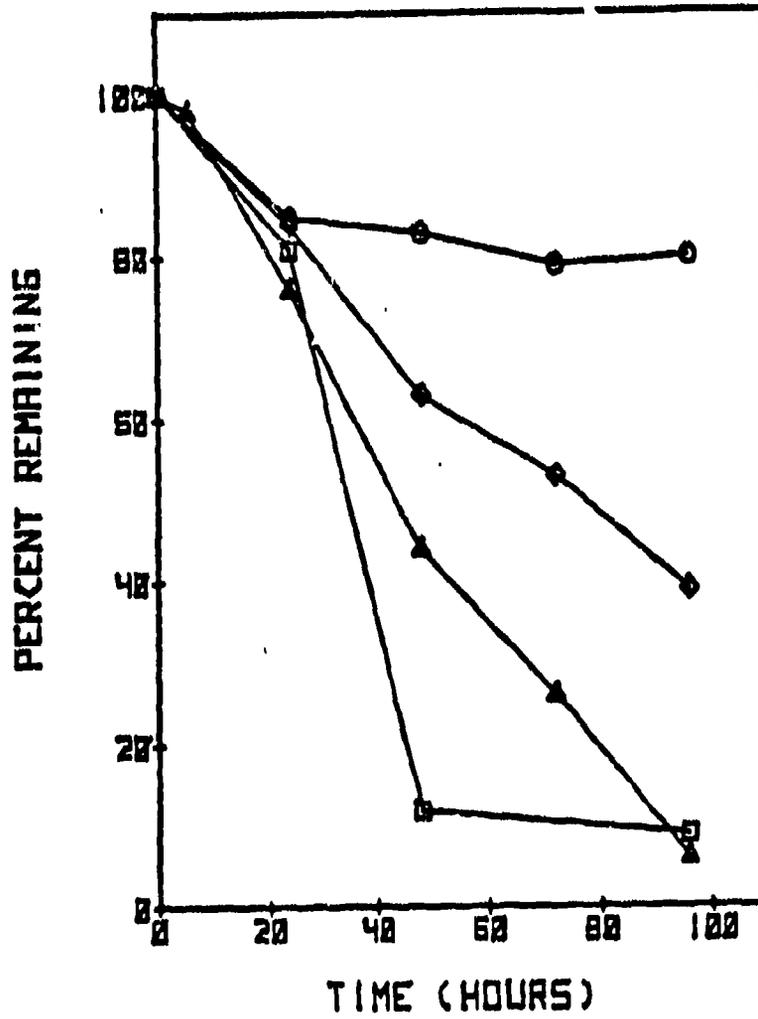


Figure 1. Percent of WR6026·2HCl remaining as a function of time. Run 1, □ ; Run 2, Δ; Run 3, ○; Run 4, ◇ .

Effect of Ethylenediaminetetracetic Acid (EDTA) on WR6026·2HCl Stability

Since heavy metal ions are often responsible for catalyzing oxidation and photochemical degradation reactions, another series of experiments was designed to observe the effect of chelating any heavy metals in the system with a 0.1% W/V solution of the tetra sodium salt of EDTA. Data are given in Table III and shown in Figure 2.

Table III: Effect of EDTA on WR6026·2HCl Stability. Percent of WR6026·2HCl Remaining with Time.

| Time (Hrs) | Run |     |     |     |
|------------|-----|-----|-----|-----|
|            | 1   | 2   | 3   | 4   |
| 0          | 100 | 100 | 100 | 100 |
| 24         | 91  | 95  | 90  | 98  |
| 72         | 76  | 82  | 65  | 98  |
| 144        | 47  | 54  | 22  | 78  |
| 168        | 38  | 49  | 12  | 70  |
| 192        | 20  | 48  | 12  | 78  |

Conditions: All runs had an air headspace and used a pH2 buffer.

- Run 1: Ascorbic acid, clear glass
- Run 2: Ascorbic acid, amber glass
- Run 3: No ascorbic acid, clear glass
- Run 4: No ascorbic acid, amber glass

It is apparent from the data in this table for Run 4 compared to Run 3 in Table II that EDTA has enhanced the stability of WR6026·2HCl during the early stages of degradation. It appears, at this point in time, that the best conditions for enhancing stability for this drug in the liquid form would be: pH 2 buffer, amber glass and the presence of EDTA. Work is continuing to determine the usefulness of a nitrogen purge. An effective antioxidant is also being sought.

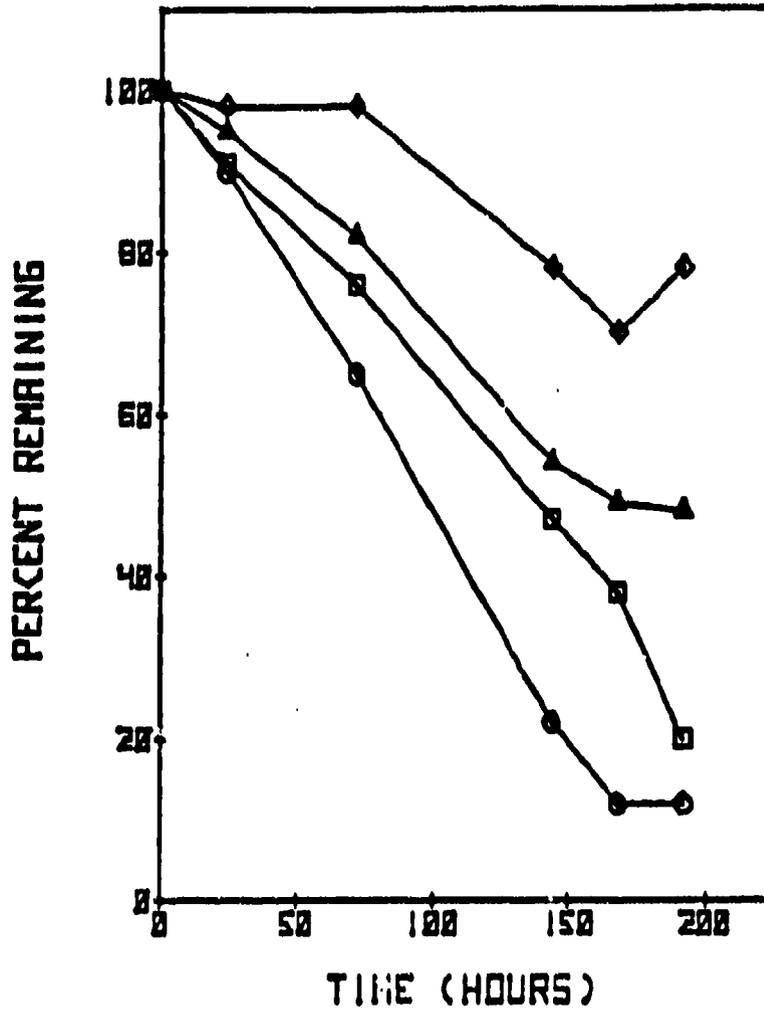


Figure 2. Effect of EDTA on WR6026-2HCl stability. Percent of WR6026-2HCl remaining as a function of time. Run 1, □; Run 2, Δ; Run 3, ○; Run 4, ◇.

Quantitative Blood Assay for WR180,409·H<sub>3</sub>PO<sub>4</sub>

An attempt was made to develop a quantitative assay for WR180,409·H<sub>3</sub>PO<sub>4</sub> in blood using high pressure liquid chromatography.

Extraction Procedure

1. A known volume of blood was mixed with a known amount of WR180,409·H<sub>3</sub>PO<sub>4</sub> and allowed to equilibrate with shaking at room temperature for one hour.
2. The blood samples (usually 1 ml) were lysed by sonification with an ultrasonic probe or by quick freezing in a dry ice/acetone bath and then thawing.
3. The lysed sample was then centrifuged at 3000 rpm in an IEC Model CS centrifuge for 10-15 minutes.
4. To 1 ml of the supernatant was added 2.5 ml of 0.05 N NaOH containing 75 mg/ml NaCl.
5. The sample was then extracted 3x with 2 ml volumes of 40% V/V diethyl ether in n-hexane. The mixture was centrifuged each time to separate the layers and the organic layer was drawn off.
6. The pooled extract was evaporated in a stream of dry nitrogen with gentle heating.
7. The dried extract was redissolved in n-hexane and back extracted 3x with 60-100 µl of 0.005 N H<sub>2</sub>SO<sub>4</sub> containing the internal standard, WR184,806·H<sub>3</sub>PO<sub>4</sub>, at a concentration of 3.71 µg/ml. The mixture was centrifuged each time to separate the layers and the aqueous layer was drawn off.
8. The entire pooled aqueous extract was injected into a 200 µl loop injector.

It is necessary to lyse and extract whole blood because the drug partitions into the red blood cells. Substantial amounts of drug are lost if these cells are not lysed prior to extraction. High pressure liquid chromatographic conditions are given in Table IV. Typical chromatograms are shown in Figure 3. Retention time for WR180,409·H<sub>3</sub>PO<sub>4</sub>

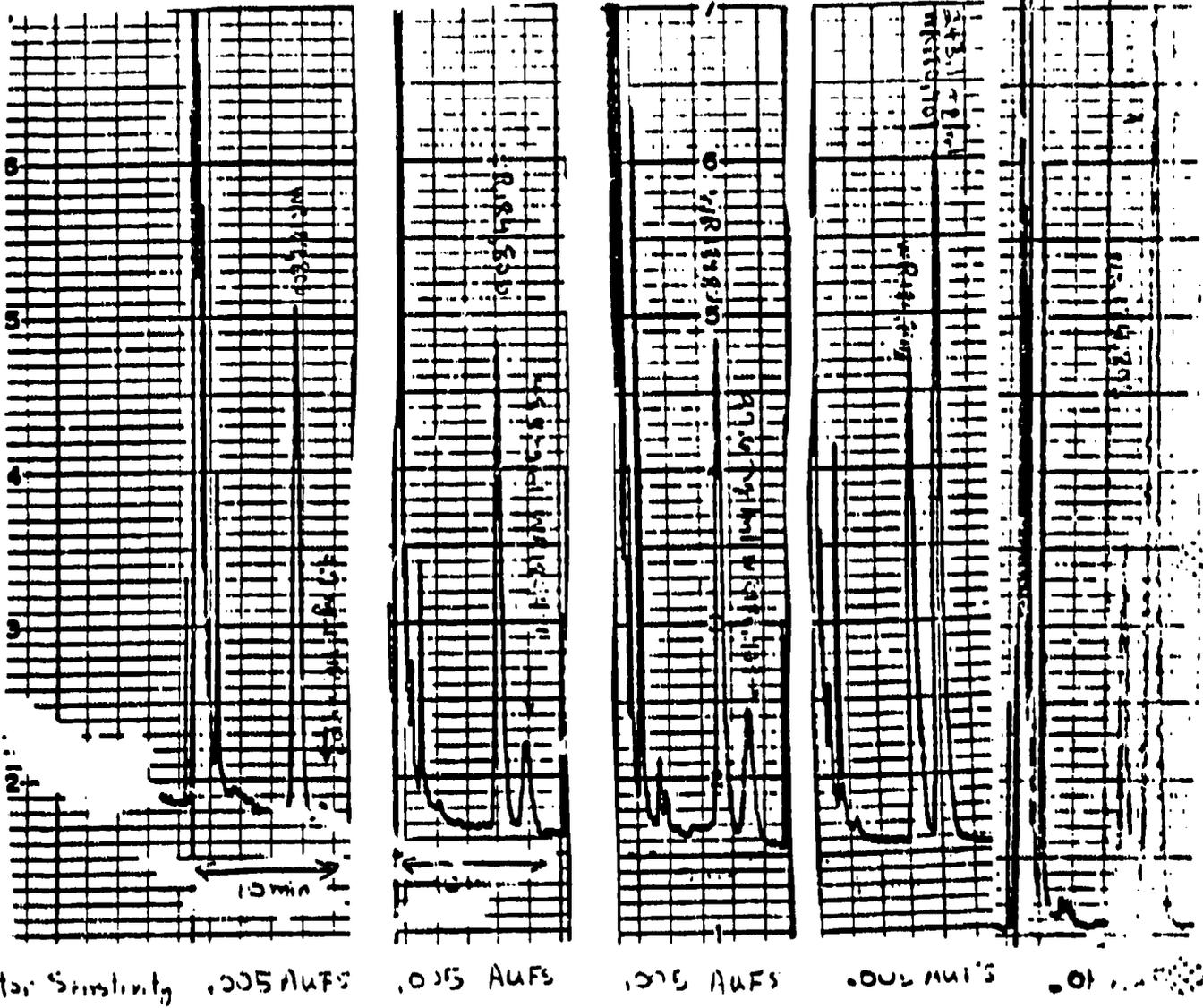


Figure 3. Typical chromatograms for  
 WR180,409·H<sub>3</sub>PO<sub>4</sub>

Table IV. High Pressure Liquid Chromatographic Conditions for the Analysis of WR180,409·H<sub>3</sub>PO<sub>4</sub> in Blood

|               |   |
|---------------|---|
| System:       | Waters M6000A pump with a Waters 440 UV detector at 254 nm and a Rheodyne 7125 injector with a 200 µl loop.   |
| Column:       | Waters CN cartridge (5mm x 10cm) in a Waters Radial Compression Module, Model RCM 100 with a CN Guard-Pak.  |
| Mobil. Phase: | 33% UV Grade Acetonitrile (Burdick & Jackson)<br>7% Methanol (Burdick & Jackson)<br>60% pH3 Phosphate buffel (.05 M) prepared using NaH <sub>2</sub> PO <sub>4</sub> and H <sub>3</sub> PO <sub>4</sub> . |
| Flow Rate:    | 3 ml/min  |

varied from 9-12 minutes, while that of the internal standard WR184,806 varied from 7-10 minutes. The retention time of the two compounds progressively shorten with time, but at least 200 samples can be analyzed before the retention times become too short and the peaks overlap.

Standard curves for WR180,409·H<sub>3</sub>PO<sub>4</sub> extracted as previously described are shown in Table V and Figure 4. Curve A represents the lysing of the red blood cells by sonification, while Curve B was done by the freeze-thaw method. Sonification appears to be more efficient.

Table V: Standard Curves for WR180,409·H<sub>3</sub>PO<sub>4</sub> Extracted as Described in the Extraction Procedure

| Concentration<br>(ng/ml) | Peak Height Ratio (180,409:184,1806) |                      |
|--------------------------|--------------------------------------|----------------------|
|                          | Curve A <sup>a</sup>                 | Curve B <sup>b</sup> |
| 9.8                      | .07                                  | .05                  |
| 48.8                     | .23                                  | .20                  |
| 97.6                     | .56                                  | .28                  |
| 243.1                    | 1.38                                 | 1.45                 |
| 484.2                    | 3.01                                 | 2.71                 |

<sup>a</sup>Lysed by sonification

<sup>b</sup>Lysed by freeze/thaw method

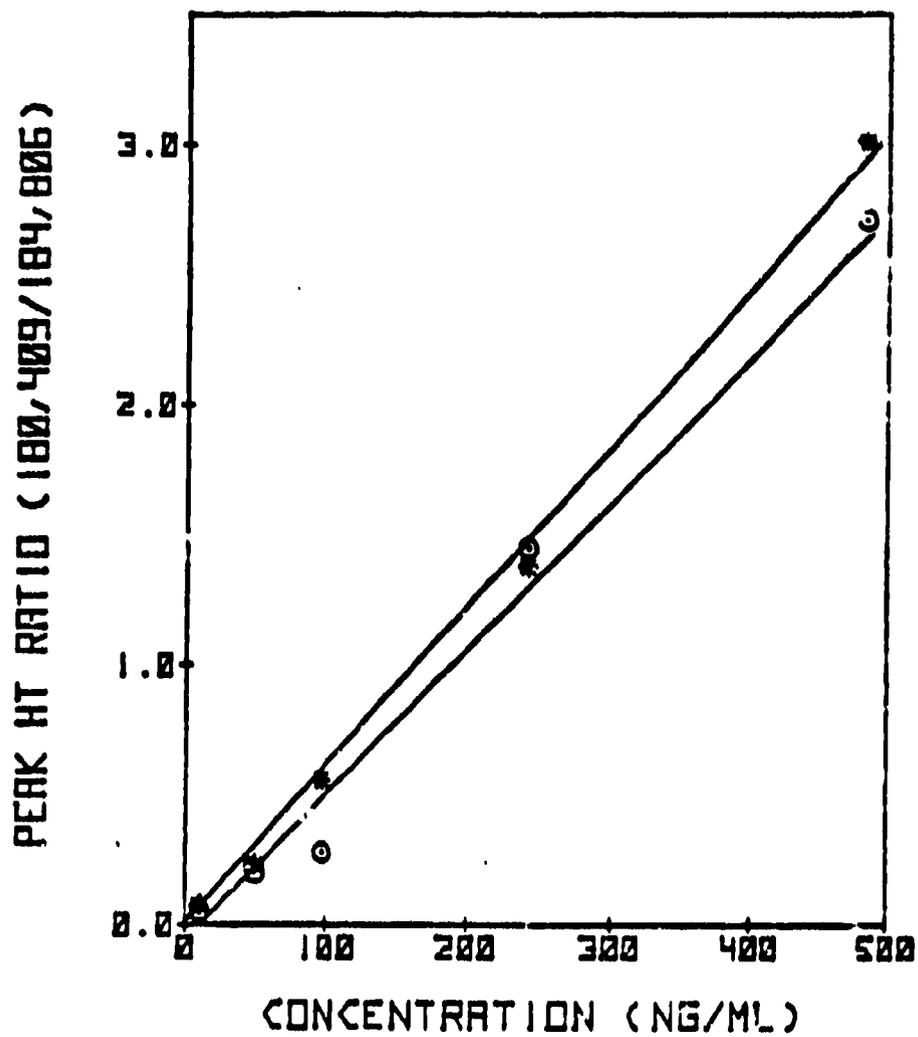


Figure 4. Standard curves for WR180.409-H<sub>3</sub>PO<sub>4</sub> extraction procedure. Curve A, ●, Curve B, ○.

The extraction procedure was modified slightly by including the internal standard in the blood sample in step one along with the  $\text{WR180,409}\cdot\text{H}_3\text{PO}_4$ . Results of this modification are presented in Table VI and the standard curve is shown in Figure 5. Inclusion of the internal standard in the blood sample appears to produce better results. The assay appears to be valid down to 10 ng/ml.

Table VI. Standard Curve for  $\text{WR180,409}\cdot\text{H}_3\text{PO}_4$  When Internal Standard Is Added to Blood

| <u>Concentration</u><br><u>(ng/ml)</u> | <u>Peak Height Ratio</u><br><u>(180,409:184,806)</u> |
|--|--|
| 9.8                                    | .035   |
| 24.3                                   | .12  |
| 54.7                                   | .27  |
| 106.4                                  | .49  |

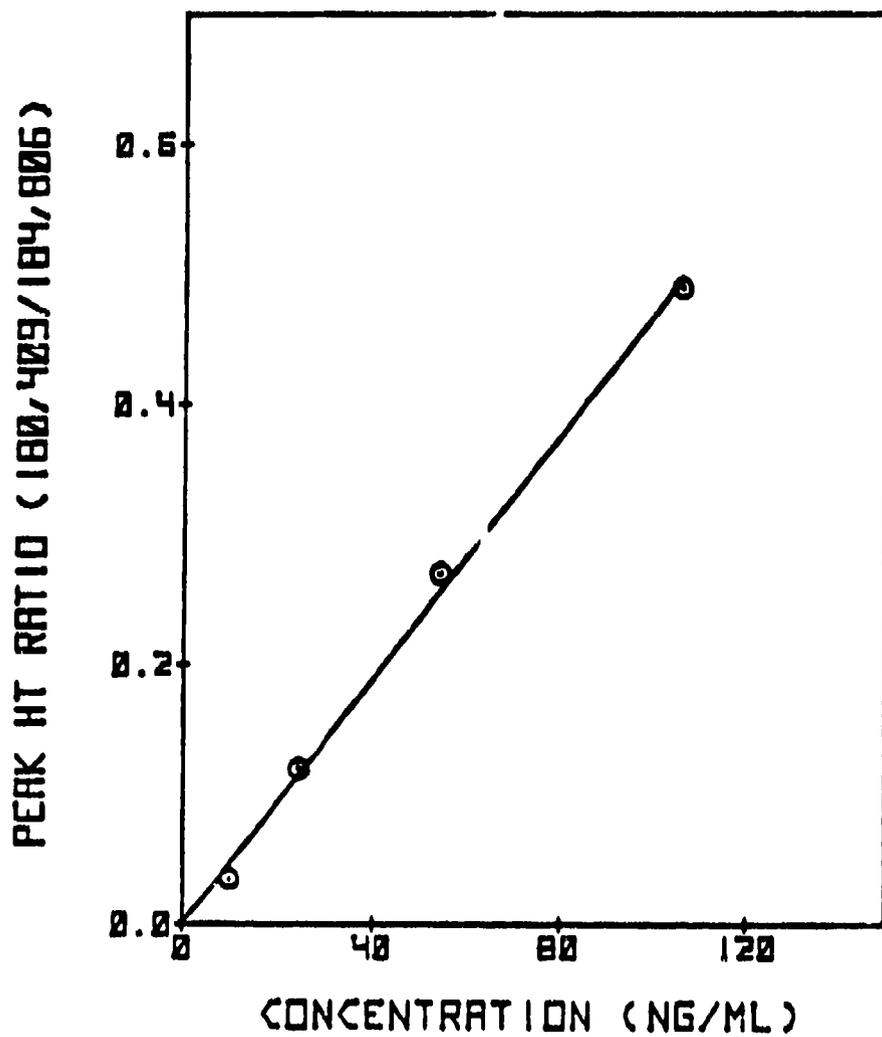


Figure 5. Standard curve for WR180,409-H<sub>3</sub>PO<sub>4</sub> when internal standard is added to the blood.

Quarterly Report Number 11  
Formulation and Production of WR6026-2HCl (Lot AF)  
One Mg Capsules (WRA-06-05182), 5 Mg Capsules  
(WRA-07-05182) and Matching Placebos (WRA-08-05182)

John L. Lach, Principal Investigator  
Douglas R. Flanagan, Assistant Principal Investigator  
Lloyd E. Matheson, Jr., Assistant Principal Investigator

July, 1982

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College of Pharmacy  
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Iowa City, Iowa 52242  
(319/353-4520)

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

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### Objective

The objective of this work is to formulate and produce capsules of WR6026·2HCl (Lot AF), an experimental anti-leishmania drug, for use in Phase I and Phase II human trials.

### Summary

One and 5 mg capsules of WR6026·2HCl, and matching placebos were formulated and produced. The basic formulation incorporates WR6026·2HCl, hydrous lactose and colloidal silicon dioxide (Cab-O-Sil M-5). For color matching purposes, D&C Yellow #10 Lake was added to the placebo formulation instead of the WR6026·2HCl. The formulations were encapsulated into #2 opaque gelatin capsule shells.

Weight variation test for twenty placebo capsules (Lot #WRA-06-5182) showed an average fill of 331.9 mg per capsule with a range of 319 to 341 mg. For twenty 1 mg capsules (Lot #WRA-06-5182) the average fill was 324.9 mg per capsule with a range of 313 to 338 mg. For twenty 5 mg capsules (Lot #WRA-07-05182) the average fill was 331.7 mg per capsule with a range of 311 to 342 mg. In all cases the balance was tared with an empty capsule shell.

The disintegration test for all lots demonstrated capsule opening in 2 minutes at 37°C in 900 ml of distilled water.

The content uniformity of 10 capsules of the one mg capsule (Lot WRA-06-5182) formulation yielded an average of 97.47% of label claim with a range of 94.4% to 103.84%. For ten 5 mg capsules (Lot WRA-07-05182) the average content uniformity was 92.75% of label claim with a range of 89.3% to 97.06%.

In all lots USP requirements were met for weight variation, content uniformity and disintegration.

### Methodology

The sample of WR6026·2HCl (Lot AF) was received on 5 May 1982 and was recorded in raw material receiving notebook number 17. The drug was assigned material lot number 386-017-386 and control number DD-052-118. The drug was stored in the original amber glass container at room temperature until use.

### Purity

The purity of the drug was taken as 99.5% as reported by SRI Analysis Report No. 394.

### Formulation Ingredients

Identification tests were carried out on the formulation excipients (i.e., hydrous lactose, USP and colloidal silicon dioxide, NF) according to compendial requirements. WR6026·2HCl (Lot AF) was identified by comparing its ultraviolet spectrum and molar absorptivity at selected wavelengths in aqueous solution with that reported previously (1). The coloring agent, D & C yellow #10 Lake, used for the placebos was identified by comparing its solution spectrum to that obtained from previous lots of the same material. Certificates of analysis from the manufacturers are included in Appendix I, pp. 79-81.

### Manufacturing Procedure

The placebo formulation (WRA-08-05182) was produced by mixing 600 gm of hydrous lactose, USP; 5 gm of D&C Yellow #10 Lake and 2 gm of colloidal silicon dioxide, NF in a one quart V-blender shell for 5 minutes. The blend was passed through a #60 mesh screen and returned to the blender for an additional 5 minutes of mixing. After color comparison with the 5 mg capsule powder blend, additional lactose (200 gm) was added and blended for another 5 minutes. No. 2 white, opaque gelatin capsules were filled with 332 mg of powder blend using a Deltay Manual Capsule Filling machine. Procedures are described in detail in Appendix I, pp. 74-75.

The 5 mg formulation (WRA-07-05182) was produced by mixing 23.8 gm of hydrous lactose, USP; 1.9 gm of colloidal silicon dioxide, NF and 8.7 gm of WR6026·2HCl (Lot AF) for 5 minutes in a one quart V-blender. The blend was passed through a #60 mesh screen and returned to the blender. Hydrous lactose, USP was added geometrically after passing through a #60 mesh screen in quantities of 75, 150 and 305 grams. Mixing was done for 5 minutes after each lactose addition. No. 2 white opaque gelatin capsules were filled with 332 mg of powder blend using a Deltay Manual Capsule Filling machine. Procedures are described in detail in Appendix II, pp. 85-88.

The 1 mg formulation (WRA-06-05182) was produced by starting with 20.3 gm of the 5 mg formulation powder blend and further diluting with 78.58 gm of hydrous lactose, USP. Blending was done for 5 minutes. No. 2 white opaque gelatin capsules were filled with 330 mg of powder blend using a Deltay Manual Capsule Filling machine. Procedures are described in detail in Appendix III, pp. 97-99.

### USP Methods and Requirements

The weight variation test for capsules is described on p. 989 of USP XX. Twenty capsules must be weighed individually and the individual weights must be within the limits of 90 to 110% of the average weight. This test was conducted on placebo, 1 mg and 5 mg capsules with a Mettler H51AR semimicro balance according to the USP XX requirements.

The disintegration test is described on pp. 958-959 of USP XX. Twelve capsules from each capsule batch were tested with 900 cc of water at 37°C as the disintegration medium.

The content uniformity test for capsules is described on p. 956 of USP XX. Ten capsules of each capsule batch were assayed individually by UV spectrophotometric methods described previously (1) after dissolving in dilute sulfuric acid and filtering off the insoluble excipients. Ten capsules of each batch were assayed individually and the content of each of not less than nine capsules were required to be within the limits of 85 to 115% of the label claim.

No dissolution test was performed on the capsules because of the high solubility of WR6026·2HCl (i.e., > 200 mg/ml). Compendial dissolution tests are required for drugs or drug formulations which have poor solubility which could result in poor dissolution characteristics.

### Results

#### Disintegration Test

In all lots the disintegration time was 2 minutes.

#### Weight Variation Test

The weight variation test for twenty placebo capsules (Lot WRA-08-05182) produced an average fill of 331.9 mg per capsule with a fill range of 319 to 341 mg. For twenty 1 mg capsules (Lot WRA-06-05182), the average fill was 324.9 mg per capsule with a range of 313 to 338 mg. For twenty 5 mg capsules (Lot WRA-07-05182), the average fill was 331.7 mg per capsule with a range of 311 to 342 mg.

#### Content Uniformity Test

The content uniformity of 10 capsules of the one mg capsule (Lot WRA-06-05182) formulation yielded an average of 97.47% of label claim with a range of 94.4% to 103.84%. For ten 5 mg capsules (Lot WRA-07-05182) the average content uniformity was 92.75% of label claim with a range of 89.3% to 97.06%.

#### Batch Size

The number of placebo capsules filled in Lot WRA-08-05182 was 1535 capsules. The number of 5 mg capsules filled in Lot WRA-07-05812 was 1512 capsules. The number of 1 mg capsules filled in Lot WRA-06-05182 was 288 capsules.

Packaging

Twenty capsules each were placed into 7 dram glass amber vials. The void space was filled with Rayon Pharmaceutical coil and shrink seals were applied.

Labels

Labels were prepared as per instructions and are shown in Appendix I, p. 73, Appendix II, p. 85, and Appendix III, p. 97 for each lot.

Conclusions

The capsule formulations of WR6026·2HCl meet all compendial requirements for capsules.

References

1. Lach, J.L., et al., Quarterly Progress Report #6, April 1981, Contract No. DAMD 17-79-C-9136, College of Pharmacy, University of Iowa, Iowa City, Iowa.

Appendix I

Manufacturing Formula and Quality Control Tests on WR6026·2HCl  
Placebo Capsules (Lot #WRA-08-05182).

University of Iowa College of Pharmacy  
MANUFACTURING FORMULA

Page 1 of \_\_\_\_\_ pages

Form CP 1  
1507

|   |  |
|---|--|
| Product <u>Placebo Capsule for WR 6026-2HCl</u> | List No. <u>WRA-08</u>                         |
| Formula <u>WR 6026-2HCl</u>                     | Batch Size <u>1700 Capsules</u>                |
| Written by <u>J. J. J.</u> Date <u>5-17-82</u>  | Checked by <u>P. G. M.</u> Date <u>5-17-82</u> |
| Production authorized by <u>John J. J.</u>      | Control No. <u>WRA-08-05182</u>                |

## Analysis

| Assay for    | Theoretical | Actual |
|--------------|-------------|--------|
| WR 6026-2HCl | 0 mg.       | 0 mg.  |
|              |             |        |
|              |             |        |
|              |             |        |
|              |             |        |

Control Assay No. IFC-A-029 Worksheet Checked by T. F. O. L. Date 6-2-82

## Specifications

|                          | Initial   | Theoretical           | Actual             |
|--------------------------|-----------|-----------------------|--------------------|
| Size                     | <u>JJ</u> | #2 Gelatin Capsules   | #2 capsule         |
| Weight                   | <u>JJ</u> | 332 mg./capsule       | 331.9 mg.          |
| Color                    | <u>JJ</u> | White opaque cap/body | White opaque cap   |
| Disintegration           | <u>JJ</u> | NMT 10 minutes        | 2 min              |
| Tablet Hardness          |           |                       |                    |
| Tablet Thickness         |           |                       |                    |
| Clarity                  |           |                       |                    |
| pH                       |           |                       |                    |
| Density                  |           |                       |                    |
| Viscosity                |           |                       |                    |
| Sedimentation            |           |                       |                    |
| Gross Appearance         |           |                       |                    |
| Sterility                |           |                       |                    |
| Pyrogen                  |           |                       |                    |
| Other : Weight Variation | <u>JJ</u> | Meet USP              | Meet USP           |
| Color of Powder Blend    | <u>JJ</u> | Match Active Blend    | Match Active Blend |

## Package and Label

Type of Container Amber Glass Rx Vial  
 Size of Container 7 Dram  
 Method of Packaging  
Hand count capsules.

Remarks  
 Void space in vial filled with Rayon  
 Pharmaceutical coil.

Shrink-seal applied to closed vial.

Packaged by J. J. J.  
 Date 6-4-82

|  |                   |
|--|-------------------|
| WALTER REED ARMY INSTITUTE OF RESEARCH<br>Division of Experimental Therapeutics Washington, D.C. 20312   |                   |
| WR 6026-2HCl AF Placebo 20 Capsules<br>6-Methoxy-8-(6-diethylaminoethylamino)lepidine<br>dihydrochloride   |                   |
| Control No. WRA-08-05182   | Manufactured 5/82 |
| Bottle   |                   |
| CAUTION: New Drug—Limited by Federal Law to Investigational Use Only<br>Manufactured by: Pharmaceutical Services, College of Pharmacy<br>The University of Iowa, Iowa City, Iowa 52242 |                   |

Product Placebo for WR 6026'2HC1 List No. WRA-08  
 Batch Size Approx. 1700 Capsules Control No. WRA-08-05182

Caution or Special Instructions

307

| BATCH CONTAINS | INGREDIENTS AND DIRECTIONS   | M. & MAT'L CONTROL NO. | INITIAL   | AMOUNT PER BATCH |
|----------------|--|------------------------|-----------|------------------|
|                | Add 20.1 gr. V-blender shell   |                        |           |                  |
|                | Lactose, USP, Hydrous<br>Mfr. <u>Sheffield 605</u><br>Mfr. Lot # <u>000-30</u><br>Material Lot #: <u>M-610-016-610</u><br>Exp. Date: <u>3-4-83</u>   | <u>Y-031-098</u>       | <u>JY</u> | 600 gm           |
|                | D & C Yellow #10 Lake, 16% Para Dye<br>Mfr. <u>Warner-Jenkinson</u><br>Mfr. Lot #: <u>AA 9727</u><br>Material Lot #: <u>M-634-016-634</u><br>Exp. Date: <u>3-25-83</u>   | <u>Y-031-122</u>       | <u>JY</u> | 5 gm             |
|                | Colloidal Silicon Dioxide, NF<br>Mfr. <u>Culbert Cd-0-EL M-5</u><br>Mfr. Lot #: <u>16181-6</u><br>Material Lot #: <u>M-036-017-036</u><br>Exp. Date: <u>10-9-82</u>  | <u>RB-101-062</u>      | <u>JY</u> | 2 gm             |
|                | Blend for 5 minutes<br>Blending Start: <u>3:15 PM</u><br>Blending Stop: <u>3:20 PM</u>   |                        | <u>JY</u> |                  |
|                | Pass blend through a #60 mesh screen; return to blender and blend for 5 minutes<br>Blending start: <u>3:27</u><br>Blending stop: <u>3:32</u>   |                        | <u>JY</u> |                  |
|                | Compare color of this blend with the color of the WRA-07-05182 blend. If the color of this blend is too dark, vs the active blend, add more lactose (subroutine A). If the color of this blend is too light vs the active blend, add more lake (subroutine B). |                        |           |                  |
|                | Color of this blend compared to the WRA-07-05182 active blend: <u>To Dark</u><br>Subroutine Chosen: <u>A</u>   |                        | <u>JY</u> |                  |

Product Placebo for WR 6026-2HCl

List No. WRA-08

Batch Size Approx. 1700 Capsules

Control No. WRA-08-05182

Indication or Special Instructions

07

| STEP CONTAINS | INGREDIENTS AND DIRECTIONS   | RAW MAT'L CONTROL NO. | INITIAL            | AMOUNT PER BATCH |
|---------------|--|-----------------------|--------------------|------------------|
|               | Subroutine A   |                       |                    |                  |
|               | Add additional Lactose, USP, Hydrated<br>Mfr.: <u>Pharmacia 605</u><br>Mfr. Lot #: <u>021-30</u><br>Material Lot #: <u>M-610-016-60</u><br>Exp. Date: <u>3-4-83</u><br>Amount of Lactose Added: <u>200 gm</u>  | <u>V-021-092</u>      | <u>[Signature]</u> | <u>200 gm</u>    |
|               | Blend for 5 minutes<br>Blending Start: <u>3:40</u><br>Blending Stop: <u>3:45</u>   |                       | <u>[Signature]</u> |                  |
|               | Subroutine B   |                       |                    |                  |
|               | Add additional D & C Yellow #10 Lake,<br>16% Pure Dye<br>Mfr.: <u>[Signature]</u><br>Mfr. Lot #: <u>Not Used</u><br>Material Lot #: <u>[Signature]</u><br>Exp. Date: <u>[Signature]</u><br>Amount of D & C Yellow #10 Lake Added: <u>[Signature]</u> |                       |                    |                  |
|               | Blend for 5 minutes<br>Blending Start: <u>5:15-87</u><br>Blending Stop: <u>[Signature]</u>   |                       |                    |                  |
|               | Weight of Total Blend: <u>805 gm</u>   |                       | <u>[Signature]</u> |                  |
|               | % Yield = $\frac{805}{807} \times 100 = 99.8\%$  |                       | <u>[Signature]</u> |                  |
|               | Acceptable Range: 90-103%  |                       |                    |                  |
|               | Fill 332 mg. of powder blend into #2 gelatin capsules white opaque cap/body.<br>Mfr.: <u>Capsugel, Parke-David</u><br>Mfr. Lot #: <u>YD-0441-Y</u><br>Material Lot #: <u>M-042-012-042</u><br>Exp. Date: <u>6-12-83</u>                              | <u>T-049-006</u>      | <u>[Signature]</u> |                  |
|               | Fill capsules, 96 at a time, using Daltav Manual Capsule Filling machine.  |                       |                    |                  |
|               | Add <u>335 gm</u> of powder blend for each set of 96 capsules.   |                       | <u>[Signature]</u> |                  |
|               | 5 gm Sample taken for Assay  |                       | <u>[Signature]</u> |                  |
|               | 5 gm Sample taken for Retained Sample  |                       | <u>[Signature]</u> |                  |

Product Placebo for WR 6026-2HC1List No. WRA-08Batch Size Approx. 1700 CapsulesControl No. WRA-08-05182

Caution or Special Instructions

1507

| EACH CONTAINS | INGREDIENTS AND DIRECTIONS  | RAW MAT'L<br>CONTROL NO. | INITIAL | AMOUNT<br>PER BATCH |
|---------------|---|--------------------------|---------|---------------------|
|               | 10 Process capsule fill weights (gross)   |                          |         |                     |
|               | Capsule Set #   |                          |         |                     |
|               | 1. 3.937 gm   | 9. 3.940                 | JH bc   |                     |
|               | 2. 3.964  | 10. 3.857                |         |                     |
|               | 3. 3.908  | 11. 3.916                |         |                     |
|               | 4. 3.949  | 12. 3.930                |         |                     |
|               | 5. 3.973  | 13. 3.931                |         |                     |
|               | 6. 3.905  | 14. 3.884                |         |                     |
|               | 7. 3.949  | 15. 3.937                |         |                     |
|               | 8. 3.958  | 16. 3.943                |         |                     |
|               | Weight of 10 empty capsules: 0.619 gm   |                          | JH Fz   |                     |
|               | # of capsules filled: 1535  |                          |         |                     |
|               | 30 capsules removed for quality control and retained sample.  |                          | JH      |                     |
|               | Capsules packaged (20 capsules/bottle)  |                          |         |                     |
|               | Void Space packed with<br>Paragon Pharmaceutical coil<br>Kendall Co.<br>Lot # 389502/722 (02/722)<br>3-2-74 | DD-032-006               | JH Fz   |                     |
|               | Bottle/Drawer:  |                          |         |                     |
|               | Owen Johnson 7da. Amber Kwick<br>Material Lot # MM 403-017-403<br>5-21-74                                   | DD-032-135               | JH Fz   |                     |
|               | 50 bottles sent to Army remainder kept in stock (24 bottles)  |                          |         |                     |

# The University of Iowa

Iowa City, Iowa 52242

77

College of Pharmacy  
Department of Pharmaceutical Service

(319) 353-4630



1847

## DISINTEGRATION TEST

Item: Placebo Capsules for WR 6026.2HCl

Noc No.: WRA-08-05182

Apparatus: USP XX, p. 958

Temperature: 37°C

Medium: 900 ml Distilled Water

Test: 2.0 minutes

# The University of Iowa

Iowa City, Iowa 52242

College of Pharmacy  
Department of Pharmaceutical Service

(319) 353-4520



1847

## WEIGHT VARIATION OF FINISHED CAPSULES

Lot # WRA-08-05182

| <u>No.</u> | <u>Mg/Capsule</u> | <u>No.</u> | <u>Mg/Capsule</u> |
|------------|-------------------|------------|-------------------|
| 1          | 333               | 11         | 338               |
| 2          | 326               | 12         | 338               |
| 3          | 332               | 13         | 335               |
| 4          | 331               | 14         | 341               |
| 5          | 335               | 15         | 337               |
| 6          | 320               | 16         | 334               |
| 7          | 337               | 17         | 319               |
| 8          | 320               | 18         | 339               |
| 9          | 338               | 19         | 336               |
| 10         | 330               | 20         | 320               |

Average fill: 331.9 mg/Capsule\*

Deviation from low (319 mg) = 3.9%

Deviation from high (341 mg) = 2.7%

\*Balance was tared with empty capsules

SHEFFIELD PRODUCTS

PROTOCOL OF ASSAY

CUSTOMER: UNIVERSITY OF IOWA  
ADDRESS: PURCHASING DEPT.  
IOWA CITY IOWA 52242  
~~ATTN: DENISE MCCLELLAN~~

Pharm. - Coll. of

PRODUCT: LACTOSE U.S.P. HYDROUS 60S

DATE SHIPPED: 2/25/81  
NUMBER OF DRUMS: 3  
INVOICE NO.: 71858

LOT NO.: 0NJ30  
CUSTOMER ORDER NO.: 04786

RESULTS OF ASSAY WHERE APPLICABLE TO PRODUCT SHIPPED:

CHEMICAL/PHYSICAL

MICROBIOLOGICAL

SOLUBILITY.....PASS  
MOISTURE %..... 5.07 - 5.07  
ASH %..... 0.022  
SPECIFIC ROTATION..... 55.21  
ACIDITY.....PASS  
PH (10% SOL.)..... 4.3 - 4.7  
ALCOHOL SOL. RESIDUE..... 1.23

STAND. PLATE COUNT... <100/GRAM  
THERMOPHILE COUNT.....PASS  
COLIFORM.....NEGATIVE  
MOLD.....<50/GRAM

RECEIVED

APR 02 1981

RECEIVED

MAR 30 1981

DATE: 3/24/1981 PURCHASING DEPT

PURCHASING DEPT.

This copy for your files

SHEFFIELD PRODUCTS, BOX 398, MEMPHIS, TENN. 38101

KRAFT INC.

The information herein is true & accurate to the best of our knowledge. However, both the information & product are offered without warranty or guarantee as to any specific use. Nothing herein shall be construed as a recommendation to use any product in violation of any patent rights.



## CABOT CORPORATION

P.O. BOX 188, TUSCOLA, ILLINOIS 61963

TELEPHONE AREA CODE 317  
TUSCOLA 313-3378  
TELEX TUSCOLA 918-663-2642

November 10, 1981

Mr. John Jordan  
University of Iowa  
College of Pharmacy  
Pharmaceutical Services Div.  
Iowa City, IA 52242

Dear Mr. Jordan:

Quality control testing gave the following average test data for the lot(s) shipped at the time of packaging at our Tuscola, Illinois plant:

|                                   |       |
|-----------------------------------|-------|
| Grade                             | M-5   |
| Lot Code                          | 11181 |
| pH (4% Aqueous Slurry)            | 3.82  |
| Surface Area (m <sup>2</sup> /g.) | 205   |
| 325 Mesh Residue (%)              | .002  |
| Moisture (% @ 105 °C)             | 0.41  |
| Density (lbs./cu.ft.)             | 2.07  |

In addition to the above data, our product meets the requirements set forth in the National Formulary XV; certified at the time of shipment as follows:

|   |          |
|---|----------|
| Silica Content (% SiO <sub>2</sub> , Ignited Basis) | 99.99%   |
| Identification Test                                 | Positive |
| pH (4% Aqueous Slurry)                              | 3.82     |
| Loss on Drying (2 hrs @ 105°C, %)                   | 0.41     |
| Loss on Ignition (1000°C, Dry Basis, %)             | 0.51     |
| Arsenic (ppm)                                       |          |

I trust this information meets your needs. If I can be of further assistance, please call.

Very truly yours,

Gabriel Paci  
Quality Assurance Manager  
CAB-O-SIL Division

GP/cjo

DATE September 18, 19

• Warner-Jenkinson Mfg. Company  
2526 Baldwin Street  
St. Louis, MO 63106

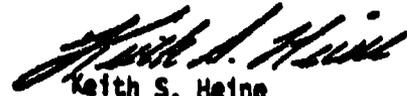
240.00

## COLOR ADDITIVE CERTIFICATE

The batch of Color Additive described below is hereby certified to you. The use of this color is subject to the terms, conditions, and restrictions set forth in the Federal Food, Drug and Cosmetic Act and the regulations thereunder.

| <u>NAME OF COLOR</u>            | <u>MFR<br/>BATCH NO.</u> | <u>QUANTITY<br/>IN LBS</u> | <u>CERT %<br/>PURE COLOR</u> | <u>CERTIFIED<br/>FOR USE IN</u>                                  |
|---------------------------------|--------------------------|----------------------------|------------------------------|--|
| D&C Yellow #10<br>Aluminum Lake | 684-R                    | 1600                       | 16                           | Drugs & Cosmetics in<br>accordance with<br>applicable limitation |

CC: KAN-00

  
Keith S. Heine  
FOR THE  
COMMISSIONER OF FOOD AND DRUGS.

FORM FDA 2008 (1/75)

PREVIOUS EDITION IS OBSOLETE.

**The University of Iowa**

Iowa City, Iowa 52242

College of Pharmacy  
Department of Pharmaceutical Service

(319) 383-4520



1847

## APPROVAL FOR SHIPMENT FORM

Product Name: WR 6026-2HCl, Placebo capsules lot WRA-08-05182Container Size: 20 capsules Dosage Form: capsuleAcceptable Container: 50 Rejects: 0Total Units Shipped: 50Date Shipped: June 7, 1982Delivery Ticket Number: N/A

Name and Address of Receiver:

Dr. Larry Fleckenstein  
Forest Glen Annex  
Building 500  
Brookville Road  
Water Reed Army Institute of Research  
Silver Springs, MD 20910Approval of Shipment by: *Rennie A. P. King*

Pharmaceutical Services  
College of Pharmacy

University of Iowa  
Iowa City, Iowa

## PRODUCT RELEASE FORM

Part A

Product: Placebo Capsule for WR 6026 2HCl

Lot No.: WRA-08-05182

Batch Size: 1700 capsules

Date Received by Warehouse: \_\_\_\_\_

| <u>Quantity</u>                       | <u>Size</u> |
|---------------------------------------|-------------|
| <u>74 bottles of 20 capsules each</u> |             |
| _____                                 | _____       |
| _____                                 | _____       |

Warehouse: Place this product in quarantine. Please match this form with the release form before placing the product in use.

Part A remains with product until released.

-----  
(Detach along dotted line)

## PRODUCT RELEASE FORM

Part B

Part B remains with Quality Control Department Analysis Sheets

Product: Placebo Capsule for WR 6026 2HCl

Lot No.: WRA-08-05182

Batch Size: 1700 capsules

Warehouse: Please release, destroy, return to mfg. this product and remove from quarantine.

Signature: *Luig Jang Ch.*

Date Released: 6-3-82

Appendix 17

Manufacturing and Quality Control Tests on WR6026·2HCl Five Mg Capsules (Lot #WRA-07-05182).

University of Iowa College of Pharmacy  
**MANUFACTURING FORMULA**

Page 1 of \_\_\_\_\_ pages

Form CP 1  
1507

|   |                                 |
|---|---------------------------------|
| Product <u>WR 6026 2HCl, 5 mg, Capsules</u>                         | List No. <u>WRA-07</u>          |
| Formula <u>WR 6026 2HCl</u>   | Batch Size <u>1700 Capsules</u> |
| Date <u>5-17-82</u> Checked by <u>J. Conroy</u> Date <u>5-17-82</u> | Control No. <u>WRA-07-05182</u> |
| Prepared by <u>J. Conroy</u>  |                                 |

## Analysis

| Assay for    | Theoretical | Actual  |
|--------------|-------------|---------|
| WR 6026-2HCl | 5.0 mg      | 4.63 mg |
|              |             |         |
|              |             |         |
|              |             |         |
|              |             |         |

Control Assay No. IPC-A-028 Worksheet Checked by J.F. O'Leary Date 6-3-82

## Specifications

|                         | Initial   | Theoretical           | Actual                |
|-------------------------|-----------|-----------------------|-----------------------|
| Size                    | <u>JF</u> | #2 Gelatin Capsules   | #2 Gelatin Capsules   |
| Weight                  | <u>JF</u> | 332 mg/capsule        | 331.7 mg capsules     |
| Color                   | <u>JF</u> | White Opaque cap/body | white opaque cap/body |
| Disintegration          | <u>JF</u> | NMT 10 minutes        | 2 min                 |
| Tablet Hardness         |           |                       |                       |
| Tablet Thickness        |           |                       |                       |
| Clarity                 |           |                       |                       |
| pH                      |           |                       |                       |
| Density                 |           |                       |                       |
| Viscosity               |           |                       |                       |
| Sedimentation           |           |                       |                       |
| Gross Appearance        |           |                       |                       |
| Sterility               |           |                       |                       |
| Pyrogen                 |           |                       |                       |
| Other: Weight Variation | <u>JF</u> | Meet USP              | Meet USP              |
| Color of Powder Blend   | <u>JF</u> | Yellow                | Yellow                |

## Package and Label

Type of Container Amber Glass Rx Vials  
 Size of Container 7 Dram  
 Method of Packaging Hand count capsules

## Remarks

Void space in vial filled with Rayon  
 Pharmaceutical Oil.

Shrink-seal applied to closed vial.

Packaged by J. Conroy  
 Date 6-4-82

|   |  |
|---|--|
| WALTER REED ARMY INSTITUTE OF RESEARCH<br>Division of Experimental Therapeutics Washington, D.C. 20312  |  |
| WR 6026-2HCl AF 5 mg 20 Capsules<br>8-Methoxy-8-(6-diethylamino)hexylamino)lepidine<br>dihydrochloride  |  |
| Control No. WRA-07-05182 Manufactured 5/82<br>Bottle  |  |
| CAUTION: New Drug--Limited by Federal Law to Investigational Use Only<br>Manufactured by: Pharmaceutical Services, College of Pharmacy<br>The University of Iowa, Iowa City, Iowa 52242 |  |

Product WR-6026 2HC1, 5 mg. Capsules

List No. WRA-07

Batch Size 1700 Capsules

Control No. WRA-07-05182

Caution or Special Instructions

1507

| EACH CONTAINS | INGREDIENTS AND DIRECTIONS   | RAW MAT'L CONTROL NO. | INITIAL   | AMOUNT PER BATCH |
|---------------|--|-----------------------|-----------|------------------|
|               | Add to a 1 qt. plastic V-blender shell.  |                       |           |                  |
|               | Lactose USP, hydrous<br>Mfr.: <u>Sheffield 605</u><br>Mfr. Lot #: <u>10AN-30</u><br>Material Lot #: <u>M-610-016-610</u><br>Exp. Date: <u>3-4-83</u>                 | <u>Y-031-098</u>      | <u>JF</u> | <u>23.8 gm</u>   |
|               | Colloidal Silicon Dioxide, NF<br>Mfr.: <u>Cabot Cab-O-Sil M-5</u><br>Mfr. Lot #: <u>16181-6</u><br>Material Lot #: <u>M-016-017-016</u><br>Exp. Date: <u>10-9-83</u> | <u>RB-101-062</u>     | <u>JF</u> | <u>1.9 gm</u>    |
|               | WR 6026-2HC1<br>Source: <u>Walter Reed Army Institute of Research</u><br>Lot #: <u>AF</u><br>Material Lot #: <u>M-386-017-386</u><br>Exp. Date: <u>5-5-84</u>        | <u>AD-052-112</u>     | <u>JF</u> | <u>8.7 gm</u>    |
|               | Blend for 5 minutes:<br>Blending start: <u>10:40</u><br>Blending stop: <u>10:45</u>  |                       | <u>JF</u> |                  |
|               | Pass blend through a #60 mesh screen.<br>Return to blender.  |                       |           |                  |
|               | Add to the same blender, through a #60 mesh screen:  |                       |           |                  |
|               | Lactose, USP, hydrous<br>Mfr.: <u>Sheffield 605</u><br>Mfr. Lot #: <u>10AN-30</u><br>Material Lot #: <u>M-610-016-610</u><br>Exp. Date: <u>3-4-83</u>                | <u>Y-031-098</u>      | <u>JF</u> | <u>15 gm</u>     |
|               | Blend for 5 minutes:<br>Blending Start: <u>10:50</u><br>Blending Stop: <u>10:55</u>  |                       | <u>JF</u> |                  |
|               | Add to the same blender through a #60 mesh screen:   |                       |           |                  |
|               | Lactose, USP, hydrous<br>Mfr.: <u>Sheffield 605</u><br>Mfr. Lot #: <u>10AN-30</u><br>Material Lot #: <u>M-610-016-610</u><br>Exp. Date: <u>3-4-83</u>                | <u>Y-031-098</u>      | <u>JF</u> | <u>150 gm</u>    |

Product WR 6026-2HCL, 5 mg., Capsules

List No. WRA-07

Batch Size 1700 Capsules

Control No. WRA-07-05182

Indication or Special Instructions

07

| BATCH CONTAINS | INGREDIENTS AND DIRECTIONS  | RAW MAT'L CONTROL NO. | INITIAL | AMOUNT PER BATCH |
|----------------|---|-----------------------|---------|------------------|
|                | Blend for 5 minutes<br>Blending Start: 11:00<br>Blending Stop: 11:05  |                       | JF      |                  |
|                | Add to the same blender through a #60 mesh screen   |                       | JF      |                  |
|                | Lactose, USP, Hydrated<br>Mfr.: <u>Sh. M. 605</u><br>Mfr. Lot #: <u>11021-30</u><br>Material Lot #: <u>M-610-016-610</u><br>Exp. Date: <u>3-4-83</u>  | <u>Y-031-008</u>      | JF      | 305 gm           |
|                | Blend for 5 minutes<br>Blending Start: 11:15<br>Blending Stop: 11:20  |                       | JF      |                  |
|                | Weight of final blend: <u>564 gm</u>  |                       | JF      |                  |
|                | Remove a sample for assay and a sample for retention. <u>Cap Sample for each removed</u>  |                       | JF      |                  |
|                | Remove <u>20.3 gm</u> for use in blending WRA-06-05182 1 mg. capsules after assay has been performed.   |                       | JF      |                  |
|                | Fill 332 mg of powder blend into:<br>#2 gelatin capsules, white opaque body/cap<br>Mfr.: <u>Capsugel, Parke Davis</u><br>Mfr. Lot #: <u>Y8-0441-Y</u><br>Material Lot #: <u>M-048-012-048</u><br>Exp. Date: <u>6-18-83</u>  | <u>T-049-006</u>      | JF      | 1500             |
|                | Fill capsules, 96 at a time, using the Deltay Capsule filling machine (manual).   |                       | JF      |                  |
|                | Add <u>33 gm</u> of powder blend for each set of 96 capsules.   |                       | JF      |                  |
|                | In-Process fill weights (gross weights)<br>Capsule Set #: 1. <u>3.943</u> 9. <u>3.900</u><br>2. <u>3.935</u> 10. <u>3.911</u><br>3. <u>3.901</u> 11. <u>3.917</u><br>4. <u>3.991</u> 12. <u>3.913</u><br>5. <u>3.971</u> 13. <u>3.893</u><br>6. <u>3.941</u> 14. <u>3.897</u><br>7. <u>3.921</u> 15. <u>3.870</u><br>8. <u>3.922</u> 16. <u>3.900 (48) cap</u><br>17. <u>3.847 (24) cap</u> |                       | JF      |                  |

Product WR 6026-2HC1, 5 mg. Capsules

List No. WRA-07

Batch Size 1700 Capsules

Control No. WRA-07-05182

Description of Special Instructions

07

| BATCH CONTAINS | INGREDIENTS AND DIRECTIONS   | RAW MAT'L CONTROL NO. | INITIAL   | AMOUNT PER BATCH |
|----------------|--|-----------------------|-----------|------------------|
|                | Weight of 10 empty capsules: <u>0.619 gm</u>   |                       | <u>JF</u> |                  |
|                | # of capsules filled: <u>1517</u>  |                       | <u>JF</u> |                  |
|                | % Yield:<br>Powder Blend:  |                       |           |                  |
|                | % Yield = $\frac{564}{566.4} \times 100 = 99.75\%$                                   |                       | <u>JF</u> |                  |
|                | Acceptable range: 90 - 110%  |                       |           |                  |
|                | 30 capsules removed for quality control and blend sample                             |                       | <u>JF</u> |                  |
|                | Capsules packaged, 30 capsules/bottle into   |                       |           |                  |
|                | 7dd. Amies Barrels.<br>Oregon - Tillamook<br>Material lot # M 403-017-403<br>5-21-84 | DD-052-135            | <u>JF</u> |                  |
|                | Void space filled with   |                       |           |                  |
|                | Ryan's Pharmaceutical Co.<br>Kendall Co.<br>Lot # 30950 21722. (021782)<br>3-2-84    | DD-032-005            | <u>JF</u> |                  |
|                | 72 bottles filled  |                       | <u>JF</u> |                  |
|                | 50 bottles sent to army  |                       | <u>JF</u> |                  |
|                | 22 bottles remain in stock   |                       | <u>JF</u> |                  |

**The University of Iowa**

Iowa City, Iowa 52242

College of Pharmacy  
Department of Pharmaceutical Service

(319) 353-4520



1847

**DISINTEGRATION TEST**

Item: WR 6026.2HCl, 5 mg. Capsules

Lot No.: WRA-07-05182

Apparatus: USP XX, p. 958

Temperature: 37°C

Medium: 900 ml Distilled Water

Test: 2.0 minutes

# The University of Iowa

Iowa City, Iowa 52242



College of Pharmacy  
Department of Pharmaceutical Service

(319) 353-4520

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## WEIGHT VARIATION OF FINISHED CAPSULES, 5 MG

WR 6026.2HCl/Capsule

Lot # WRA-07-05182

| <u>No.</u> | <u>Mg/Capsule</u> | <u>No.</u> | <u>Mg/Capsule</u> |
|------------|-------------------|------------|-------------------|
| 1          | 341               | 11         | 329               |
| 2          | 326               | 12         | 333               |
| 3          | 332               | 13         | 311               |
| 4          | 322               | 14         | 331               |
| 5          | 330               | 15         | 329               |
| 6          | 338               | 16         | 326               |
| 7          | 332               | 17         | 332               |
| 8          | 332               | 18         | 339               |
| 9          | 340               | 19         | 336               |
| 10         | 342               | 20         | 333               |

Average fill: 331.7 mg/Capsule\*

Deviation from low (311 mg) = 6.0%

Deviation from high (342 mg) = 3.1%

\*Balance was tared with empty capsules

# The University of Iowa

Iowa City, Iowa 52242

College of Pharmacy  
Department of Pharmaceutical Service

(319) 353-4520



1847

## FORMULATED PRODUCT

WRA-07-05182  
5.0 mg/Capsule

| <u>No.</u> | <u>Mg/Capsule</u> | <u>% of Label</u> |
|------------|-------------------|-------------------|
| 1          | 4.660             | 93.20%            |
| 2          | 4.623             | 92.46             |
| 3          | 4.696             | 93.92             |
| 4          | 4.635             | 92.70             |
| 5          | 4.672             | 93.44             |
| 6          | 4.623             | 92.46             |
| 7          | 4.853             | 97.06             |
| 8          | 4.623             | 92.46             |
| 9          | 4.502             | 90.04             |
| 10         | 4.465             | 89.30             |
| Average =  | 4.635 mg/Capsule  | 92.75%            |

Deviation from low (4.465) = 3.66%

Deviation from high (4.853) = 4.70%

T.F.C. hi





CABOT CORPORATION

P.O. BOX 188, TUSCOLA, ILLINOIS 61963

TELEPHONE AREA CODE 217  
TUSCOLA 253-2176  
TOLL FREE TUSCOLA 810-663-2142

November 10, 1981

Mr. John Jordan  
University of Iowa  
College of Pharmacy  
Pharmaceutical Services Div.  
Iowa City, IA 52242

Dear Mr. Jordan:

Quality control testing gave the following average test data for the lot(s) shipped at the time of packaging at our Tuscola, Illinois plant:

|                                   |       |
|-----------------------------------|-------|
| Grade                             | M-5   |
| Lot Code                          | 1I181 |
| pH (4% Aqueous Slurry)            | 3.82  |
| Surface Area (m <sup>2</sup> /g.) | 205   |
| 325 Mesh Residue (%)              | .002  |
| Moisture (% @ 105 °C)             | 0.41  |
| Density (lbs./cu.ft.)             | 2.07  |

In addition to the above data, our product meets the requirements set forth in the National Formulary XV; certified at the time of shipment as follows:

|   |          |
|---|----------|
| Silica Content (% SiO <sub>2</sub> , Ignited Basis) | 99.99%   |
| Identification Test                                 | Positive |
| pH (4% Aqueous Slurry)                              | 3.82     |
| Loss on Drying (2 hrs @ 105°C, %)                   | 0.41     |
| Loss on Ignition (1000°C, Dry Basis, %)             | 0.51     |
| Arsenic (ppm)                                       |          |

I trust this information meets your needs. If I can be of further assistance, please call.

Very truly yours,

Gabriel Paci  
Quality Assurance Manager  
CAB-O-SIL Division

GP/cjb

**The University of Iowa**

Iowa City, Iowa 52242

College of Pharmacy  
Department of Pharmaceutical Service

(319) 353-4520



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## APPROVAL FOR SHIPMENT FORM

Product Name: WR 6026-2HCl 5 mg capsules, lot WPA-07-05182Container Size: 20 caps. Dosage Form: capsuleAcceptable Container: 50 Rejects: 0Total Units Shipped: 50Date Shipped: June 7, 1982Delivery Ticket Number: N/A

Name and Address of Receiver:

Dr. Larry Fleckenstein  
Forest Glen Annex  
Building 500  
Brookville Road  
Walter Reed Army Institute of Research  
Silver Springs, MD 20910Approval of Shipment by: *Kevin O'Connell*

Pharmaceutical Services  
College of Pharmacy

University of Iowa  
Iowa City, Iowa

## PRODUCT RELEASE FORM

Part A

Product: WR 6026 2 HCl, 5 mg capsules

Lot No.: WRA-07-05182

Batch Size: 1700 capsules

Date Received by Warehouse: 5-20-82

Quantity \_\_\_\_\_ Size \_\_\_\_\_

72 bottles of 20 capsules each

Warehouse: Place this product in quarantine. Please match this form with the release form before placing the product in use.

Part A remains with product until released.

(Detach along dotted line)

## PRODUCT RELEASE FORM

Part B

Part B remains with Quality Control Department Analysis Sheets

Product: WR 6026 2HCl, 5 mg capsules

Lot No.: WRA-07-05182

Batch Size: 1700 capsules

Warehouse: Please (release, destroy, return to mfg.) this product and remove from quarantine.

Signature: raig-jong chin

Date Released: 6-2-82

## Appendix III

Manufacturing and Quality Control Tests on WR6026-2HCl One Mg Capsules (Lot #WRA-06-05182).

Form CP 1  
1507

|   |   |
|---|---|
| Product: <u>WR 6026-2 HCl, 1 mg. Capsules</u>     | List No. <u>WRA-06</u>                            |
| Formula <u>[Signature]</u>                        | Batch Size <u>300 Capsules</u>                    |
| Written by <u>[Signature]</u> Date <u>5-17-82</u> | Checked by <u>[Signature]</u> Date <u>5-17-82</u> |
| Production authorized by <u>[Signature]</u>       | Control No. <u>WRA-06-05182</u>                   |

**Analysis**

| Assay for     | Theoretical | Actual    |
|---------------|-------------|-----------|
| WR 6026-2 HCl | 1.0 mg      | 0.9747 mg |
|               |             |           |
|               |             |           |
|               |             |           |
|               |             |           |

Control Assay No. FFA-A-026 Worksheet Checked by [Signature] Date 6-3-82

**Specifications**

|                         | Initial      | Theoretical           | Actual                |
|-------------------------|--------------|-----------------------|-----------------------|
| Size                    | <del> </del> | #2 Galatin Capsules   | #2 Galatin Capsules   |
| Weight                  | <del> </del> | 330 mg/capsule        | 324.9 mg              |
| Color                   | <del> </del> | White Opaque Cap/Body | white opaque cap/body |
| Disintegration          | <del> </del> | NMT 10 min.           | 2.0 minutes           |
| Tablet Hardness         |              |                       |                       |
| Tablet Thickness        |              |                       |                       |
| Clarity                 |              |                       |                       |
| pH                      |              |                       |                       |
| Density                 |              |                       |                       |
| Viscosity               |              |                       |                       |
| Sedimentation           |              |                       |                       |
| Gross Appearance        |              |                       |                       |
| Sterility               |              |                       |                       |
| Pyrogen                 |              |                       |                       |
| Other: Weight Variation | <del> </del> | Meet USP              | Meet USP              |
| Color of Powder Blend   | <del> </del> | Light Yellow          | lt yellow             |

**Package and Label**

Type of Container Amber Glass Rx Vials  
 Size of Container 7 Dram  
 Method of Packaging Hand count capsules

**Remarks**

Void space in vial filled with rayon pharmaceutical coil.

Shrink seals applied to closed vials.

Packaged by [Signature]  
 Date 6-4-82

WALTER REARMY INSTITUTE OF RESEARCH  
 Division of Experimental Pharmacology Washington, D.C. 20012

**WR 6026-2HCl AF 1 mg 20 Capsules**  
**6-Methoxy-8-(6-diethylaminohexylamino)lepidine dihydrochloride**

Control No. **WRA-06-05182** Manufactured 5/82  
 Bottle

CAUTION: New Drug—Limited by Federal Law to Investigational Use Only  
 Manufactured by: Pharmaceutical Services, College of Pharmacy  
 The University of Iowa, Iowa City, Iowa 52242

Product WR-6026 2HCl, 1 mg. CapsulesList No. WRA-06Batch Size 300 CapsulesControl No. WRA-06-05182

Caution or Special Instructions

1507

| EACH CONTAINS | INGREDIENTS AND DIRECTIONS   | RAW MAT'L CONTROL NO. | INITIAL   | AMOUNT PER BATCH |
|---------------|--|-----------------------|-----------|------------------|
|               | Add to a 1-qt. plastic V-blender shell.  |                       |           |                  |
|               | Blend from WRA-07-05182 after assay.<br>Assay results: <u>4.996 mg / 332 mg of blend</u><br>Amount of blend needed is the amount necessary to obtain 300 mg. of active drug.   |                       | <i>JF</i> | <u>20.32 gm</u>  |
|               | Add to the same blender through a #60 mesh screen  |                       |           |                  |
|               | Lactose, USP, hydrated<br>Mfr.: <u>Sheffield 605</u><br>Mfr. Lot #: <u>YDNI-30</u><br>Material Lot #: <u>M-610-016-610</u><br>Exp. Date: <u>3-4-83</u><br><u>1-15-82</u>   | <u>Y-031-098</u>      | <i>JF</i> | <u>78.58</u>     |
|               | Blend for 5 minutes:<br>Blending Start: <u>1:15</u><br>Blending Stop: <u>1:20</u>  |                       | <i>JF</i> |                  |
|               | Weight of final blend <u>99 gm.</u>  |                       | <i>JF</i> |                  |
|               | Remove samples for assay and for retained sample. <u>1 gm Sample removed</u>   |                       | <i>JF</i> |                  |
|               | Fill 330 mg. of powder blend into #2 gelatin capsules, white opaque body/cap.<br>Mfr.: <u>Capsugel - Parke Davis</u><br>Mfr. Lot #: <u>YCA-0441-V</u><br>Material Lot #: <u>M-048-017-048</u><br>Exp. Date: <u>6-12-83</u> | <u>T-048-006</u>      | <i>JF</i> |                  |
|               | Fill capsules, 96 at a time, using the Daltay Manual Capsule filling machine.  |                       |           |                  |
|               | Add <u>37 gm.</u> of powder blend for each set of 96 capsules.   |                       | <i>JF</i> |                  |
|               | In process capsule fill weights (gross weight)<br>Capsule Set #<br>1. <u>3.899 gm</u><br>2. <u>3.891</u><br>3. <u>3.860</u>  |                       | <i>JF</i> |                  |

Product WR-6026 2HCl, 1 mg. Capsules

List No. WRA-06

Batch Size 300 Capsules

Control No. WRA-06-05182

Caution or Special Instructions

1507

| EACH CONTAINS | INGREDIENTS AND DIRECTIONS                                   | RAW MAT'L<br>CONTROL NO. | INITIAL   | AMOUNT<br>PER BATCH |
|---------------|--|--------------------------|-----------|---------------------|
|               | Weight of 10 empty capsules <u>0.619</u>                     |                          | <u>JF</u> | <u>62</u>           |
|               | # of Capsules filled: <u>288</u>                             |                          |           |                     |
|               | % Yield  |                          |           |                     |
|               | Powder blend   |                          |           |                     |
|               | % Yield = $\frac{99}{99} \times 100 = 100\%$                 |                          | <u>JF</u> |                     |
|               | Capsules filled  |                          |           |                     |
|               | % Yield = $\frac{288}{293} \times 100 = 98.3\%$              |                          | <u>JF</u> |                     |
|               | Acceptable range 90 - 103%                                   |                          |           |                     |
|               | 20 capsules removed for quality control and retained samples |                          | <u>JF</u> |                     |
|               | Capsules packaged 20/bottle in                               |                          |           |                     |
|               | 7 dram amber Rx vials  | <u>00-052-135</u>        | <u>JF</u> | <u>62</u>           |
|               | Quinn-Tillman  |                          |           |                     |
|               | M 403-017-403  |                          |           |                     |
|               | 5-21-84  |                          |           |                     |
|               | Void space filled with                                       |                          |           |                     |
|               | Ryan Pharmaceutical Co                                       | <u>00-082-006</u>        | <u>JF</u> | <u>6</u>            |
|               | Ketchell Co  |                          |           |                     |
|               | Lot # 3695021722 (021782)                                    |                          |           |                     |
|               | 3-7-84   |                          |           |                     |
|               | 11 filled bottles  |                          | <u>JF</u> | <u>65</u>           |
|               | 5 bottles sent to army                                       |                          | <u>JF</u> | <u>6</u>            |
|               | 6 bottles remain in inventory                                |                          | <u>JF</u> | <u>6</u>            |

**The University of Iowa**

Iowa City, Iowa 52242

College of Pharmacy  
Department of Pharmaceutical Service

(319) 353-4520



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**DISINTEGRATION TEST****Item:** WR 6026.2HCl, 1 mg. Capsules**Lot No.:** WRA-06-05182**Apparatus:** USP XX, p. 958**Temperature:** 37°C**Medium:** 900 ml Distilled Water**Test:** 2.0 minutes

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**WEIGHT VARIATION OF FINISHED CAPSULE, 1 MG****WR 6026.2HCl/Capsule****Lot # WRA-06-05182**

| <u>No.</u> | <u>Mg/Capsule</u> | <u>No.</u> | <u>Mg/Capsule</u> |
|------------|-------------------|------------|-------------------|
| 1          | 322               | 11         | 335               |
| 2          | 322               | 12         | 331               |
| 3          | 330               | 13         | 329               |
| 4          | 321               | 14         | 315               |
| 5          | 327               | 15         | 320               |
| 6          | 330               | 16         | 313               |
| 7          | 338               | 17         | 322               |
| 8          | 329               | 18         | 320               |
| 9          | 325               | 19         | 325               |
| 10         | 319               | 20         | 324               |

Average fill: 324.9 mg/Capsule\*

Deviation from low (313 mg) = 3.7%

Deviation from high (338 mg) = 4.0%

\*Balance was tared with empty capsule

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**FORMULATED PRODUCT**WRA-06-05182  
1.0 mg/Capsule

| <u>No.</u>                  | <u>Mg/Capsule</u> | <u>% of Label</u> |
|-----------------------------|-------------------|-------------------|
| 1                           | 0.9754            | 97.54%            |
| 2                           | 0.9510            | 95.10             |
| 3                           | 1.0093            | 100.93            |
| 4                           | 0.9924            | 99.24             |
| 5                           | 0.9440            | 94.40             |
| 6                           | 0.9440            | 94.40             |
| 7                           | 1.0384            | 103.84            |
| 8                           | 0.9513            | 95.13             |
| 9                           | 0.9633            | 96.33             |
| 10                          | 0.9770            | 97.79             |
| Average = 0.9747 mg/Capsule |                   | 97.47%            |

Deviation from low (0.9440) = 3.14%

Deviation from high (1.0384) = 6.53%

T. F. C. L.

SHEFFIELD PRODUCTS

PROTOCOL OF ASSAY

CUSTOMER: UNIVERSITY OF IOWA  
ADDRESS: PURCHASING DEPT.  
IOWA CITY IOWA 52242  
~~ATTN: DENISE MOSEMAN~~

Pharm. - Coll. of

PRODUCT: LACTOSE U.S.P. HYDROUS 68S

LOT NO.: 0NJ30  
CUSTOMER ORDER NO.: U14706

DATE SHIPPED: 2/25/81  
NUMBER OF DRUMS: 3  
INVOICE NO.: 71858

RESULTS OF ASSAY WHERE APPLICABLE TO PRODUCT SHIPPED:

CHEMICAL/PHYSICAL

SOLUBILITY.....PASS  
MOISTURE %..... 5.07 - 5.07  
ASH %..... 0.022  
SPECIFIC ROTATION..... 55.21  
ACIDITY.....PASS  
PH (10% SOL.)..... 4.3 - 4.7  
ALCOHOL SOL. RESIDUE..... 1.23

MICROBIOLOGICAL

STAND. PLATE COUNT... <100/GRAM  
THERMOPHILE COUNT.....PASS  
COLIFORM.....NEGATIVE  
MOLD.....<50/GRAM

RECEIVED

APR 02 1981

RECEIVED

MAR 30 1981

PURCHASING DEPT.

DATE: 3/24/1981 PURCHASING DEPT.

THIS COPY FOR YOUR FILE

SHEFFIELD PRODUCTS, BOX 398, MEMPHIS, TENN. 38101

KRAFT INC.

The information herein is true & accurate to the best of our knowledge. However, both the information & product are offered without warranty or guarantee as to any specific use. Nothing herein shall be construed as a recommendation to use any product in violation of any patent rights.

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## APPROVAL FOR SHIPMENT FORM

Product Name: WR 6026-2HC! 1 mg capsules, lot WRA-06-05182Container Size: 20 capsules Dosage Form: capsuleAcceptable Container: 5 Rejects: 0Total Units Shipped: 5Date Shipped: June 7, 1982Delivery Ticket Number: N/A

Name and Address of Receiver:

Dr. Larry Fleckenstein  
Forest Glen Annex  
Building 500  
Brookville Road  
Walter Reed Army Institute of Research  
Silver Springs, MD 20910

Approval of Shipment by: *Dennis A. Bell*

Pharmaceutical Services  
College of Pharmacy

University of Iowa  
Iowa City, Iowa

## PRODUCT RELEASE FORM

Part A

Product: WR 6026 2HCl, 1 mg capsulesLot No.: WRA-06-05182Batch Size: 300 capsulesDate Received by Warehouse: 5-20-82

| Quantity                              | Size |
|---------------------------------------|------|
| <u>11 bottles of 20 capsules each</u> |      |
|                                       |      |
|                                       |      |

Warehouse: Place this product in quarantine. Please match this form with the release form before placing the product in use.

Part A remains with product until released.

(Detach along dotted line)

## PRODUCT RELEASE FORM

Part B

Part B remains with Quality Control Department Analysis Sheets

Product: WR 6026 2HCl, 1 mg capsulesLot No.: WRA-06-05182Batch Size: 300 capsules

Warehouse: Please (release, destroy, return to mfg.) this product and remove from quarantine.

Signature: [Signature]Date Released: 6-3-82

## DISTRIBUTION LIST

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