AD NUMBER

ADB081073

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

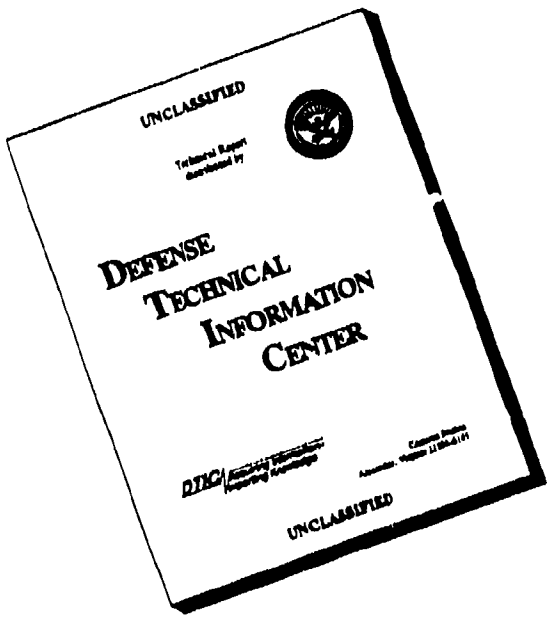
FROM

Distribution authorized to U.S. Gov't. agencies only; contractor performance evaluation; July 1982. Other requests shall be referred to the Commander, U.S. Army Medical Research and Development Command, Attn: SGRD-RMS, Fort Detrick, Frederick, MD 21701.

AUTHORITY

Dep. CS for Info. Mgt, Ft. Detrick, SGRD-RMI-S [70-1y], Memo. 7 Jun 1994

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

AD _____

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

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-B082 0736	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Preformulation Studies of Selected Pretreatment and Therapeutic Compounds		5. TYPE OF REPORT & PERIOD COVERED Annual 7/1/81 - 6/30/82
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) John L. Lach, Douglas R. Flanagan, Lloyd E. Matheson		8. CONTRACT OR GRANT NUMBER(s) DAMD17-79-C-9136
9. PERFORMING ORGANIZATION NAME AND ADDRESS College of Pharmacy, University of Iowa, Iowa City, IA 52242		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A.3M162770A871.AF
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Fort Detrick, ATTN: SGRD-RMS, Frederick, MD 21701		12. REPORT DATE July, 1982
		13. NUMBER OF PAGES 111
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution limited to U.S. Government agencies only; contractor performance evaluation; July, 1982. Other requests for this document must be referred to the Commander, U.S. Army Medical Research and Development Command (Attn: SGRD-RMS) Fort Detrick, Frederick, MD 21701

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Antimalarials; preformulations; antileishminiasis; capsule production; tablet production; placebo; chromatography; WR6026·2HCl; WR180,409·H_2PO_4; WR171,669·HCl; WR142,490·HCl; liposomes.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This annual report contains:

1. Quarterly Report No. 8 (1 August 1981 - 30 September 1981)
2. Quarterly Report No. 9 (1 October 1981 - 31 December 1981)
3. Quarterly Report No. 10 (1 January 1982 - 31 March 1982)
4. Quarterly Report No. 11 (1 April 1981 - 30 June 1982)

AD _____

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

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_3PO_4

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_3PO_4 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.

Table of Contents for Annual Report

AD _____

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

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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 dicetylphosphate (DCP) and α-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.. 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 (ε = 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 (ε = 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

Sample #	Entrapped Conc (mg/ml)
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 wiith 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.

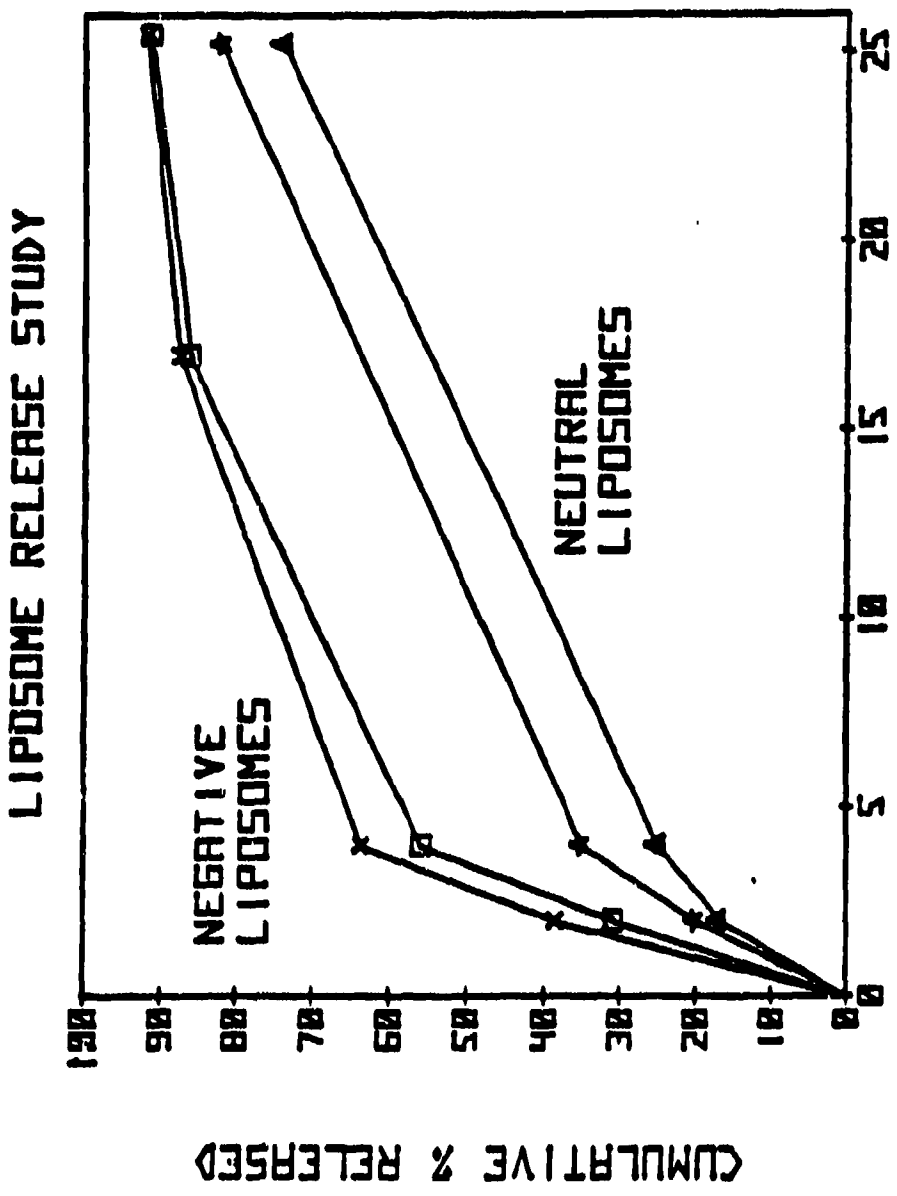


Figure 1. Release of VR5026-2HCl from Liposomes. Key: x, ■ - Negative Liposomes; *, Δ - Neutral Liposomes

Table I
Percent Release of WR6026
from Washed Liposomes

	Sample No.	2 hours	4 hours	17 hours	25.5 hours
Negative Liposomes	1	30.9	55.9	86.3	91.4
	2	38.6	63.7	87.7	91.9
Neutral 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

	Temperature		
Time (Days)	4°C(Refrigerator)	-10°C(Freezer)	40°C
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

Time (Days)	-10°C(Freezer)	40°C[a]	R.T.(20-25°C)[a]
0	4.51 mg/ml	4.51 mg/ml	4.05 mg/ml
0 (freeze & thaw 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

[a]Liposomes dry out, become solid and are difficult to redisperse.

These studies demonstrate that freezing has a deleterious effect on liposome entrapment while refirgerator, room temperture 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 unentrapped 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 unentrapped 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 monoprotonated 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

Batch Size (ml)	Swelling Time (hours)	Entrapped Conc (mg/ml)[a]	% Entrapped
255	4	2.08 (2.04, 2.04, 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, 5.25, 5.39)	4.72
15	17.5	3.39 (4.00, 3.70, 4.08)	3.77

[a]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 given 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

Batch No.	Entrapped Conc. (mg/ml)	% Entrapped
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 molydate, 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)

Table of Contents

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, Pluron.c 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 tabletting 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-10[illegible], 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 distintegration 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 indistinquishable 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

University of Iowa College of Pharmacy

Form CP 1
1507

MANUFACTURING FORMULA

Product: Placebo Capsules for WR 171,669 HCl
List No.: WRA 2
Formula Written by: L. Gwinn Date: 12/29/81
Formula Checked by: [illegible] Date: 12/31/81
Production authorized by: [illegible]
Batch Size: 1500
Control No.: WRA-2-12291

Analysis

Assay for	Theoretical	Actual
WR 17669 HCl	None	None

Control Assay No. _____ Worksheet Checked by _____ Date _____

Specifications

	Initial	Theoretical	Actual
Size	[illegible]	#0 Capsules	# 0 Capsules
Weight	[illegible]	390 ± 25 mg.	406.2 mg.
Color	[illegible]	Body: White op. 999 Cap: White op. 999	Body: White op. 999 Cap: White op. 999
Disintegration (See attached sheet)	[illegible]	NMT 15 minutes	4 minutes.
Tablet Hardness			
Tablet Thickness			
Clarity			
pH			
Density			
Viscosity			
Sedimentation			
Gross Appearance			
Sterility			
Pyrogen			
Other : Weight Variation (See attached sheet)	[illegible]	USP	USP

Package and Label

Type of Container: Amber Glass Vials * with Standard Closures
Size of Container: 11 dram & shrink seals
Method of Packaging: Using Drug-O-Matic automatic filling machine.
Remarks: * Owens-Illinois (FW-4011)

Packaged by: M. Matt
Date: 1-8-82

WR 171,669 HCL AD
HALOFANTRINE HYDROCHLORIDE
PLACEBO
20 CAPSULES/BOTTLE
LOT NO.: WRA-2-12291 BOTTLE NO.: 79
MANUFACTURE DATE: 12/29/81
CAUTION: NEW DRUG. LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE ONLY. PREPARED FOR: WALTER REED ARMY INSTITUTE OF RESEARCH BY:

Pharmaceutical Services • College of Pharmacy
The University of Iowa • Iowa City, Iowa 52242

roduct Placebo Capsules for WR 171,669 HCl List No. WRA 2

atch Size 1500 Control No. WRA-2-12291

aution or Special Instructions

507

ACH CONTAINS	INGREDIENTS AND DIRECTIONS	RAW MAT'L CONTROL NO.	INITIAL	AMOUNT PER BATCH
350 mg.	1. Weigh Corn Starch.	Staley A.E. lot: G 737 81		
	2. Fill #0 White Opaque Capsules on Parke-Davis capsule machine. Weigh 10 capsules from each ring (the weight should be 3.9 gm).	Parke-Davis lot: TSD 8491-3		
	3. Clean capsules with salt.			
	4. Yield: 1560 Capsules			
	# of capsules packaged: 1440			
	Retain sample: 120 Capsules			
	# (30 Capsules per 11 dram bottle)			

Product Placebo Capsules for WR 171,669 HCl List No. WRA 2

Batch Size 1500 Control No. WRA-2-12291

Caution or Special Instructions

1507

EACH CONTAINS	INGREDIENTS AND DIRECTIONS	RAW MAT'L CONTROL NO.	INITIAL	AMOUNT PER BATCH
	In. Process weight variation.			
	420 415			
	400 405			
	418 410			
	418			
	414			
	420			
	390			
	415			
	410			
	415			
	Total yield: 1560 Capsules			

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-2-12291

No.	Mg/Capsule	No.	Mg/Capsule
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

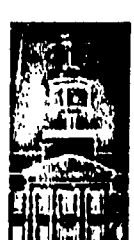
Deviation from low (390) = 4.0%
Deviation from high (426) = 4.8%

Balance was tared with empty capsule.

The University of Iowa
Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4520



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

Moisture, %	11.5
Iron - PPM	0
Ash, %	.06
pH	6.2
Color	2.7
Flavor	OK
Odor	OK
Screen Analysis:	
On #80(%)	.92
On #325 (%)	.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

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

APPENDIX II

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

University of Iowa College of Pharmacy
MANUFACTURING FORMULA

Form CP 1
1507

Product Placebo Tablets for WR 142,490 HCl	List No. WRA 3
Formula Written by R. Grover Date 12/29/81 Formula Checked by Overholt Date 12/24/81	Batch Size 1500
Production authorized by Emma A. Elliott	Control No. WRA-3-12291

Analysis

Assay for	Theoretical	Actual
WR 142,490 HCl	None	none

Control Assay No. _____ Worksheet Checked by _____ Date _____

Specifications

	Initial	Theoretical	Actual
Size	[illegible]	7/16 inch S.C.	7/16 inch S.C.
Weight (see attached sheet)	[illegible]	552.7 ± 27.6 mg	563.9 mg
Color	[illegible]	White	white
Disintegration (see attached sheet)	[illegible]	NMT 30 Minutes	35 seconds
Tablet Hardness (see attached sheet)	[illegible]	> 4 kg	4.6 kg
Tablet Thickness (see attached sheet)	[illegible]	5.9 ± 0.5 mm	5.9 mm
Clarity			
pH			
Density			
Viscosity			
Sedimentation			
Gross Appearance			
Sterility			
Pyrogen			
Other Weight Variation	[illegible]	U.S.P.	U.S.P.

Package and Label

Type of Container: Amber glass vials* with standard closures
Size of Container: 11 dram with shrink seals
Method of Packaging: Using Drug-O-Matic automatic filling machine
Remarks:

*Owens-Illinois (PW-4011)

WR 142,490 HYDROCHLORIDE
MEFLOQUINE HYDROCHLORIDE
PLACEBO
20 TABLETS/BOTTLE
LOT NO.: WRA-3-12291 BOTTLE NO. 79
MANUFACTURE DATE: 12/29/81
CAUTION: NEW DRUG. LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE ONLY. PREPARED FOR: WALTER REED ARMY INSTITUTE OF RESEARCH BY:

Pharmaceutical Services • College of Pharmacy
The University of Iowa • Iowa City, Iowa 52242

Packaged by M. McMath
Date 1-8-82

Product Placebo Tablets for WR 142,490 HCl List No. WRA 3

Batch Size 1500 Control 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 Lot # INC 19		632 gm
60.0 mg	2. Weigh Avicel PH 101.	FMC Lot # 1114		90 gm
55.0 mg	3. Weigh Sta-Rx 1500.	Colorcon Lot 908A9		82.5 gm
	4. Blend Lactose, Avicel and Sta-Rx in a V-blender for 10 minutes.			
	Mixing started at: 10[illegible] am ; Stopped at 10[illegible] am	12/29/81		
5.5 mg	5. Weigh Magnesium Stearate and transfer it to the V-blender (4).	Mallinckrodt Lot# KMKH		8.25 gm
	6. Blend it for 5 minutes.			
	Time: Mixing started: 10[illegible] am ; mixing stopped: 10[illegible] am	12/29/81		
11.0 mg	7. Weigh Talc and transfer it to the same V-blender (4).	[illegible] Lot# 9646		16.5 gm
	8. Blend it for an additional 5 minutes.			
	Time: Mixing started: 10[illegible] am ; mixing stopped at [illegible] am	12/29/81		
	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.			
	10. Vacuum tablets, inspect and package.			
	11. Package 20 tablets in 11 dram amber glass vials using Drug-O-Matic automatic filling machine.			
	12. Yield: 1225 tablets *			
	# of tablets packaged : 1200			
	Retain sample : 25 tablets			
	* (20 tablets per 11 dram bottles)			

The University of Iowa
Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4820

WEIGHT VARIATION OF FINISHED TABLETS

Lot No: WRA-3-12291

No.	Mg/Tablet	No.	Mg/Tablet
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%

The University of Iowa
Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4820

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: 35 seconds (average of six tablets)

The University of Iowa

Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4820

IN-PROCESS CONTROL FOR PLACEBO TABLETS

Lot # WRA-3-12291

Hardness (> 4 kg)	Thickness (5.9 ± 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

SHEFFIELD PRODUCTS — PROTOCOL OF ASSAY

CUSTOMER: UNIVERSITY OF IOWA
ADDRESS: [redacted]
IOWA CITY IOWA 52242

ATTN: DENISE MCLELLEN

PRODUCT: LACTOSE U.S.P. ANHYDROUS DIRECT TABLETING

LOT NO.: 1NC19
CUSTOMER ORDER NO.: V57239

DATE SHIPPED: 5/ 1/81
NUMBER OF DRUMS: 1
INVOICE NO.: 72308

RESULTS OF ASSAY WHERE APPLICABLE TO PRODUCT SHIPPED:

CHEMICAL/PHYSICAL		MICROBIOLOGICAL	
SOLUBILITY	PASS	STAND. PLATE COUNT	<100/GRAM
MOISTURE %	0.37 - 0.37	THERMOPHILE COUNT	PASS
ASH %	0.076	COLIFORM	NEGATIVE
HEAVY METALS	<5 PPM	SALMONELLA	NEGATIVE
SPECIFIC ROTATION	55.03	MOLD	<50/GRAM
ACIDITY	PASS		
PH [10% SOL.]	4.4 - 4.9		
ALCOHOL SOL. RESIDUE	2.83		

DATE: 5/ 1/1981

SHEFFIELD PRODUCTS, BOX 330, MEMPHIS, TENN. 38101 — KRAFT INC.

FMC CORPORATION
Food & Pharmaceutical Products Division
1301 Ogletown 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
Starch Test	negative
Retained on a screen having 37 um openings, wt. %	>5
Identification	passes

RB Worth

R. B. Worth
Quality Control Manager

CHEMICAL ENG DEPT.

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

STA-RX 1500 STARCH PROTOCOL

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 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.

PARIS BY-PASS • P.O. BOX M • PARIS, KENTUCKY 40361 • (606) 987-7000

ITEM - MAGNESIUM STEARATE NF

CODE 2256

LOT KHKH

TESTS	RESULTS
Identification	Passes Test
Loss on drying	3.72%
Lead (Pb)	0.0001%
Assay (MgO)	7.72%
Sieve test US Standard #325 Mesh	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

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_3PO_4

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

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

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_3PO_4 using a high pressure liquid chromatographic procedure.

Table of Contents

List of Tables

List of Figures

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

	Run			
Condition	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 µl 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 WR6026·2HCl remaining over time is given in Table II and shown in Figure 1 for the conditions described in Table I.

Table II: Percent of WR6026·2HCl Remaining as a Function of Time.

	Run			
Time (Hrs)	1	2	3	4
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

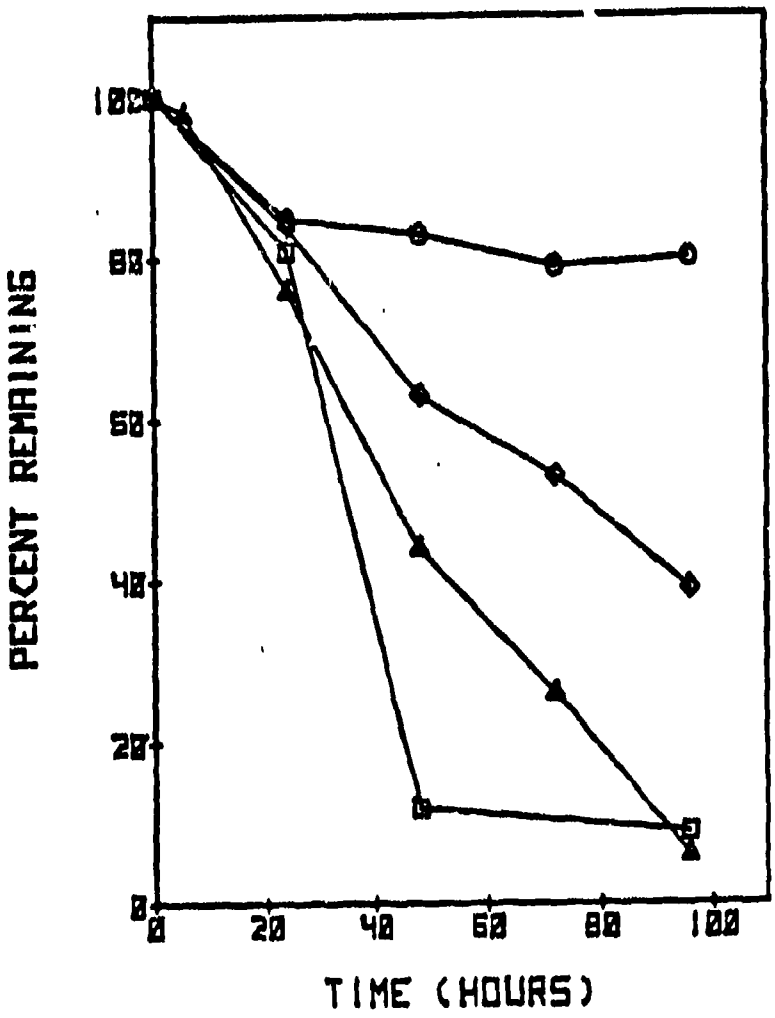


Figure 1. Percent of WR6026·2HCl remaining as a function of time. Run 1, □ ; Run 2, Δ; Run 3, O; Run 4, ◇ .

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.

	Run			
Time (Hrs)	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.

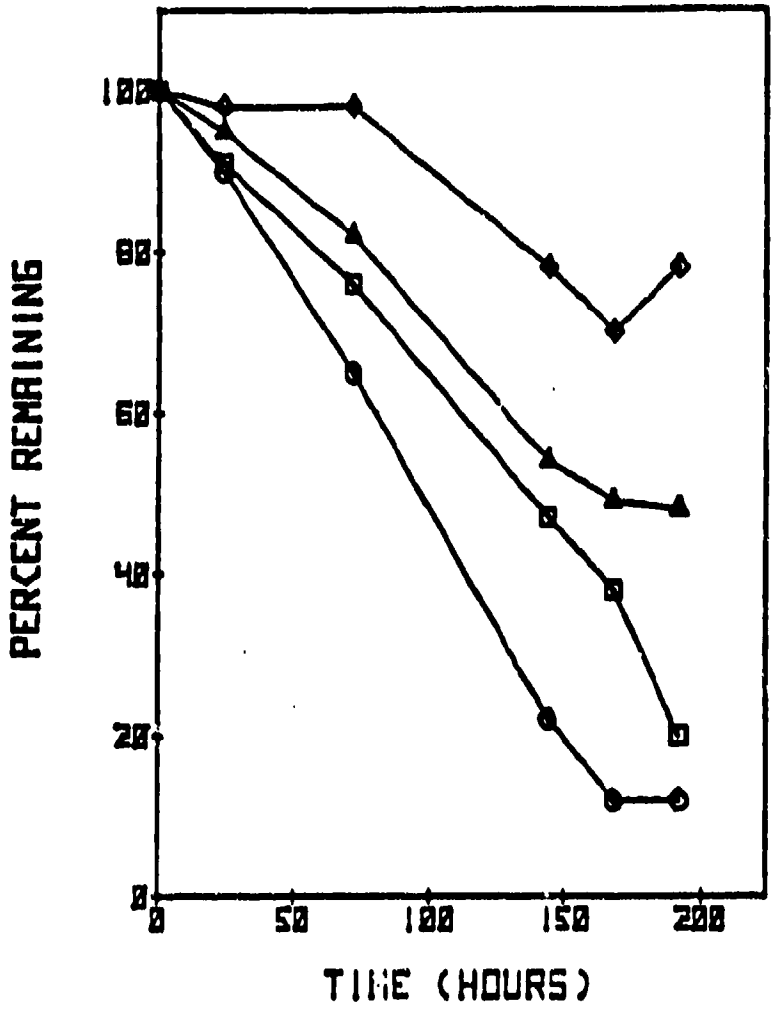


Figure 2. Effect of EDTA on WR6026·2HCl stability. Percent of WR6026·2HCl remaining as a function of time. Run 1, □; Run 2, Δ; Run 3, O; Run 4, ◇.

Quantitative Blood Assay for WR180,409·H_3PO_4

An attempt was made to develop a quantitative assay for WR180,409·H_3PO_4 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_3PO_4 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_2SO_4 containing the internal standard, WR184,806·H_3PO_4, 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_3PO_4

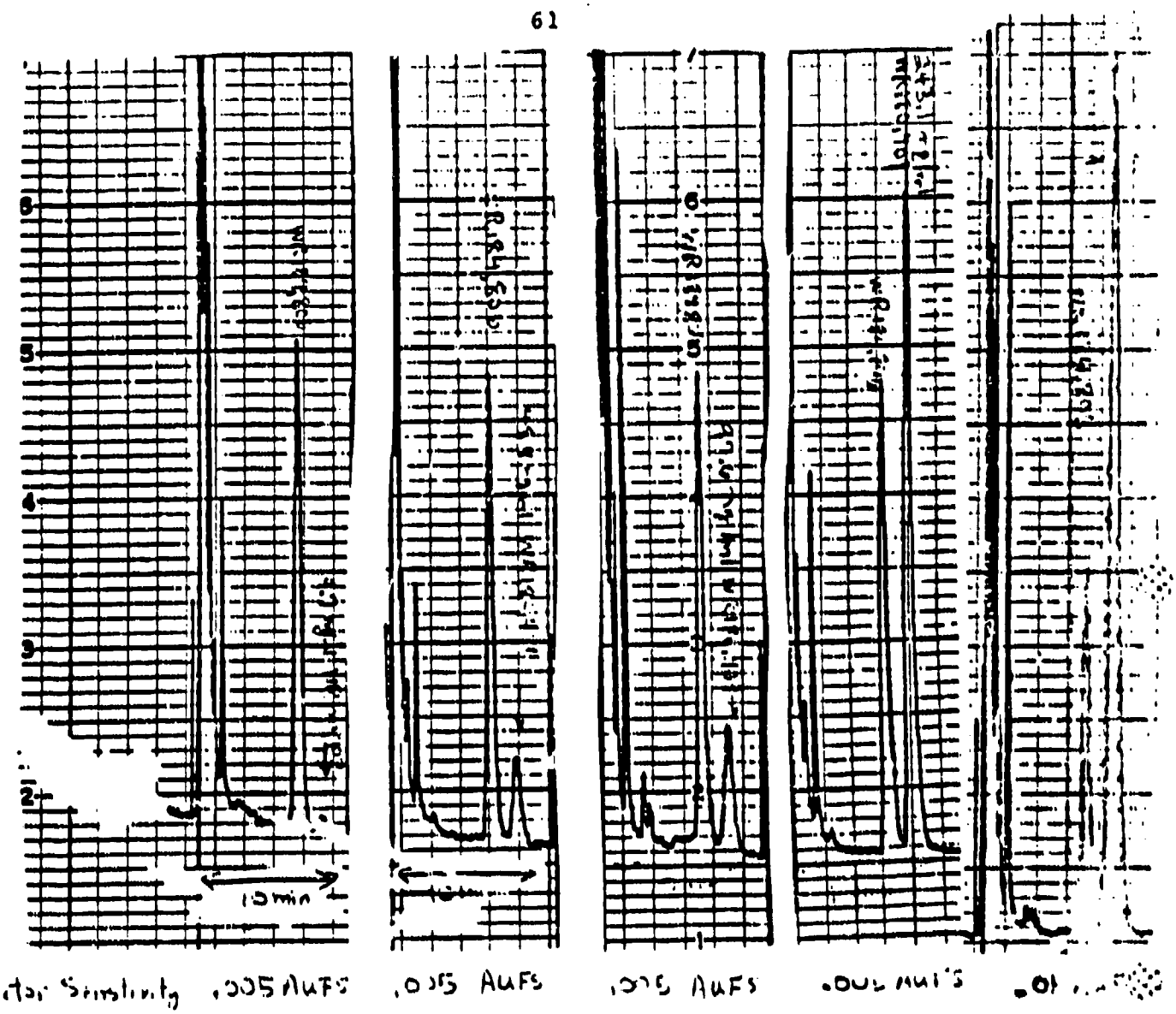


Figure 3. Typical chromatograms for WR180,409·H_3PO_4

Table IV. High Pressure Liquid Chromatographic Conditions for the Analysis of WR180,409·H_3PO_4 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.
Mobile Phase:	33% UV Grade Acetonitrile (Burdick & Jackson) 7% Methanol (Burdick & Jackson) 60% pH3 Phosphate buffel (.05 M) prepared using NaH_2PO_4 and H_3PO_4.
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_3PO_4 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_3PO_4 Extracted as Described in the Extraction Procedure

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

[a]Lysed by sonification
[b]Lysed by freeze/thaw method

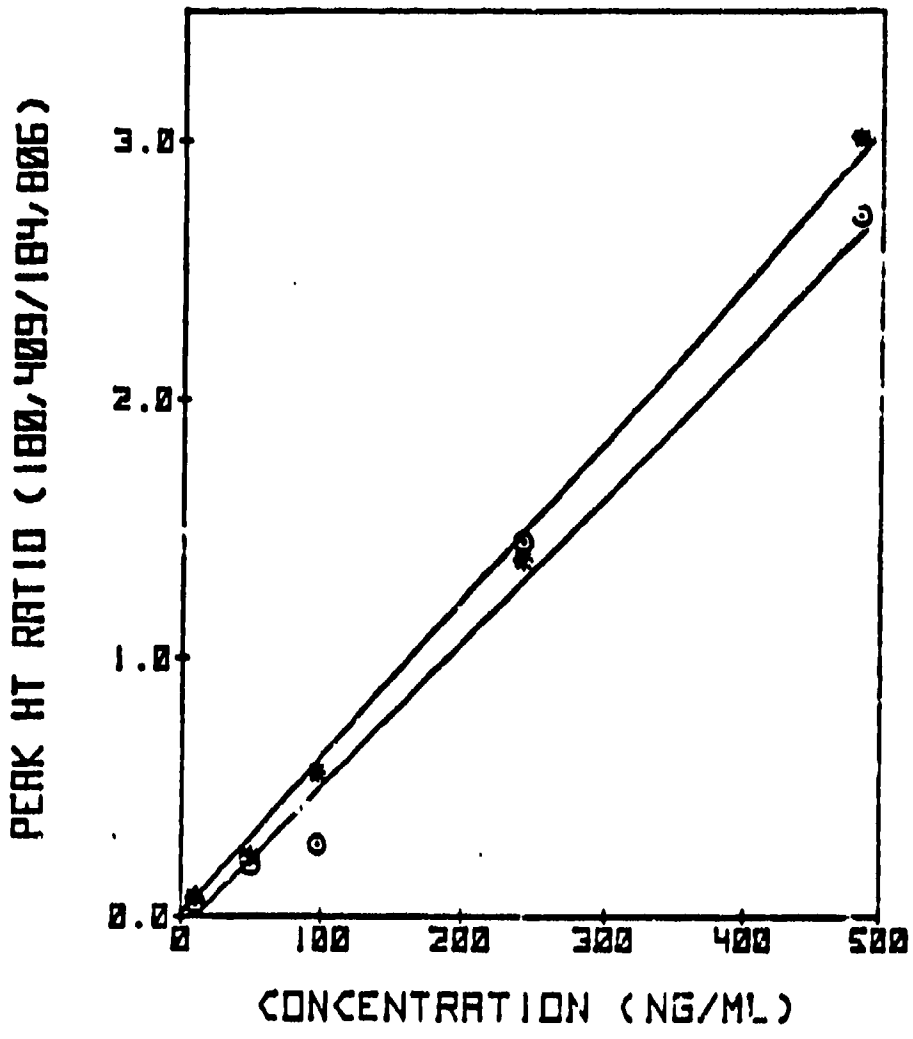


Figure 4. Standard curves for WR180,409·H_3PO_4 extraction procedure. Curve A, *, Curve B, O.

The extraction procedure was modified slightly by including the internal standard in the blood sample in step one along with the WR180,409·H_3PO_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 WR180,409·H_3PO_4 When Internal Standard Is Added to Blood

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

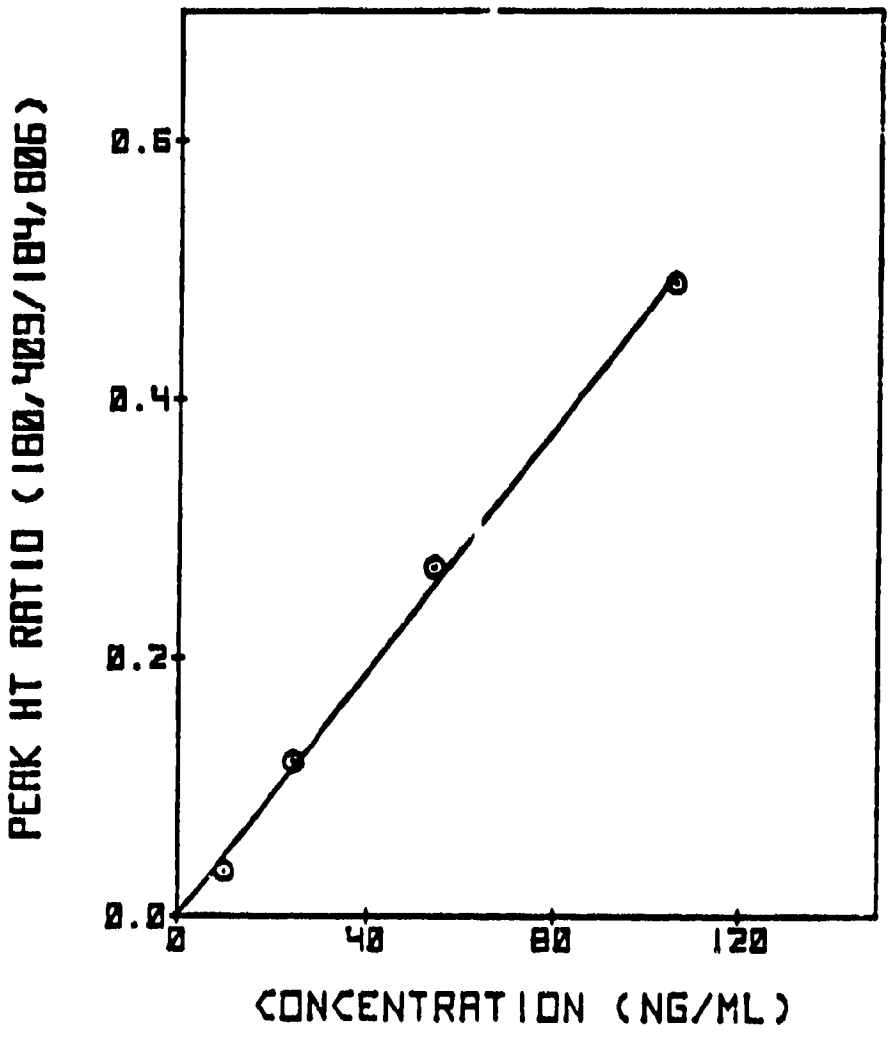


Figure 5. Standard curve for WR180,409·H_3PO_4 when internal standard is added to the blood.

AD _____

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

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)

Table of Contents

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 white opaque gelatin capsule shells.

Weight variation test for twenty placebo capsules (Lot #WRA-07-05182) 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

Page 1 of _____ pages

Form CP 1
1507

MANUFACTURING FORMULA

Product: Placebo Capsule for WR 6026·2HCL
Formula Written by: [signature] Date: 5-17-82
Formula Checked by: [signature] Date: 5-17-82
Production authorized by: John L Lasch

List No.: WRA-08
Batch Size: 1700 Capsules
Control No.: WRA-08-05182

Analysis

Assay for	Theoretical	Actual
WR 6026·2HCl	0 mg.	0 mg

Control Assay No. IFC-A-029 Worksheet Checked by: [signature] Date: 6-3-82

Specifications

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

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: [signature]
Date: 5-4-82

WALTER REED ARMY INSTITUTE OF RESEARCH
Division of Experimental Therapeutics Washington, D.C. 20012
WR 6026·2HCl AF Placebo 20 Capsules
6-Methoxy-8-(6-diethylaminohexylamino)lepidine dihydrochloride
Control No. WRA-08-05182 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

roduct Placebo for WR 6026·2HCl List No. WRA-08

ıcch Size Approx. 1700 Capsules Control No. WRA-08-05182

ıution or Special Instructions

307

ACH CONTAINS	INGREDIENTS AND DIRECTIONS	L.# MAT'L CONTROL NO.	INITIAL		AMOUNT PER BATCH
	Add to 1 qt. V-blender shell				
	Lactose, USP, Hydrous	Y-031-098			600 gm
	Mfr.: Sheffield 605				
	Mfr. Lot #: ONJ-30				
	Material Lot #: M-610-016-610				
	Exp. Date: 3-4-83				
	D & C Yellow #10 Lake, 16% Pure Dye	Y-031-122			5 gm
	Mfr.: Warner-Jenkinson				
	Mfr. Lot #: AA 9727				
	Material Lot #: M614-016-634				
	Exp. Date: 3-25-83				
	Colloidal Silicon Dioxide, NF	RB-101-052			2 gm
	Mfr.: Cabot Cab-O-Sil M-5				
	Mfr. Lot #: 16131-L				
	Material Lot #: M-036-017-036				
	Exp. Date: 10-9-83				
	Blend for 5 minutes				
	Blending Start: 3:15 PM				
	Blending Stop: 3:20 PM				
	Pass blend through a #60 mesh screen; return to blender and blend for 5 minutes				
	Blending start: 3:27				
	Blending stop: 3:32				
	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: Too Dark				
	Subroutine Chosen: A				

oduct Placebo for WR 6026·2HCl List No. WRA-08

tch Size Approx. 1700 Capsules Control No. WRA-08-05182

uction or Special Instructions

07

CH CONTAINS	INGREDIENTS AND DIRECTIONS	RAW MAT'L CONTROL NO.	INITIAL	AMOUNT PER BATCH
	Subroutine A			
	Add additional Lactose, USP, Hydrous Mfr.: Sheffield 605 Mfr. Lot #: DNJ-30 Material Lot #: M-610-016-60 Exp. Date: 3-4-83 Amount of Lactose Added: 200gm	Y-021-092		200gm
	Blend for 5 minutes Blending Start: 3:40 Blending Stop: 3:45			
	Subroutine B			
	Add additional D & C Yellow #10 Lake, 16% Pure Dye Mfr.: Mfr. Lot #: Not Used Material Lot #: Exp. Date: Amount of D & C Yellow #10 Lake Added:			
	Blend for 5 minutes Blending Start: Blending Stop: 5-18-82			
	Weight of Total Blend: 805 gm			
	% Yield = 805 / 807 x 100 = 99.8 % Acceptable Range: 90-103%			
	Fill 332 mg. of powder blend into #2 gelatin capsules white opaque cap/body. Mfr.: Capsugel, Parke-Davis Mfr. Lot #: YD-0441-Y Material Lot #: M-048-013-048 Exp. Date: 6-18-83	T-049-006		
	Fill capsules, 96 at a time, using Daltay Manual Capsule Filling machine.			
	Add 335 gm of powder blend for each set of 96 capsules.			
	6gm Sample Taken for Assay			
	6gm Sample Taken for Retained Sample			

Product Placebo for WR 6026·2HCl List No. WRA-08

Batch Size Approx. 1700 Capsules Control No. WRA-08-05182

Caution or Special Instructions

1507

EACH CONTAINS	INGREDIENTS AND DIRECTIONS	RAW MAT'L CONTROL NO.	INITIAL	AMOUNT PER BATCH
	In Process capsule fill weights (gross)			
	Capsule Set #			
	1. 3.937 gm 9. 3.940			
	2. 3.964 10. 3.857			
	3. 3.908 11. 3.916			
	4. 3.949 12. 3.938			
	5. 3.973 13. 3.931			
	6. 3.905 14. 3.884			
	7. 3.949 15. 3.937			
	8. 3.958 16. 3943			
	Weight of 10 empty capsules: 0.619 gm			
	# of capsules filled: 1535			
	30 capsules removed for quality control and retain samples			
	Capsules packaged (30 capsules/bottle)			
	Vial Space packaging with Lannett Pharmaceutical Cont Kendall Co. Lot # 369502/732 (02/182) 3-2-84	DD-032-006		
	Bottle/Closure:			
	Owens Illinois 7dr. Amber Round Natural Lot # MM 403-017-403 5-21-84	DD-052-135		
	50 bottles sent to Ann, remainder kept in stock. (24 Bottles)			

The University of Iowa
Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4520

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

No.	Mg/Capsule	No.	Mg/Capsule
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 MCGEEGAN~~ Pharm - Coll. of

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

LOT NO.: 0NJ30
CUSTOMER ORDER NO.: 044786

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

RESULTS OF ASSAY WHERE APPLICABLE TO PRODUCT SHIPPED:

CHEMICAL/PHYSICAL		MICROBIOLOGICAL	
SOLUBILITY	PASS	STAND. PLATE COUNT	<100/GRAM
MOISTURE %	5.07 - 5.07	THERMOPHILE COUNT	PASS
ASH %	0.022	COLIFORM	NEGATIVE
SPECIFIC ROTATION	55.21	MOLD	<50/GRAM
ACIDITY	PASS		
PH C10% SOL.]	4.3 - 4.7		
ALCOHOL SOL. RESIDUE	1.23		

RECEIVED
APR 02 1981
PURCHASING DEPT

RECEIVED
MAR 30 1981
PURCHASING DEPT.

DATE: 3/27/1981 This copy for your files

SHEFFIELD PRODUCTS, BOX 398, MEMPHIS, TENN. 38101 KRAFT INC.

CAB-O-SIL® DIVISION

CABOT CORPORATION P.O. BOX 188, TUSCOLA, ILLINOIS 61953

TELEPHONE AREA CODE 217
TUSCOLA 253-2170
TELEX TUSCOLA 910-663-2043

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 ($m^2/g.$)	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_2, 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

Gabriel Paci
Quality Assurance Manager
CAB-O-SIL Division

GP/cjo

WASHINGTON, D.C. 20204

81

LOT NO. AA- 512.

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.

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

cc: KAN-DO

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

FORM FDH 2000 (1/75)

PREVIOUS EDITION IS OBSOLETE.

The University of Iowa
Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4520

1847

APPROVAL FOR SHIPMENT FORM

Product Name: WR 6026-2HCl, Placebo capsules lot WRA-08-05182

Container Size: 20 capsules Dosage Form: capsule

Acceptable Container: 50 Rejects: 0

Total Units Shipped: 50

Date Shipped: June 7, 1982

Delivery 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 20910

Approval of Shipment by: Dennis A Clikeman

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: _____

Quantity	Size
74 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: 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: [signature]

Date Released: 6-3-82

Appendix II

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

University of Iowa College of Pharmacy MANUFACTURING FORMULA

Form CP 1
1307

Product WR-6026 2HCl, 5 mg, Capsules
Formula
Weighed by [illegible] Date 5-17-82 Checked by [illegible] Date 5-17-82
Processed by [illegible]

List No. WRA-07
Batch Size 1700 Capsules
Control No. WRA-07-05182

Analysis

Assay for	Theoretical	Actual
WR 6026·2HCl	5.0 mg	4.63 mg

Control Assay No. IFC-A-028 Worksheet Checked by [illegible] Date 6-3-82

Specifications

	Initial	Theoretical	Actual
Size	[illegible]	#2 Gelatin Capsules	#2 Gelat. Capsules
Weight	[illegible]	332 mg/capsule	331.7 mg capsules
Color	[illegible]	White Opaque cap/body	white/opaque caps
Disintegration	[illegible]	NMT 10 minutes	2 min
Tablet Hardness			
Tablet Thickness			
Clarity			
pH			
Density			
Viscosity			
Sedimentation			
Gross Appearance			
Sterility			
Pyrogen			
Other : Weight Variation	[illegible]	Meet USP	Met USP
Color of Powder Blend		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 Coil.

Shrink-seal applied to closed vial.

Packaged by [illegible]
Date 5-24-82

WALTER REED ARMY INSTITUTE OF RESEARCH
Division of Experimental Therapeutics Washington, D.C. 20012
WR 6026·2HCl AF 5 mg 20 Capsules
6-Methoxy-8-(6-diethylaminohexylamino)lepidine dihydrochloride
Control No. WRA-07-05182 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, 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 Mfr.: Sheffield 605 Mfr. Lot #: ONJ-30 Material Lot #: M-610-016-610 Exp. Date: 3-4-83	Y-031-098		23.8 gm
	Colloidal Silicon Dioxide, NF Mfr.: Cabot Cab-O-Sil M-5 Mfr. Lot #: ~~M-036-~~ 16181-L Material Lot #: M-036-017-036 Exp. Date: 10-9-83	RA-101-062		1.9 gm
	WR 6026·2HCl Source: Walter Reed Army Institute of Research Lot #: AF Material Lot #: M-386-017-386 Exp. Date: 5-5-84	AA-052-182		8.7 gm
	Blend for 5 minutes: Blending start: 10:40 Blending stop: 10145			
	Pass blend through a #60 mesh screen. Return to blender.			
	Add to the same blender, through a #60 mesh screen:			
	Lactose, USP, hydrous Mfr.: Sheffield 605 Mfr. Lot #: ONJ-30 Material Lot #: M-610-016-610 Exp. Date: 3-4-83	Y-031-098		71 gm
	Blend for 5 minutes: Blending Start: 10:50 Blending Stop: 10:55			
	Add to the same blender through a #60 mesh screen:			
	Lactose, USP, hydrous Mfr.: Sheffield 605 Mfr. Lot #: ONJ-30 Material Lot #: M-610-016-610 Exp. Date: 3-4-83	Y-031-098		150 gm

oduct WR 6026·2HCl, 5 mg., Capsules List No. WRA-07

tch Size 1700 Capsules Control No. WRA-07-05182

uction or Special Instructions

07

CH CONTAINS	INGREDIENTS AND DIRECTIONS	RAW MAT'L CONTROL NO.	INITIAL	AMOUNT PER BATCH
	Blend for 5 minutes Blending Start: 11:00 Blending Stop: 11:05			
	Add to the same blender through a #60 mesh screen			
	Lactose, USP, Hydrous Mfr.: Sheffield 605 Mfr. Lot #: 10A1-30 Material Lot #: M-610-016-610 Exp. Date: 3-4-83	Y-031-09R		305 gm
	Blend for 5 minutes Blending Start: 11:15 Blending Stop: 11:20			
	Weight of final blend: 564 gm			
	Remove a sample for assay and a sample for retention. Each Sample Assayed Approved			
	Remove 20.3 gm for use in blending WRA-06-05182 1 mg. capsules after assay has been performed.			
	Fill 332 mg of powder blend into: #2 gelatin capsules, white opaque body/cap Mfr.: Capsugel, Parke Davis Mfr. Lot #: YA-0441-Y Material Lot #: M-048-017-048 Exp. Date: 6-18-83	T-049-006		1600
	Fill capsules, 96 at a time, using the Deltay Capsule filling machine (manual).			
	Add 33 gm of powder blend for each set of 96 capsules.			
	In-Process fill weights (gross weights). Capsule Set #: 1. 3.943 9. 3.900 2. 3.935 10. 3.911 3. 3.901 11. 3.917 4. 3.991 12. 3.913 5. 3.871 13. 3.893 6. 3.941 14. 3.897 7. 3.921 15. 3.890 8. 3.929 16. 3.900 (48) Caps 17. 3.947 (74) Caps			

roduct WR 6026·2HCl, 5 mg. Capsules List No. WRA-07

tch Size 1700 Capsules Control No. WRA-07-05182

ution or Special Instructions

07

CH CONTAINS	INGREDIENTS AND DIRECTIONS	RAW MAT'L CONTROL NO.	INITIAL	AMOUNT PER BATCH
	Weight of 10 empty capsules: 0.619 gm			
	# of capsules filled: 1517			
	% Yield: Powder Blend:			
	% Yield = 564 x 100 = 99.9% 564.4 gm			
	Acceptable range: 90 - 103%			
	30 capsules removed for quality control and stored samples			
	Capsules packaged, 20 capsules/bottle into			
	7 dr. Amber vials Owens-Illinois Material Lot# M 403-017-403 5-21-84	DD-052-135		
	Void space filled with			
	Rayon Pharmaceutical Coil Kendall Co. Lot# 36350 21722 (021782) 3-2-84	DD-032-006		
	72 bottles filled			
	50 bottles sent to army 22 bottles remain in stock			

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

1847

WEIGHT VARIATION OF FINISHED CAPSULES, 5 MG

WR 6026.2HCl/Capsule

Lot # WRA-07-05182

No.	Mg/Capsule	No.	Mg/Capsule
1	341	11	329
2	326	12	333
3	332	13	311
4	322	14	[illegible]
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

No.	Mg/Capsule	% of Label
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.

SHEFFIELD PRODUCTS — PROTOCOL OF ASSAY

CUSTOMER: UNIVERSITY OF IOWA
ADDRESS: PURCHASING DEPT.
IOWA CITY IOWA 52242
~~ATTN: DENISE MCLEEAN~~ Pharm. - Coll. of

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

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		MICROBIOLOGICAL	
SOLUBILITY	PASS	STAND. PLATE COUNT	<100/GRAM
MOISTURE %	5.07 - 5.07	THERMOPHILE COUNT	PASS
ASH %	0.022	COLIFORM	NEGATIVE
SPECIFIC ROTATION	55.21	MOLD	<50/GRAM
ACIDITY	PASS		
PH [10% SOL.]	4.3 - 4.7		
ALCOHOL SOL. RESIDUE	1.23		

RECEIVED
APR 02 1981
PURCHASING DEPT.

RECEIVED
MAR 30 1981
PURCHASING DEPT.

DATE: 3/24/1981 This copy for your files

SHEFFIELD PRODUCTS, BOX 398, MEMPHIS, TENN. 38101 KRAFT INC.

CAB-O-SIL® DIVISION

CABOT CORPORATION P.O. BOX 188, TUSCOLA, ILLINOIS 61953

TELEPHONE AREA CODE 217
TUSCOLA 253-2175
TELEX TUSCOLA 910-663-1042

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 ($m^2/g.$)	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_2, 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

1847

APPROVAL FOR SHIPMENT FORM

Product Name: WR 6026-2HCl 5 mg capsules, lot WRA-07-05182

Container Size: 20 caps. Dosage Form: capsule

Acceptable Container: 50 Rejects: 0

Total Units Shipped: 50

Date Shipped: June 7, 1982

Delivery 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: [signature]

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: Ling-Tong Chi

Date Released: 6-3-82

Appendix III

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

University of Iowa College of Pharmacy

Page 1 of _____ pages

Form CP 1
1507

MANUFACTURING FORMULA

Product: WR 6026-2 HCl, 1 mg. Capsules
Formula Written by: [signature] Date: 5-17-82
Formula Checked by: [signature] Date: 5-17-82
Production authorized by: John F Lasch

List No.: WRA-06
Batch Size: 300 Capsules
Control No.: WRA-06-05182

Analysis

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

Control Assay No. TFe-A-026 Worksheet Checked by: [signature] Date: 6-3-82

Specifications

	Initial	Theoretical	Actual
Size		#2 Gelatin Capsules	#2 Gelatin Capsules
Weight		330 mg/capsule	324.9 mg
Color		White Opaque Cap/Body	white opaque capsule
Disintegration		NMT 10 min.	2.0 minutes
Tablet Hardness			
Tablet Thickness			
Clarity			
pH			
Density			
Viscosity			
Sedimentation			
Gross Appearance			
Sterility			
Pyrogen			
Other: Weight Variation		Meet USP	Meet USP
Color of Powder Blend		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 REED ARMY INSTITUTE OF RESEARCH
Division of Experimental Therapeutics 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

Page _____ of _____ pages

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 CONTROL NO.	INITIAL	AMOUNT PER BATCH
	Add to a 1 pt. plastic V-blender shell.			
	Blend from WRA-07-05182 after assay. Assay results: 4.996 mg / 332 mg of Blend Amount of blend needed is the amount necessary to obtain 300 mg. of active drug.			20.32 gm
	Add to the same blender through a #60 mesh screen			
	Lactose, USP, hydrous Mfr.: Sheffield 605 Mfr. Lot #: DNL-30 Material Lot #: M-610-016-610 Exp. Date: ~~5-1-83~~ 3-4-83 ~~M-610-016~~	Y-031-098		78.68
	Blend for 5 minutes: Blending Start: 1:15 Blending Stop : 1:20			
	Weight of final blend 99 gm.			
	Remove samples for assay and for retained sample. 1gm Samples Removed			
	Fill 330 mg. of powder blend into #2 gelatin capsules, white opaque body/cap. Mfr.: Capsugel - Parke Davis Mfr. Lot #: YC-0441-V Material Lot #: M-048-017-048 Exp. Date: 6-18-83	T-049-006		
	Fill capsules, 96 at a time, using the Daltay Manual Capsule filling machine.			
	Add 32 gm. of powder blend for each set of 96 capsules.			
	In process capsule fill weights (gross weight) Capsule Set # i. 3.899 gm ii. 3.891 g iii. 3.860			

81496

Page _____ of _____ pages

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 CONTROL NO.	INITIAL	AMOUNT PER BATCH
	Weight of 10 empty capsules 0.619			
	# of Capsules filled: 288			
	% Yield Powder blend			
	% Yield = 99/99 x 100 = 100 %			
	Capsules filled			
	% Yield = 288/293 x 100 = 98.3 %			
	Acceptable range 90 - 103%			
	20 capsules removed for quality control and retained samples			
	Capsules packaged 20/bottle in			
	7dram amber Reveals	NA-052-135		
	Owens-Illinois			
	M 407-017-407			
	5-21-84			
	Void Space filled with			
	Kamo Pharmaceutical Cork	NA-827-006		
	Kendall Co			
	Lot# 3695021727 (02/782)			
	3-2-84			
	11 filled bottles			
	5 bottles sent to qmy			
	6 bottles remain in Operations			

F1888

The University of Iowa
Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4620

1847

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

The University of Iowa
Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4620

1847

WEIGHT VARIATION OF FINISHED CAPSULE, 1 MG

WR 6026.2HCl/Capsule

Lot # WRA-06-05182

No.	Mg/Capsule	No.	Mg/Capsule
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

The University of Iowa
Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4520

1847

FORMULATED PRODUCT

WRA-06-05182
1.0 mg/Capsule

No.	Mg/Capsule	% of Label
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. Chi

SHEFFIELD PRODUCTS — PROTOCOL OF ASSAY

CUSTOMER: UNIVERSITY OF IOWA
ADDRESS: PURCHASING DEPT.
IOWA CITY IOWA 52242
~~ATTN: DENISE MCLELLAN~~ Pharm - Coll. of

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

LOT NO.: ONJ30
CUSTOMER ORDER NO.: U94786

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

RESULTS OF ASSAY WHERE APPLICABLE TO PRODUCT SHIPPED:

CHEMICAL/PHYSICAL		MICROBIOLOGICAL	
SOLUBILITY	PASS	STAND. PLATE COUNT	<100/GRAM
MOISTURE %	5.07 - 5.07	THERMOPHILE COUNT	PASS
ASH %	0.022	COLIFORM	NEGATIVE
SPECIFIC ROTATION	55.21	MOLD	<50/GRAM
ACIDITY	PASS		
PH (10% SOL.)	4.3 - 4.7		
ALCOHOL SOL. RESIDUE	1.23		

RECEIVED
APR 02 1981
PURCHASING DEPT.

RECEIVED
MAR 30 1981
Pharmacology DEPT.

DATE: 3/24/1981 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.

The University of Iowa
Iowa City, Iowa 52242

College of Pharmacy
Department of Pharmaceutical Service

(319) 353-4620

1847

APPROVAL FOR SHIPMENT FORM

Product Name: WR 6026-2HCl 1 mg capsules, lot WRA-06-05182

Container Size: 20 capsules Dosage Form: capsule

Acceptable Container: 5 Rejects: 0

Total Units Shipped: 5

Date Shipped: June 7, 1982

Delivery 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. Ellard

Pharmaceutical Services
College of Pharmacy

University of Iowa
Iowa City, Iowa

PRODUCT RELEASE FORM — Part A

Product: WR 6026 2HCl, 1 mg capsules

Lot No.: WRA-06-05182

Batch Size: 300 capsules

Date Received by Warehouse: 5-20-82

Quantity	Size
11 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, 1 mg capsules

Lot No.: WRA-06-05182

Batch 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

12 copies	Director Walter Reed Army Institute of Research Walter Reed Army Medical Center ATTN: SGRD-UWZ-C Washington, DC 20012
4 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, MD 21701
2 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234