2.7.1 Summary of Biopharmaceutic Studies and Associated Analytical Methods

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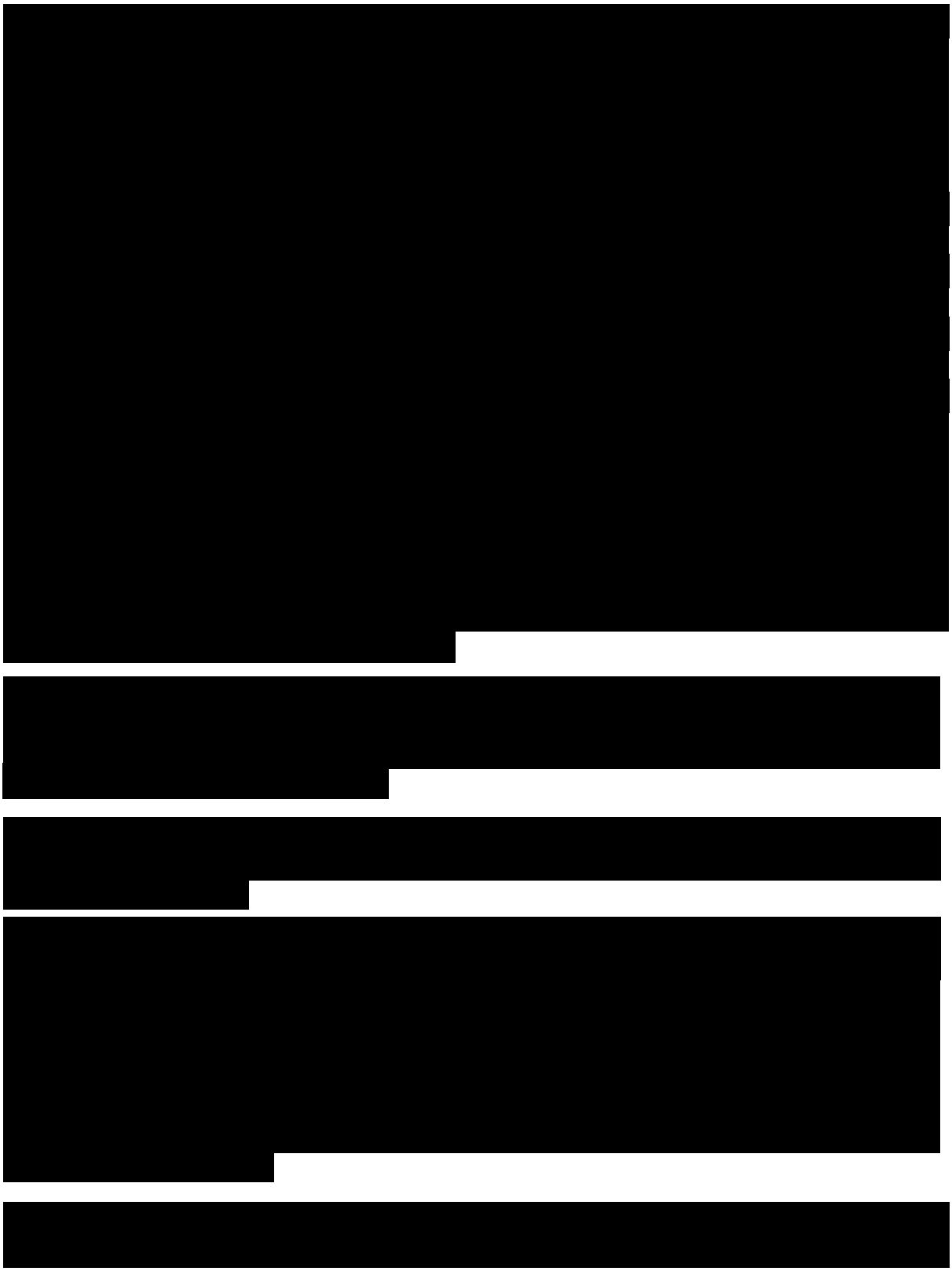
Abbreviations

6HTS	6β-hydroxy-7α-thiomethyl-spironolactone
7TS	7α-thiomethyl-spironolactone
ANOVA	Analysis of variance
AUC	Area under the curve
CAN	Canrenone
Cmax	Maximum concentration
CV	Coefficient of variation
EMA	European Medicines Agency
EU	European Union
GCP	Good clinical practice
HPLC	High performance liquid chromatography
IMP	Investigational medicinal product
kg	kilograms
LC-MS/MS	liquid chromatography with tandem mass spectrometry detection
LLOQ	Lower limit of quantification
MAA	Marketing authorisation application
mg	milligrams
mL	millilitres
ng	nanograms
PK	pharmacokinetic
QC	quality control
SD	Standard deviation
SPIR	spironolactone
STDs	Calibration standards
Tmax	Time of maximal concentration
UK	United Kingdom
ULOQ	Upper limit of quantification
VAS	Visual analogue scale
WHO	World Health Organisation

2.7.1.1 Background and Overview

Nova Laboratories has developed an oral liquid formulation of spironolactone, a mineralocorticoid-receptor antagonist, for use in adults, adolescents and children. The indications of spironolactone 10 mg/mL oral suspension are proposed to be identical to those of the reference spironolactone 100 mg tablet, Aldactone, approved in the EU.



[redacted].

Therefore, the key issue with this development is whether the oral liquid formulation of spironolactone, spironolactone 10 mg/mL oral suspension, is bioequivalent with the approved tablet formulation Aldactone, or, if not, whether its pharmacokinetic (PK) profile will ensure equivalent efficacy, without increasing the risk of adverse events.

The biopharmaceutics for this development has been limited to a single bioequivalence study: "A single centre, single-dose, open-label, randomized, four period crossover study to assess the bioequivalence of an oral spironolactone suspension 10 mg/mL (Kayraas™) and an oral spironolactone tablet 100 mg (Aldactone®, Pfizer healthcare, Ireland) in a replicate design in healthy males and females under fed conditions." The bioequivalence study was conducted in the fed state to reflect the administration instructions i.e. doses are to be taken with a meal.

Venous blood samples were collected into labelled dipotassium ethylenediaminetetraacetic acid (K_2EDTA) plastic tubes. Following centrifugation, the supernatant was divided into 2 aliquots (of at least 0.8 mL plasma each) and frozen. For analysis, analytes were extracted from the biological matrix with a mixture of hexane and dichloromethane using a liquid-liquid extraction technique. The extracts were dried under nitrogen and reconstituted in a mixture of formic acid solution and methanol.

The method employed to simultaneously determine concentrations of spironolactone (SPIR) and metabolites 7α-thiomethyl-spironolactone (7TS), 6β-hydroxy-7α-thiomethyl-spironolactone (6HTS) and canrenone (CAN) in the human plasma samples was high performance liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). [redacted] A copy of the bioanalytical protocol and amendment (study number INV684), validation protocol [redacted] and the resulting method validation report are presented in Module 5.3.1.4.

A total of 6576 samples, as primary and duplicate sets, (excluding the concentration range estimation samples) were sent for analyses between 31 August and 18 September 2020. Samples were stored at ~ -20°C in freezers until analysis between 9 October and 10 November 2020. The number of results generated for each analyte was 3288. In addition, a total of 224 samples from the duplicate set of aliquots were re-analysed, as incurred sample reanalysis, in a total of four runs (one run near the middle and three runs near the end of the study) to confirm that the method performed consistently. The percentage of evaluated samples within 20% acceptance criteria were 96.0% for SPIR, 98.2% for 7TS, 87.1% for 6HTS and 97.3% for CAN.

Samples were assayed in runs where the unknown samples were interspersed with calibration standards (STDs) and quality control (QC) samples, a blank extract (to monitor carry-over) and a zero sample (to monitor interference by the internal standard). The samples of all treatment periods of a particular participant were analysed together in one run. Each run included one standard calibration curve constructed from STDs from at least 6 different concentration levels, ranging from the lower limit of quantification (LLOQ) to the upper limit of quantification (ULOQ), assayed in duplicate. The regression model that was determined and used during the method validation was applied. Quality control samples were assayed in duplicate using at least 3 different concentration levels (one at ~ 3 x LLOQ [QC_{low}], one QC at mid-range (~ 50 % of the ULOQ [QC_{medium}]) and one within ~ 20 - 25 % of the ULOQ [QC_{high}]), or at least 5 % of the number of study samples, whichever was higher. The calibration concentrations ranged from LLOQ to ULOQ and were: 3.13, 6.25, 12.5, 25.0, 50.0, 100, 200 and 400 ng/mL for SPIR, 6HTS and CAN and 6.26, 12.5, 25.0, 50.0, 100, 200, 400 and 800 ng/mL for 7TS.

Twelve samples (from 8 subjects) were reanalysed for SPIR, 12 samples (from 8 subjects) were reanalysed for both SPIR and 7TS, 105 samples (from 24 subjects) were reanalysed for 6HTS and 30 samples (from 13 subjects) were reanalysed for CAN. The main reasons for reanalysis were samples were above the limit of quantification, lost in process or the peak appeared in the first timepoint sample. It was found that 6HTS analysis was affected by lipemia and therefore samples with moderate grades of lipemia had to be diluted with water before analysis, resulting in the high number of retests required.

The method validation (and the partial validation runs) met the acceptance criteria as defined by the regulatory guidelines and as detailed in the standard operating procedures (SOPs) of BASD, the Validation Protocol and Amendment to the Validation Protocol. The validation data are summarised below:

Table 2.7.1.1: Validation data summary

<table>
<tr><th>Test</th><th colspan="3">Results</th></tr>
<tr><td rowspan="6">Accuracy</td><td colspan="3">Accuracy is expressed as %Bias (the difference between the true nominal value and the calculated value, expressed as a percentage). The mean calculated concentration of each QC level over three consecutive accuracy and precision runs must be within +/- 15% (20% at the LLOQ) of the respective nominal concentration.
Within-Run and Between-Run Accuracy (3 runs):</td></tr>
<tr><td rowspan="2">Analyte</td><td colspan="2">% Bias range (mean results)</td></tr>
<tr><td>Calibration standards</td><td>Control samples</td></tr>
<tr><td>Spironolactone</td><td>-4.3 to 3.2</td><td>-4.1 to 1.2</td></tr>
<tr><td>6β-hydroxy-7α-thiomethyl-spironolactone</td><td>-5.8 to 5.8</td><td>-4.8 to 8.3</td></tr>
<tr><td>Canrenone</td><td>-2.0 to 2.8</td><td>-3.9 to 2.2</td></tr>
<tr><td>7α-thiomethyl-spironolactone</td><td>-2.4 to 6.4</td><td>-1.9 to 2.0</td></tr>
</table>

<table>
<tr><th>Test</th><th colspan="3">Results</th></tr>
<tr><td>Precision</td><td colspan="3">The %CV of each QC level over three consecutive accuracy and precision runs must be ≤ 15% (20% at the LLOQ).
Within-Run and Between-Run Precision (3 runs):</td></tr>
<tr><td rowspan="6"></td><td rowspan="2">Analyte</td><td colspan="2">% CV range (mean results)</td></tr>
<tr><td>Calibration standards</td><td>Control samples</td></tr>
<tr><td>Spironolactone</td><td>3.5 to 8.2</td><td>4.3 to 8.1</td></tr>
<tr><td>6β-hydroxy-7α-thiomethyl-spironolactone</td><td>2.2 to 8.1</td><td>4.3 to 12.1</td></tr>
<tr><td>Canrenone</td><td>1.7 to 7.5</td><td>2.2 to 8.7</td></tr>
<tr><td>7α-thiomethyl-spironolactone</td><td>4.5 to 6.4</td><td>5.1 to 13.3</td></tr>
<tr><td>Dilution Integrity</td><td colspan="3">A 2-fold dilution was successfully validated.</td></tr>
<tr><td>Carry-over</td><td colspan="3">No inherent carry-over was detected in the blank samples.</td></tr>
<tr><td>Reinjection Reproducibility</td><td colspan="3">A run that could not be completed due to a technical reason can be reinjected.</td></tr>
<tr><td>Maximum Batch Size</td><td colspan="3">A total of 224 samples can be analysed in a batch.</td></tr>
<tr><td>Recovery</td><td colspan="3">Absolute Recovery (extraction efficiency):
SPIR: 88.6% (mean %CV = 4.9)
7TS: 86.4% (mean %CV = 4.9)
6HTS: 86.1% (mean %CV = 6.1)
SPIR-d7: 89.7% (mean %CV = 6.4)
CAN: 85.1% (mean %CV = 4.3)
CAN-d6: 86.7% (mean %CV = 5.6)

Relative Recovery:
SPIR: 0.988 (mean %CV = 5.2)
7TS: 0.964 (mean %CV = 4.3)
6HTS: 0.961 (mean %CV = 6.6)
CAN: 0.982 (mean %CV = 4.2)

The results indicate that the extraction efficiency is consistent at all concentration ranges.</td></tr>
<tr><td>Analyte Stock Solution Stability</td><td colspan="3">SPIR, 7TS, 6HTS and CAN (high concentration): At least 22 hours at RT and at least 2 days and 23 hours at ~ 5°C and ~ -20°C in methanol in glass containers.
Note: No stock solution stability assessment was required for the internal standards (ISs), since stable isotope-labelled ISs were used in this study.</td></tr>
<tr><td>Internal Standard Working Solution Stability</td><td colspan="3">SPIR-d7 and CAN-d6: At least 20 hours at RT and at least 6 days at ~ 5°C in water in polypropylene containers.</td></tr>
<tr><td>Analyte stability in the matrix</td><td colspan="3">Bench Top / Short-Term Stability:
SPIR, 7TS, 6HTS and CAN:
At least 22 hours and 7 minutes at 2-8°C.

Freeze-Thaw Stability:
SPIR, 7TS, 6HTS and CAN:
At least 4 freeze-thaw cycles at ~ -20°C.

Formal Long-Term Stability:
SPIR, 7TS, 6HTS and CAN:
At least 141 days in human K_2EDTA plasma when stored at ~ -20°C in polypropylene containers.</td></tr>
</table>

Test	Results
Processed Sample Stability / On-Instrument Stability	**6HTS and CAN:** At least 70 hours and 46 minutes at 2-8°C. **SPIR and 7TS:** At least 123 hours and 15 minutes at 2-8°C.
Whole Blood Stability	**SPIR and CAN:** At least 1 hour and 14 minutes at RT and on ice. **7TS and 6HTS:** At least 1 hour and 2 minutes at RT and on ice.
Effect of Co-Medication	**Over-the-Counter Drugs:** SPIR, 7TS, 6HTS and CAN can be accurately quantified in the presence of commonly used over-the-counter drugs (paracetamol, ibuprofen, cyclizine, cetirizine, pseudoephedrine, codeine and diclofenac). **Contraceptive Drugs:** SPIR, 7TS, 6HTS and CAN can be accurately determined in the presence of contraceptive compounds (ethinylestradiol, drospirenone, medroxyprogesterone 17-acetate, gestodene, cyproterone acetate and levonorgestrel). **Co-Administered Medications:** SPIR, 7TS, 6HTS and CAN can be accurately determined in the presence of other compounds (amoxicillin and clavulanic acid).
Effect of Analytes on Each Other	SPIR, 7TS, 6HTS and CAN can be accurately determined in the presence of each other.
Blank Selectivity	No interfering peaks from endogenous and other matrix components were observed at the retention times of SPIR, 7TS, 6HTS, CAN or the ISs.
Internal Standard Interference	No contamination of the analytes by the ISs were observed in the zero samples.
Matrix Effects	The variability of the IS-normalised matrix factor was ≤ 15% at both low and high analyte concentration levels, indicating that the reproducibility of analysis was acceptable in the various matrices. **Effect of Haemolysed Matrix:** SPIR, 7TS, 6HTS and CAN can be accurately quantified when samples are severely haemolysed. **Effect of Lipemic Matrix:** SPIR, 7TS and CAN can be accurately quantified when the matrix is severely lipemic. 6HTS is affected by lipemic matrix and samples with moderate or lower grades of lipemia must be diluted with water before analysis.

2.7.1.2 Summary of Results of Individual Studies

The bioequivalence study (INV684) was carried out on 36 subjects to assess whether the Nova spironolactone 10 mg/mL oral suspension and the reference marketed tablet formulation (Aldactone 100 mg tablet®) are bioequivalent. The study was conducted in accordance with Good Clinical Practice (GCP).

This was a single-dose, open-label, laboratory-blind, randomized, four period crossover, replicate study with orally administered spironolactone 100 mg conducted under fed

conditions in 36 healthy males and females at a single study centre. In order to evaluate the taste acceptability of the test product, subjects completed a palatability questionnaire after administration of the second dose of the test product (spironolactone 10 mg/mL oral suspension).

Based on previously conducted PK and bioequivalence studies which demonstrated high (>30% CV) intra-subject variability in Cmax, spironolactone was assumed to be a highly variable drug. Hence, a full replicate design was employed in this study. Bioequivalence of the test and reference products was assessed on the basis of the 90% confidence intervals (CIs) for estimates of the geometric mean ratios between the primary PK parameters for spironolactone in relation to the conventional acceptance range of 70.00% to 143.00% for Cmax and 80.00% to 125.00% for AUC(0-t) following the guidance on bioequivalence [redacted] in relation to high variable drug products. The parent analyte (spironolactone) [redacted] were measured, however bioequivalence assessment was conducted only on the parent spironolactone.

The key PK results for spironolactone [redacted] are summarised in Table 2.7.1.2, Table 2.7.1.3, Table 2.7.1.4 and Table 2.7.1.5 respectively. Table 2.7.1.6 shows the ANOVA for the spironolactone parameters and the confidence intervals for the point estimate of the ratio. Table 2.7.1.7 shows the intra-individual variability in spironolactone parameters.

The mean plasma concentration profiles for spironolactone with both treatments are shown in Figure 2.7.1.1.

Table 2.7.1.2: Summary of Plasma PK Parameters for Spironolactone (INV684)

Pharmacokinetic parameter	Arithmetic Means (±SD)	
	Test product	Reference Product
$AUC_{(0-t)}$ (h*ng/mL)	464.6 (224.7)	451.7 (222.6)
$AUC_{(0-\infty)}$ (h*ng/mL)	505.2 (238.3)	496.8 (239.6)
Cmax (ng/mL)	160.7 (82.66)	242.2 (122.3)
Tmax[1] (h)	0.750 (0.25, 2.33)	1.250 (0.50, 5.00)
$T_{1/2,Z}$ (h)	5.23 (2.17)	4.60 (2.66)

[1] Median (Min, Max)

[redacted]

[redacted]

[redacted]

[redacted]

Table 2.7.1.6: Summary of Statistical Analyses of Plasma Spironolactone Primary Pharmacokinetic Parameters

Parameter (Unit)	n / N (Test)	n / N (Reference)	LS Means		Test / Reference Geometric Mean Ratio (%)		Model Intra CV%
			Treatment B (Test)	Treatment A (Reference)	Estimate	90% CI	
C_{max} (ng/mL)	72 / 36	71 / 36	142.24	215.03	66.15	(61.62 ; 71.01)	25.95
AUC_{0-t} (h*ng/mL)	72 / 36	71 / 36	417.56	411.36	101.51	(98.11 ; 105.02)	12.30

CI = confidence interval; CV% = coefficient of variation percentage; n = number of samples; N = number of subjects

Estimates were obtained using an ANOVA with sequence, period, product and subject within sequence as fixed effects, after logarithmic transformation of the data.

Treatment A (reference): Aldactone® 100 mg tablet

Treatment B (test): Kayraas™ 10 mL oral suspension

Table 2.7.1.7: Intra-CV Percentage between Periods for Spironolactone

Product	Parameter (Unit)	Model Intra CV%
Treatment A to Treatment A	C_{max} (ng/mL)	24.65
	AUC_{0-t} (h*ng/mL)	10.91
	$AUC_{0-\infty}$ (h*ng/mL)	10.85
Treatment B to Treatment B	C_{max} (ng/mL)	19.92
	AUC_{0-t} (h*ng/mL)	12.94
	$AUC_{0-\infty}$ (h*ng/mL)	13.21

CV% = coefficient of variation percentage

Treatment A (reference): Aldactone® 100 mg tablet

Treatment B (test): Kayraas™ 10 mL oral suspension

Figure 2.7.1.1:Plot of Geometric Mean Plasma Concentrations over time (Study INV684)

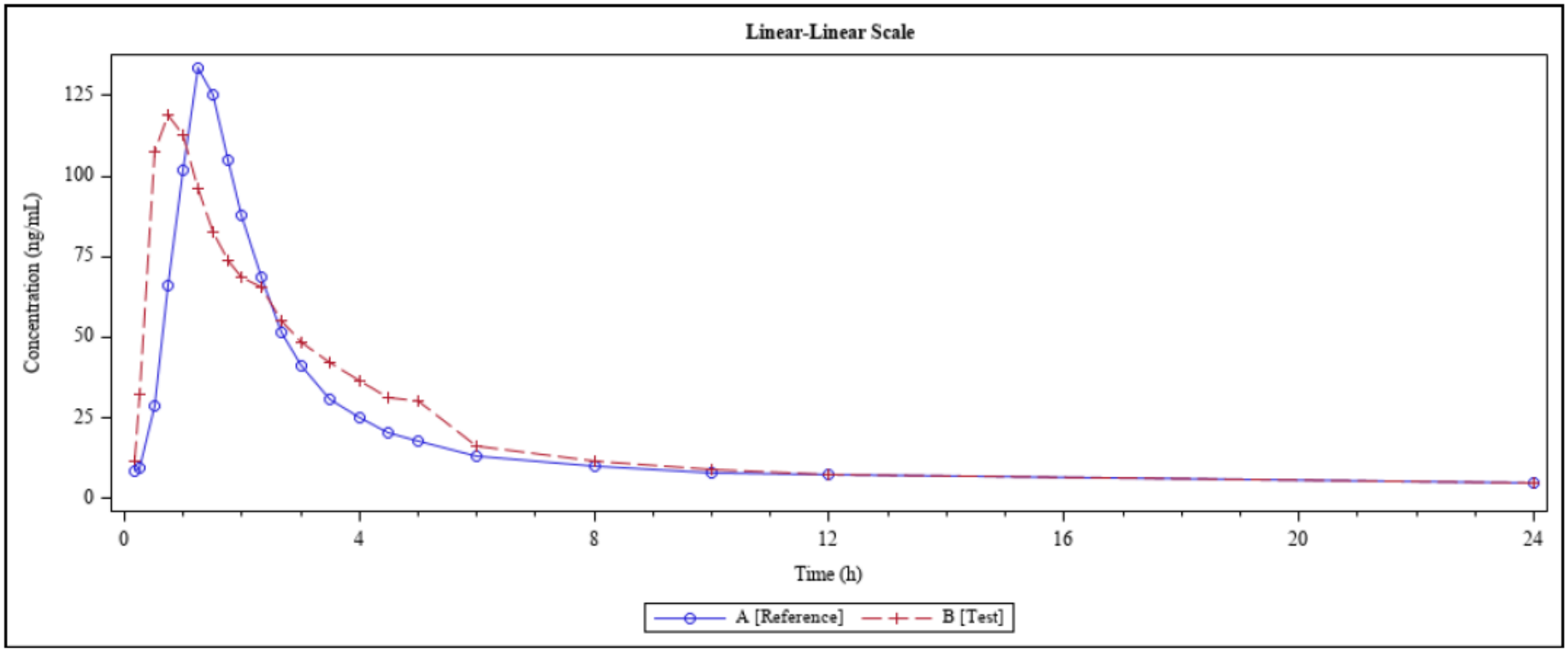


Figure 2.7.1.2: Grouped Individual Subject Plot of Plasma Concentrations of Sprionolactone Tablets 100 mg over time (Study INV684)

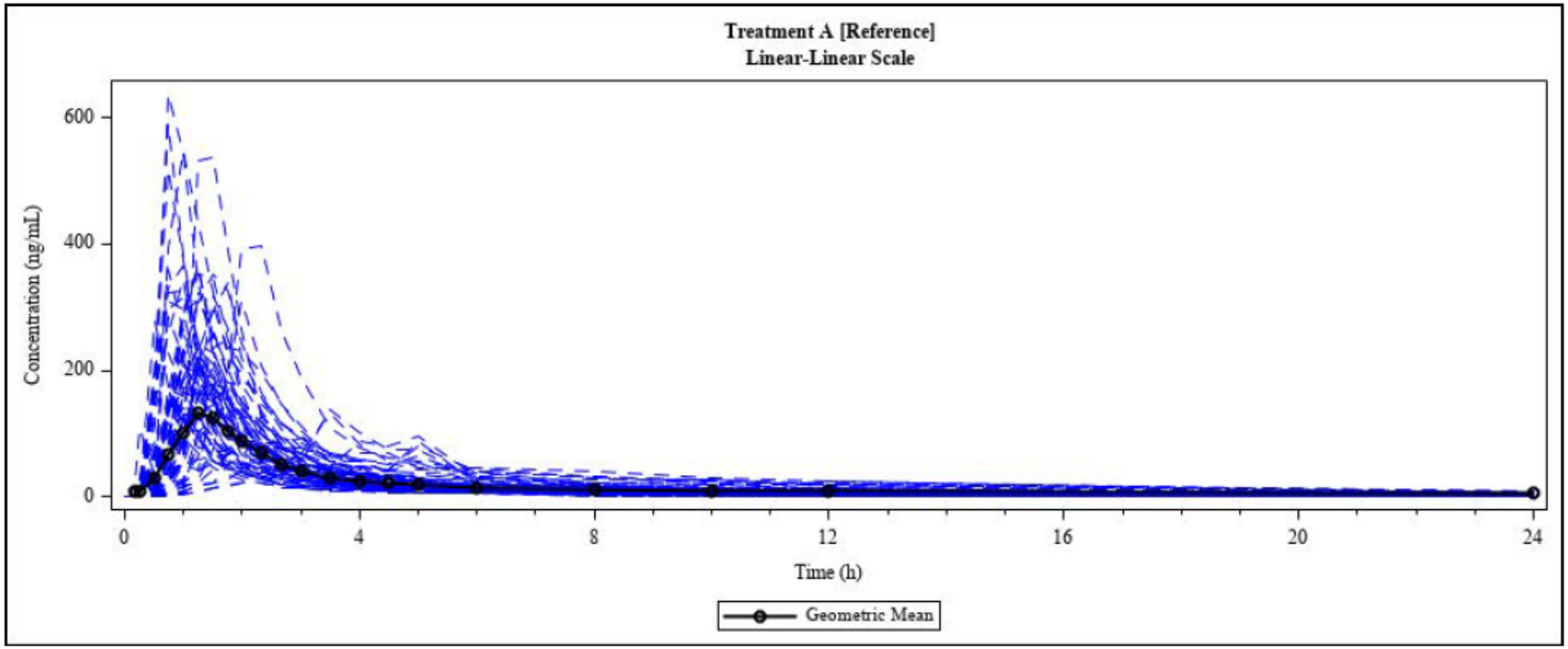
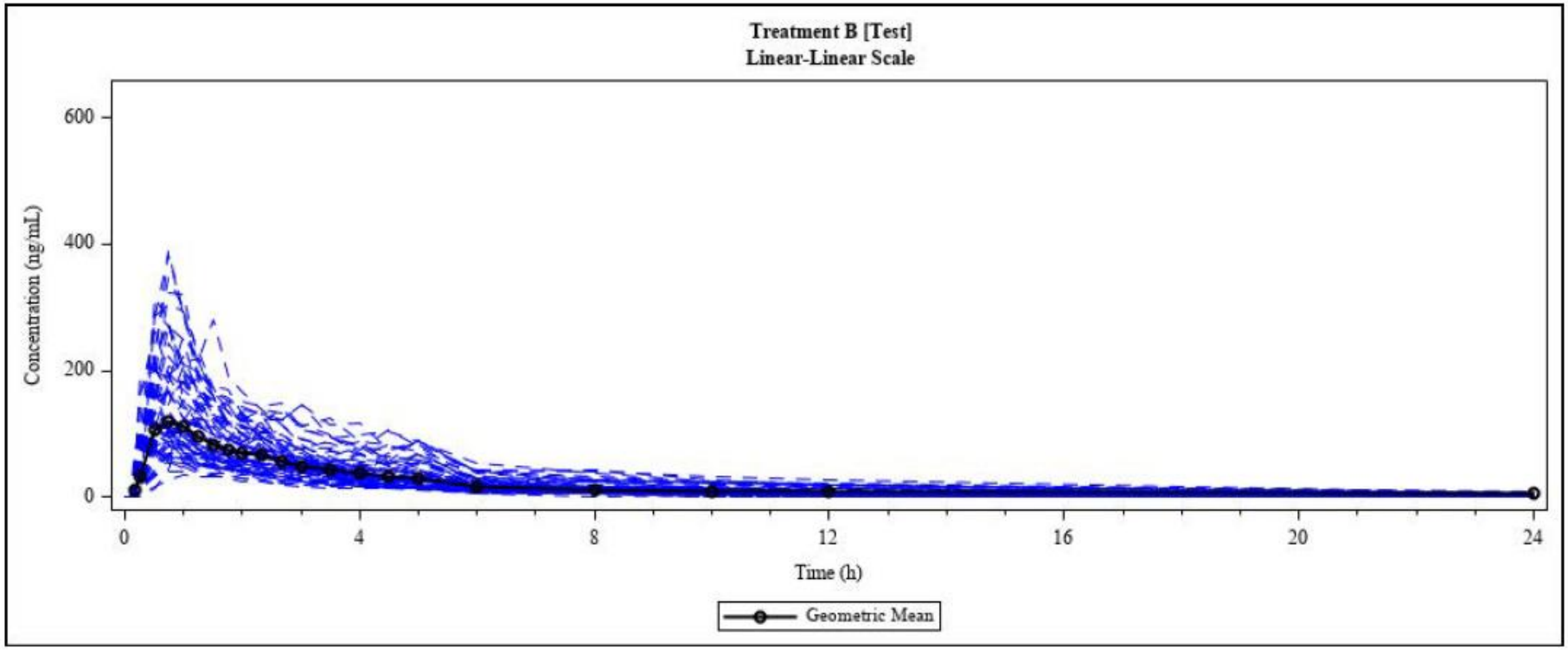


Figure 2.7.1.3: Grouped Individual Subject Plot of PK Plasma Concentrations of Spironolactone Suspension 10 mg/mL over time (Study INV684)



The mean AUC_{0-t} and $AUC_{0-\infty}$ were similar for both formulations. Table 2.7.1.6 shows that both treatments can be considered bioequivalent in terms of AUC_{0-t} and $AUC_{0-\infty}$. However, the mean Cmax values of spironolactone were higher for the tablet formulation, with the 90% confidence limits falling below the lower 70% limit, demonstrating that the two formulations are not bioequivalent for this parameter. The model intra-subject variability in Cmax was higher than AUC_{0-t}; 25.9% CV versus 12.30 % CV respectively. Figure 2.7.1.2 and Figure 2.7.1.3 show the individual plasma level plots for the tablet and the oral suspension, respectively. The intra-subject variability in Cmax was below the threshold for classification as a highly variable drug (<30% CV). It is likely that, unlike the previous studies, the replicate study design employed in the current investigation has allowed the intra-subject variability to be determined with greater precision.

The median Tmax was prolonged for the tablet 1.25 versus 0.75 hours for the oral suspension. For both formulations, in all subjects, plasma concentrations fell below 10 ng/mL at 24 hours post-dose. The mean half lives were 5.2 and 4.6 hours for the tablet and oral suspension respectively.

[redacted]

The PK conclusions from this study are:

- The test product is equivalent to the reference product with respect to the extent of absorption of spironolactone, but the test product is not equivalent to the reference product with respect to the rate of absorption of spironolactone.

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2.7.1.3 Comparison and Analyses of Results Across Studies

Not applicable.

2.7.1.4 Appendix

Not applicable.