CHAPTER 6

BIOEQUIVALENCE STUDY OF

DEFLAZACORT TABLET 30mg
STUDY TITLE
Open labeled, balanced, randomized, two-treatment, two-period, two-sequence, crossover, comparative, single dose bioequivalence study of Deflazacort tablets 30 mg, of M/s Aristo Pharmaceuticals Pvt. Ltd., India, with Calcort 30 mg Tablet (containing Deflazacort 30 mg) of Aventis Pharma, Italy, on healthy, adult, male, human subjects under fasting conditions.

SUMMARY
On the basis of the pharmacokinetic parameters $C_{\text{max}}$, $T_{\text{max}}$, AUC $0-t$, $t_{1/2}$ and $k_{\text{d}}$ studied, it can be concluded that the Test preparation of DEFLAZACORT 30mg, mfg. by ARISTO PHARMACEUTICALS LTD., is bioequivalent with the Reference preparation, Tablet Calcort mfg. by Aventis Pharma, Italy.

STUDY SITE:

M/s AnaZeal Analyticals & Research Pvt. Ltd.

C-404, TTC, MIDC, Pawane,
Pfizer Mahape Road, Off. JISL Co.,
Navi Mumbai - 400 705. INDIA
Aims and Objective
To compare the single dose oral bioavailability of Deflazacort 30 mg Tablet of M/s Aristo Pharmaceuticals Pvt. Ltd., India with Calcort 30 mg Tablets (containing Deflazacort 30 mg) of M/s Aventis Pharma, Italy in 12 healthy, adult, male, human subjects under fasting conditions.

Clinical Applications
Rheumatoid arthritis, cryoglobulinemia, idiopathic thrombocytopenic purpura, prophylaxis of interferon toxicity, juvenile chronic arthritis, nephritic syndrome, polymyalgia rheumatica, sarcoidosis, systemic lupus erythematosus, post transplant immuno-suppression.

Rationale
The sponsor, Aristo Pharmaceuticals Pvt. Ltd., India has developed a generic alternative of Deflazacort to the innovator brand CALCORT (of Aventis Pharma, Italy). Thus, a comparative study has been conducted to assess the bioequivalence between test and reference Deflazacort Tablets 30 mg

Study Design

12. DESIGN DESCRIPTION
Open label, balanced, randomized, two-treatment, two-sequence, two-period, single dose comparative bioavailability study of Deflazacort 30 mg Tablet of Aristo Pharmaceuticals Pvt. Ltd., India, in 12 healthy, adult, male, human subjects under fasting condition.

13. EVALUATION PARAMETERS
Pharmacokinetic parameters $C_{\text{max}}$, $T_{\text{max}}$, $AUC_{0-t}$, $K_{el}$ $t_{1/2}$ and $AUC_{0-\text{inf}}$ of 21-OH-deflazacort were calculated using the plasma profile of all the two formulations in individual subjects. An analysis of variance on the untransformed and log transformed pharmacokinetic parameters $C_{\text{max}}$,
AUC_{24h} and AUC_{0-inf} were performed. Wilcoxon Rank Sum test was performed to analyze T_{max}. The 90% confidence interval for the difference in the means of the log transformed data using the two one-sided hypotheses was calculated.

14. RANDOMIZATION

The order of receiving two different formulations for each subject during both the periods of the study was determined according to randomization schedules. The randomization was balanced and the code was kept under controlled access. The Principal Investigator, Co-Investigator and QA Manager who were involved in dispensing of study drugs have ensured compliance to randomization schedule.

15. HANDLING, STORAGE, DISPENSING AND ACCOUNTABILITY PROCEDURE FOR INVESTIGATIONAL NEW DRUG PRODUCTS

- **Drug Receipt & Storage**

  Adequate drug supplies, for dose administration and sample retention purposes, of the reference and test Deflazacort 30 mg formulations were supplied to the investigator by Aristo Pharmaceuticals Pvt. Ltd., India. The two formulations were supplied in sealed package labeled with Product name / Code, Strength, No. of dosage units, Manufacturer, Lot No. or Batch No., and Expiration date. The drug products with certificate of analysis (COA) were received by the Principal Investigator. The drug product were transferred to the drug store, after labeling it for Project No., Product type, Composition, Dosage form, Quantity and Date of Receipt, Manufacturer's Name, Batch No. or Lot No., Manufacturing date, Expiry date and Storage Condition.
16. STUDY SUBJECTS

- **Disposition of subjects**
  The investigational products were administered on 08/10/2006 during the period one and 15/08/2006 in period two as per the randomization table. For this study, 12 subjects were enrolled in the study and all twelve subjects completed both the periods of the study according to the protocol.

- **Data sets analyzed**
  Relevant data recorded during the pre-study examination of each subject enrolled in the study. All 12 subjects, who received the study medication and completed both the study periods were analyzed and evaluated.

17. RESTRICTIONS

- **Medications**
  Subjects were asked about their medication history in the past, particularly two weeks before screening and was instructed not to take any medication (either prescribed or over the counter drug) to prior to dosing till completion of the study.
o **Diet**

All subjects were instructed to abstain from any xanthine containing food or beverages (Chocolates, tea, coffee or cola drinks) or alcoholic products for 48 hours prior to dose administration and throughout their stay at the clinical facility. Cigarettes and tobacco products were not allowed throughout their stay at the clinical facility.

o **Activity**

The test or reference formulation was administered to the subjects in sitting posture and they were instructed to remain seated for the first two hours following the administration of drug. Thereafter, the subjects were allowed to engage only in normal activities avoiding severe physical exertion.

o **Housing**

Subjects were housed in the clinical from not less than 14 hours before administration of the dose and were discharged 24 hours after administration of the dose during each period.

o **Washout Period**

Wash out period of 07 days was maintained between two periods.

o **Duration of Fasting or Distribution of Meals**

All subjects have fasted overnight at least 10 hours before dose administration and for four hours post-dose. A standardized lunch, snacks, and dinner was served at 4.0, 8.0, and 13.0 hours post-dose and then subsequently on the second day. Standardized meal was served at the same time in each period. In case meals and blood sample collection coincide, samples were collected first and then meals.
were provided. Drinking water was not allowed from one hour pre-
dose and 2 hour post dose, accept 240 ml of water given during
administration of dose.

18. SELECTION OF SUBJECTS
All subjects have undergone physical and clinical screening
procedures within three weeks (21 days) prior to the first dose
administration. The subjects were selected based on the following
inclusion and exclusion criteria.

19. INCLUSION CRITERIA
Subjects included into study with the following criteria:
1. Subject, provided written informed consent.
2. Healthy subject aged between 18 and 45 years (both
   inclusive).
3. Subjects weight within 15% of the ideal height-weight
   chart of Life Insurance Corporation of India, and not less
   than 50 kg.
4. Normal health as determined by medical history, physical
   examination, and laboratory examinations within the
   normal range.
5. Had a normal 12 lead ECG
6. Willingness to adhere to the protocol and provided
   written informed consent to participate in the study.
7. Availability of subject for the entire study period.

20. EXCLUSION CRITERIA
1. Subjects excluded from study with the following criteria:
2. History of hypersensitivity to Olopatadine HCl tablet or related
   drugs.
3. History or presence of cardiovascular, pulmonary, hepatic,
renal, gastrointestinal, endocrine, immunological, dermatological, neurologic or psychiatric disease.

4. History or presence of alcoholism or drug abuse within the last year.

5. History or presence of ophthalmological disturbances

6. History or presence of thyroid disease, adrenal dysfunction, organic intracranial lesion such as pituitary tumor.

7. History or presence of cancer

8. Use of any medication, prescribed or over the counter, during 2 weeks preceding the study.

9. Use of enzyme modifying drugs during the 30 days before the screening period

10. Major illness during 3 months before the screening period.

11. Subjects who have participated in another clinical trial within 3 months of study start

12. Subjects who have been on abnormal diet (for whatever reason) during the four weeks preceding the study.

13. Smokers, who smoke more than 10 cigarettes per day

14. Subjects who have donated in excess of 75ml of blood in last 14 days, 750ml in last 3 months, 1000ml in last 6 months, 1750ml in last 9 months or 2000ml in last one year.

• DOSING AND ASSESSMENT OF EFFICACY

• Dose

Dose administration as described below was done under the supervision of Principal Investigator, Co-investigator and QA-manager.

• Reference Product

Calcort 30 mg Tablets (containing Deflazacort 30 mg) manufactured
by Aventis Pharma, Italy, Batch No. D006 was administered to the subjects fasted 10 to 12 hours overnight, with 240 mL of drinking water at ambient temperature as per the randomization table.

• **Test Product**

One Deflazacort 30 mg Tablet, manufactured by Aristo Pharmaceuticals Pvt. Ltd., India, Batch No. A/RD/1090, was administered to the subjects fasted 10 to 12 hours overnight, with 240 ml of drinking water at ambient temperature as per the randomization table.

• **Cautions**

Adverse effects include headache, insomnia, restlessness, hypomania, depression, moon face, central obesity, diabetes mellitus, hirsutism, growth suppression in children, hypertension, muscle weakness, bone damage (osteoporosis, pathologic long bone fractures, aseptic necrosis especially femoral and humeral heads), peptic ulcers with perforation, large and small bowel perforation, pancreatitis, petechiae, ecchymosis, delayed increased intraocular pressure, increased risk of infection, thromboembolism, vasculitis, acne, atrophy and striae.

• **Assessment of compliance for dosing**

Compliance for dosing was assessed by examination of the oral cavity with wooden spatula and torch light after dose administration in each period and by measurement of plasma concentration of 21-OH-Deflazacort during analysis of samples.
• BLOOD SAMPLING AND HANDLING

○ Sampling schedule

A total of sixteen 06-ml blood samples were collected during the period. The venous blood samples (06 ml) were withdrawn at pre-dose (prior to dosing) and at 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0 and 24.0 hours post dose following drug administration in each period.

○ Safety Precautions & Vitals

In order to avoid severe adverse events and to check, vital were taken over a period of pre-dose and post dose 1h, 2h, 3h, 6h and 24h.

○ Blood sampling

Samples were collected through an indwelling cannula placed in a forearm vein. The pre-dose blood sample was collected within a period of one hour before the dosing and post-dose samples were collected within two minutes of the Scheduled sampling time of sampling till check out of the subjects. The actual mid-point time of collection of each blood sample (to the nearest minute) was recorded on the appropriate data sheet.

Intravenous indwelling cannula was kept in situ as long as possible by injecting 0.5 ml of normal saline solution to maintain the cannula patent. Blood samples were collected after discarding the first 0.5 ml of blood from the tubing.

Alternatively, in some instances, blood samples were withdrawn by a fresh clean venipuncture using a disposable sterile syringe and a needle at each time of collection.

The blood samples were collected in pre-labeled (mentioning project no, subject no, period and sampling time point) 06-ml vacutainers
containing EDTA as the anticoagulant.

After collection of blood samples from all the subjects at each time point, one of the designated study personnel transferred all vacutainers to a sample processing section where the blood samples were centrifuged as soon as possible to separate plasma (within 30 minutes). The plasma was separated and transferred to pre-labeled tubes and promptly frozen at -20 ± 2 °C till analysis.

Discussion of study design

Open labeled, balanced, randomized, two-treatment, two-sequence, two-period, single dose bioequivalence study was chosen for getting a reliable basis for further investigation of pharmacokinetic properties of a therapeutically important drug, as required by international regulatory guidelines. Furthermore, pharmacokinetics parameters of 21-OH-Deflazacort was investigated for test formulation against reference formulation. Twelve subjects were considered to be sufficient to get reliable data for this bio study that also investigates the feasibility and accuracy of determination of 21-OH-Deflazacort levels in plasma.

Assessment of Safety

Eligibility Assessments

The following assessments were conducted before the entry of the subjects into the study:

1. Demographic data
   Age, Height and weight (as per LIC chart for healthy subject)

2. Vital Signs
   Blood pressure, Radial pulse, Oral temperature

3. Medical History, Laboratory Tests and Current Status
   The subject's status as a healthy volunteer was confirmed.

4. Medication and Therapy History
Subjects had not taken any investigational medication and concomitant therapy in last 14 days prior to dosing.

5. Physical examination
A standard physical examination was conducted. Clinically significant finding was documented.

6. Clinical Laboratory Screening
Blood and urine samples were tested for standard parameters.

Trial Assessments
The following were recorded during the conduct of the study:

1. Time of dose administration.
2. Scheduled and actual blood sampling times.
3. Concomitant therapy changes.
4. Adverse events as described below in point no. 11.3.2

Safety Assessments

- **Recording of vital signs and clinical examination**
  Vital signs like oral temperature, sitting blood pressure and radial pulse were measured and recorded during subject check-in, (in the morning of the day of dosing) prior to dose administration of study drug, 1 hr, 2 hr, 3 hr, 6 hr and 24 hr i.e., at the time of checkout Vital sign measurements were obtained with the subject in the sitting position and within half an hour of the scheduled time. Clinical examinations of all the subjects were done at the time of check-in and checkout of the study.

- **Summary of Adverse Events and Case Report Forms.**
  No serious adverse events occurred in both the periods of the study.

- **Protocol deviations**
  The study was conducted in accordance with the approved study protocol.
Protocol Deviation Form

<table>
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<th>No.</th>
<th>Activity</th>
<th>Deviation Found</th>
<th>Remarks (If any)</th>
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<tbody>
<tr>
<td>1</td>
<td>Drug Storage Activity</td>
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</tr>
<tr>
<td>2</td>
<td>Subject</td>
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<tr>
<td></td>
<td>Eligibility/Inclusion &amp;/Exclusion Criteria)</td>
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<td>3</td>
<td>Demographic Data</td>
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<td>4</td>
<td>Physical Examination</td>
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<tr>
<td>5</td>
<td>Subject Check in</td>
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<td>6</td>
<td>Subject Housing</td>
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</tr>
<tr>
<td>7</td>
<td>Informed Consent Activity</td>
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<tr>
<td>8</td>
<td>Catheter Insertion &amp; Removal</td>
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</tr>
<tr>
<td>9</td>
<td>Dose Administration</td>
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<tr>
<td>10</td>
<td>Blood Sample Collection Data</td>
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<td></td>
<td>(Delay in blood sample collection in some of the blood sample collection points in some of the subjects.)</td>
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<td>11</td>
<td>Any sample Loss</td>
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<td>15</td>
<td>Symptoms check list</td>
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<td>Adverse Event Recording Form</td>
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<td>Drop out: Refusal or withdrawal</td>
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<td>18</td>
<td>Sample Transfer to Analytical Department</td>
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<tr>
<td>19</td>
<td>Sample Adverse Activity</td>
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</table>

= Deviation Found. Please refer attached sheet

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Ethical Considerations

Institutional Review Board

The Study Protocol and the corresponding informed consent form (ICF) used to obtain informed consent of study subjects was reviewed and approved by the Institutional Review Board.

Informed Consent

The Principal Investigator and Clinical custodian had informed the subjects (in English and Hindi) before initiation of study through an oral presentation regarding the purpose, procedures to be carried out, potential hazards and rights of the subjects. Subjects have signed a consent form summarizing the discussion; one photocopy of the form was given to the subject after the procedure was over, while the original signed copy was filed in the study binder.

Ethical and Legal Aspects of the Study

The study was conducted according to the guidelines of the World Medical Assembly of Helsinki, Finland, June 1964, in their amended and revised version of, Edinburgh, Scotland, October 2000, concerning ethics in experimentation in humans and under consideration of the recommendations of Indian Council of Medical Research: "Ethical Guidelines for Biomedical Research on Human Subjects", New Delhi, 2000, and CPMP Working Party on Efficacy of Medicinal Products, the ICH-Guidance: "Good Clinical Practice: Consolidated Guideline.", Document number: CPMP/ICH/135/95, dated January 17, 1997.

Subject Participation Fees

The subjects were paid an adequate participation fee on account of their participation in the study. In case of drop-out / withdrawal of a subject before completion of the study, the guidelines of the IRB on participation fees of the withdrawn subjects were final and binding on both AnaZeal Analyticals & Research Pvt. Ltd. and the study subjects.
Analytical Procedures

Pre-study Validation

HPLC-UV method was validated for the sensitivity, specificity, linearity, accuracy & precision (repeatability and reproducibility), percent extraction yield and stability of samples (freeze-thaw stability, short-term stability, long term stability). The results of which can be verified in Validation Report.

Assay of Test Samples

Samples from subjects were assayed for 21-OH-Deflazacort in plasma using validated HPLC-UV method. During analysis, standard and quality control samples were distributed throughout each batch of study samples analyzed. The analyst worked blinded to the randomization scheme during the course of analysis. Samples of subjects who have completed the cross over dosing were analyzed.

All concentration values below the limit of quantification were set to zero for all pharmacokinetic and statistical evaluation. Missing sample and Non reportable concentration value due to poor chromatography was reported as 'M' and was not included for pharmacokinetic and statistical analysis.

Quality Assurance Audits

The raw data generated during the course of the study, including the clinical and analytical operations and the final reports were liable for inspection and quality audit in conformance to study protocol and all the governing SOPs by an auditor from the Quality Assurance Unit of AnaZeal Analyticals & Research Pvt. Ltd.

Data Handling & Record Keeping -

All clinical and analytical data generated during the conduct of the study were directly entered in the respective raw data recording forms. The computer-generated chromatograms were treated as raw data. AH raw data and transcribed data forms were compiled by the study personnel assisting in the study and were checked wherever applicable for completeness. All data related to the project were transferred to archives.
Archiving

A representative sample of the drug supplies used in the study was provided by the sponsor company. All data generated in connection with this study, together with the copy of study protocol, informed consent forms (ICFs) and the final report, were archived.