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**AUTHORITY**

USAMRMC Ltr., 10 Aug 98
TITLE: Analytical, Characterization and Stability Studies of Chemicals, Bulk Drugs and Drug Formulations

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PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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<td>Analytical, Characterization and Stability Studies of Chemicals, Bulk Drugs and Drug Formulations</td>
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**Sponsoring/Monitoring Agency Name(s) and Address(es):**
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Fort Detrick, Maryland 21702-5012

**Abstract:**
The overall purpose of this contract was to perform chemical/physical analyses on bulk pharmaceutical substances and formulated drug products of interest to the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense, etc. Specific objectives were to design, develop, validate, and apply methods to determine chemical and physical characteristics on bulk drug and drug products.

For the entire contract period, 1 August 1991 to 30 June 1997, 125 samples of bulk drugs and dosage formulations were analyzed for identity, purity or potency; 133 samples were studied for stability and solubility. Four chiral separation methods were developed and validated, and 11 other chemical assay methods were validated. Special projects included the development and application of assays for determining protein content, residual solvents, and other relevant components in microsphere vaccine preparations. A second special project was the development and application of an assay for determining bis(chloromethyl) ether in HI-6 bulk drugs. Posters were presented in the 1993 and 1996 Medical Defense Bioscience Reviews. One publication appeared in press and a second has been accepted for publication.
Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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In conducting research using animals, the investigator(s) adhered to the “Guide for the Care and Use of Laboratory Animals,” prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature Oct. 1997
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INTRODUCTION

This final report for Contract DAMD17-91-C-1135 covers the period from 1 August 1991 to 30 June 1997. The report consists of a listing of the compounds/samples analyzed and a summary of the number of the types of studies performed. The report also includes a listing of personnel receiving pay from this effort and a bibliography of all publications and meeting abstracts that resulted from this contract.

This contract was concerned with the analytical, characterization, and stability studies of chemicals, drugs, and drug formulations. The work was monitored by Mr. William Y. Ellis, the Contracting Officer Representative (COR), Chief, Chemical Handling and Data Analysis Branch, Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR).

The overall objective of this project, a continuation of one that started in 1966, was the operation of an analytical laboratory to determine the identity, purity, strength, quality, physical and chemical properties, and stability of bulk pharmaceutical substances and formulated drug products of interest to the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense, anti-viral studies, etc. Specific objectives were to: design, develop, validate, and execute methods to determine the following characteristics of candidate bulk pharmaceutical substances and formulated drugs:

- Identity, purity, and strength.
- Stability.
- Other physical and chemical characteristics, including weight variation, content uniformity, and other such compendial requirements.
- Qualitative and quantitative identity of impurities.
- Special projects not covered by the above headings.
Sample Analyses

During the contract period, 1 August 1991 to 30 June 1997, analyses of the following samples were completed and the reports sent to the COR.

1. WR302AG, BM11449; -AH, BM11458, p-aminopropiophenone, bulk assays, Report No. 768.

2. WR448AG, AG28874; -BB, AX09212; -BC, AY38994; -BD, AY39000; and -BH, BN34670; dapsone, semi-quantitative assays, Report No. 842.

3. WR2976AY, AW23860; and -CJ, BN34885; quinine sulfate, semi-quantitative assays, Report No. 836.

4. WR3091AG, BN34894; and -AC, AG64932, proquanil hydrochloride, semi-quantitative assays, Report No. 843.


6. WR6798AL, AF50013; -AT, BN34689; -AM, -AT68417; and -AM, ZP19685, diformyl dapsone, semi-quantitative assay, Report No. 844.

7. WR7557AP, BB59190; -AX, BN34849; -AZ, BN34858; and -BA, BN34876, sulfadiazine, semi-quantitative assay, Report No. 837.

8. WR35928AQ, BM08915, O-2-amino-2-deoxy-α-D-glucopyranosyl-(1→4)-O-[O-2,6-diamino-2,6-dideoxy-β-L-idopyranosyl-(1→3)-β-D-ribofuranosyl-(1→5)]-2-deoxy-D-streptamine sulfate (paromomycin sulfate cream), formulation assay, Report No. 753; -AR, BM12053, paromomycin sulfate, bulk assay by pre-column derivatization with 2,4-dinitro-1-fluorobenzene (DNFB), Report No. 772; -AR, BM12053, paromomycin sulfate, bulk assay using an OPA derivatization, Report No. 780; -AV, BM17861, paromomycin sulfate,
9. WR46234AY, BM08782 (1% w/v active) and -AZ, BM08791 (placebo), 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide (niclosamide), Report No. 743; -BA, BM09994 (active, 1%), and -BB, BM10004 (placebo), formulation assay, Report No. 754; -BC, BM11412 (active, 1%), and -BB, BM11421 (placebo), formulation assay, Report No. 764; -BF, BM13710 (active, 1%), niclosamide, formulation assay, Report No. 773; -AE, BM13569 (placebo), Report No. 781; -BC and -BF, BM11412 and BM13710, Topical anti-penetrant (TAP), assay, Report No. 830.


11. WR100553, BN71271, doxycycline hyclate, bulk assay, Report No. 905.

12. WR142490BL, BM04391 (250 mg/tablet), erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol hydrochloride (mefloquine), Report No. 742; -BQ, BM13774 and -BR, BM13783, tablet formulation assays, Report No. 774; -BS, BM18368, assay, Report No. 845.


17. WR233602AH, BM11243, floxacrine, bulk assay; Report No. 761.


20. WR243251AB and -AC, BJ45753 and -BL21100, 7-chloro-3-(2,4-dichlorophenyl)-1-[[3-(dimethylamino)propyl]imino]-1,2,3,4-tetrahydro-9(10H)acridone, assays, Report Nos. 832 and 833.


22. WR250710BF, BN39808, lot no. 32053 & -BG, BN39817, lot no. 105525 (30 mg/tablet), and -BH, BN41077, lot no.C181554-01 (placebo), 3-[(dimethylamino)carbonyl]oxy]-1-methylpyridinium bromide, assay, dissolution and disintegration, Report No. 862 and amendment; -BJ, BN51162, lot no. 94-083, bottle A (30 mg/tablet), assay, Report No. 876.


24. WR255663AK, BM04131, artelinic acid, bulk assay, Report No. 703.


27. WR268172AB, BM08871, 3-(4-carbamoyl-1-pyridino)-1-(2,4-bis(hydroxyiminomethyl)-1-pyridino)-2-oxopropane dimethanesulfonate; and -AC, BM10406, the dichloride, physical characterizations and comparison, Report No. 757.


31. WR272677AC, BM17076, p-hydroxylaminoheptanophenone, bulk assay, Report 804.


33. Microencapsulated of colonization factor antigen (CFA) vaccine, Report No. 821. During the report period, a colorimetric protein assay based on complexation between bicinchoninic acid and cuprous ion, which results from reduction of cupric ion by a protein, was developed. The assay and its several refinements were applied to more than a hundred samples of microencapsulated CFA vaccine material to determine their protein contents. Additionally, the acetonitrile, heptane, and sucrose contents in the microspheres were also determined. Moreover, the proteins in some of these samples were also characterized by electrophoresis. These analyses were performed for Col. Robert Reid of the Department of Gastroenterology, with authorization from the Project COR.

### Stability and Solubility Studies

Stability and solubility studies on the following samples have been completed and their reports submitted to the COR.


4. WR46234AU, BM08139, 1% w/v solution and AV, BM08148, placebo solution, 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide (niclosamide), stored at 35°, 45° and 55 °C, Report No. 739; AJ, BL44970, JD-10-58, 51-month sampling, Report No. 744; 5-year sampling, Report No. 783; BC, BM11412; room-temperature stability study with camouflage paint (MIL-P-2019F, lot No. G-016) and sun screen lotion (NSN6505-01-121-


11. WR250710BB, BM03509, Lot No. 038641, 30 mg/tablet, pyridostigmine bromide, accelerated stability studies at 35°, 50°, and 60°C for one year, Report No. 749; at 35°C for

12. WR253997AH, BL51037, dihydroartemisinin; bulk solubility in 13 media, Report No. 802.


15. WR268172AB, BM08871, 3-(4-carbamoyl-1-(2,4-bis(hydroxylimethyl)-1-pyridino)-2-oxapropane dimethanesulfonate and AC, BM10406, the dichloride, solubility study and comparison, Report No. 757.


21. WR279436AA, -AB, and -AC, sodium nitrite injection solutions, 300 mg/10 mL, Lot Nos. 85186, 85187, and 85188, shelf-life stability, 6.5-year samplings, Report No. 763.

**Chiral Separations**

Chiral separations on the following samples have been completed and the reports have been sent to the project COR.

1. WR238605AC, BK73252, N-(2,6-dimethoxy-4-methyl-5-(3-(trifluoromethyl)phenoxy)-8-quinolinyl)-1,4-pentanediamine succinate, and its R-(−)- and S-(+)-(4-amino-1-methylbutyl)amino)2,6-dimethoxy-4-methyl-5-(3-trifluoromethylphenoxy)quinoline fumarate, WR280407AA, BN57422 and WR280408AA, BN57431, chiral separation and circular dichroism determinations, Report No. 909.

2. WR242511AF, BM19356, 8-((4-amino-1-methylbutyl)amino)-5-(1-hexyloxy)-6-methylquinoline DL tartrate, chiral separation, Report No. 888.

3. WR243251, 7-chloro-3-(2",4"-dichlorophenyl)-1-((3'-(dimethylamino)propyl)imino)-1,2,3,4-tetrahydro-9-(10H)acridone, chiral separation, Report No. 915.

4. WR250547, [R]-7-chloro-3-(2",4"-dichlorophenyl)-1-((3'-(dimethylamino)propyl)imino)-1,2,3,4-tetrahydro-9-(10H)acridone and the [S]-enantiomer, chiral separation, Report Nos. 904 and 919.

**Method Validations**

Validations of assay procedures for the following compounds and their formulated products have been completed and the validation reports have been sent to the project COR.

1. WR6026, 6-methoxy-8-(6-diethylaminohexylamino)lepidine dihydrochloride, bulk drug and formulated drug product, Report No. 865.

2. WR35928AV, BM17861, paromomycin sulfate, Report No. 838; and sulfate assay addendum, Report No. 839.

3. WR73633, gentamicin sulfate, Report No. 841.

4. WR142490AU, BK11592, erythro-α-(2-piperidyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol hydrochloride (Mefloquine HCl), Report No. 889.

5. WR171669, halofantrine, Report No. 869.

6. WR178460, BM08577, desbutylhalofantrine, chemical assay validation, Report No. 873; chiral separation validation, Report No. 918.

7. WR238605, Report No. 890; C-E-based assay for succinate validation, Report No. 932.
8. WR242511, 8-[(4-amino-1-methylbutyl)amino]-5-(1-hexyloxy)-6-methoxy-4-methylquinoline DL-tartrate, Report No. 878.

9. WR243251, racemic 7-chloro-3-(2"",4""-dichlorophenyl)-1-{[3'- (dimethylamino)propyl]imino}-1,2,3,4-tetrahydro-9-(10H)acridone, validation of chiral assay, Report No. 915.

9. WR250547, [R]-7-chloro-3-(2"",4""-dichlorophenyl)-1-((3'-(dimethylamino)propyl)imino)-1,2,3,4-tetrahydro-9-(10H)acridone, chiral separation validation, Report No. 904; WR250548, (S)-enantiomer, chiral separation validation, Report No. 919.


11. WR255663, artelinic acid, Report No. 872.

Reference Sample Preparation

A reference sample for the following compound has been established.

WR35928, BM17861, paromomycin sulfate, established as an independent reference standard, Report No. 856.

Standard Operating Procedures (SOPs)

During the entire project report period, 55 SOPs were updated.

Presentations

The following presentations have been made.

1. A poster on the room-temperature solution stability of WR-272677, p-hydroxylaminoheptanophenone was presented at the 1993 Medical Defense Bioscience Review Conference held in Baltimore, MD.

Abstract: WR272677 has protected mice against cyanide challenge at the Battelle Laboratory. Single dose oral and intravenous pharmacokinetic and pharmacodynamic studies performed in the division of Experimental Therapeutics at the Walter Reed Army Institute of Research have shown this compound to be a potent former of methemoglobin, or ferrihemoglobin, which selectively binds cyanide. One of the steps in the development of this anticyanide agent is to determine its solubility and solution stability.
The apparent room-temperature solubilities of WR272677AB in aqueous media, ethanol, polyethylene glycol (PEG) 400, and aqueous ethanol have been determined. WR272677 is insoluble (<0.1 mg/mL) in the aqueous media and aqueous ethanol, but is freely soluble (≥400 mg/mL) in either ethanol or PEG 400.

WR272677AB dissolved in ethanol or PEG 400 under ordinary atmospheric conditions rapidly decomposes, mainly to p-azoxyheptanophenone. In the absence of molecular oxygen, however, ethanol solutions of WR272677 are stable for at least 24 hours.

2. A poster on a determination of bis(chloromethyl) ether (BCME) in bulk HI-6 was presented at the 1996 Medical Defense Bioscience Review held in Baltimore, MD.

Abstract: A specific, sensitive method for determining trace quantities of BCME in a solid matrix has been developed and validated. Under standard conditions, the lower quantifiable level is < 0.5 nanogram BCME. When the method was applied to bulk samples of HI-6, no measurable amount of BCME could be found, unless the sample was anhydrous. Although BCME is formed from HI-6 even at room temperature, the rate is very slow, and the BCME that is formed is hydrolyzed by the water in the sample. Rate information on BCME formation from anhydrous HI-6 at four temperatures have been determined; from this information, the formation of BCME over time/temperature can be estimated.

Publications

The following publications have resulted from the performance of this contract.


Abstract: This paper describes the compositional analyses of four paromomycin sulfate samples and features a validated HPLC assay method that relies on the fluorescence detection of isoindole derivatives of paromomycin, formed by post-column reaction with o-phthalaldehyde and 2-mercaptoethanol.

2. “Development and Validation of A Method to Extract and Quantitate Paromomycin and gentamicin from An Aquaphilic® Cream Formulation” by John Pick, Lori L. Olson, William Y. Ellis, and Peter Lim has been accepted for publication in Journal of Pharmaceutical and Biomedical Analysis, in December 1996.

Abstract: Butanol and dilute sulfuric acid were used to extract paromomycin and gentamicin from Aquaphilic®-based formulated creams. The extraction procedure was validated over different antibiotic concentration ranges for linearity, precision, accuracy, limited specificity, sensitivity and solution stability.
Personnel

A listing of personnel who received major contract support is as follows:

Peter Lim, P.I.
Lori Olson, Assistant P.I.
Robert Petesch, Chemist
Lee D. Nguyen, Chemist
John Pick, Chemist
Tina Nguyen, Chemist
Shane Ridge, Chemist
Gwen Wilkins, Secretary

Summary/Conclusion

During the entire contract period, 125 samples of bulk drugs and dosage formulations were analyzed for identity, purity or potency; 133 samples were studied for stability and solubility. Four chiral separation methods were developed and validated, and 11 other assay methods were validated. Special projects included the development and application of assays for determining protein content, residual solvents, and other components in microsphere vaccine preparations. A second special project was the development and application of an assay for determining BCME in HI-6 bulk drugs. Poster presentations were made at the 1993 and the 1996 Bioscience Reviews. One publication appeared in press and a second has been accepted for publication.

Respectfully submitted,

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2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or email: judy_pawlus@ftdetrck-ccmail.army.mil.

FOR THE COMMANDER:

[Signature]

PHYLIS M. RINEHART
Deputy Chief of Staff for Information Management