**Synopsis**

**TITLE OF TRIAL**
Single dose, three-way, cross-over, relative bioavailability study of three oral formulations for Hormone Replacement Therapy in postmenopausal women: 0.5 mg estradiol + 0.1 mg norethisterone acetate, 0.5 mg estradiol + 0.25 mg norethisterone acetate, and 1.0 mg estradiol + 0.5 mg norethisterone acetate

**INVESTIGATOR**
One investigator for this trial.

**TRIAL SITE**
One trial site in Germany.

**PUBLICATIONS**
None

**TRIAL PERIOD**

**DEVELOPMENT PHASE**
Phase 1

**OBJECTIVES**

Primary Objective:
The primary objective of this study was to determine the extent of bioavailability of the two Activelle Low Dose preparations and Activelle® as represented by the area under the concentration-time curves (AUC(0-∞)) and the rate and extent of absorption as represented by the maximal concentration (Cmax) and the time of maximum (tmax).

Secondary Objective:
To evaluate other pharmacokinetic parameters, safety and tolerability of the two different Activelle Low Dose (ALD) tablets.

The safety endpoints in this trial were the adverse event incidence during the whole study and vital signs, ECG, physical examination, and laboratory investigations during pre-study and end-of-trial visit.

**METHODOLOGY**
The trial was an open, randomised, single-dose, single-centre, three-way cross-over trial where three single doses of 17ß-estradiol/NETA (two tablets of each test preparation of ALD or one tablet of the reference product Activelle®) was administered orally in the morning of each period to postmenopausal women in a randomised order. The three in-house periods were separated by a three-week wash-out period.

The trial compromised the following visits:
- Visit 1 (Screening)
- Visit 2 (Dosing of first dose): in-house with 4 nights, within 28 days after Visit 1
- Visit 3 (Dosing of second dose): in-house with 4 nights, 3 weeks + (0 - 2 days) after drug administration of Visit 2
- Visit 4 (Dosing of third dose): in-house with 4 nights, 3 weeks + (0 - 5 days) after drug administration of Visit 3
- Visit 5 (Follow-up): 0 – 14 days after end of confinement of Visit 4.

Blood sampling took place over a period of 72 hours after single dosing in order to determine the concentration-time profiles of estradiol, estrone (E2/E1, 18 samples) and estrone sulfate (E1S, 17 samples) in plasma.

**NUMBER OF SUBJECTS PLANNED AND ANALYSED**
Number of subjects planned: 24
Number of subjects enrolled: 24
Number of subjects dropped: 0
Number of subjects finalised: 24 analysed for safety, 24 analysed for PK.

**DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION**
Healthy, postmenopausal, female, smoking (up to 5 cig. / per day) and non-smoking subjects of Caucasian race, 50 through 70 years of age, were recruited from the local population. First invasive pre-study (screening) examination procedure was performed only after the subjects having given voluntary written informed consent.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

A 2 tablets of “Activelle Low Dose”
[0.5 mg estradiol + 0.1 mg norethisterone acetate (NETA)]
Batch No.: PBBA044

B 2 tablets of “Activelle Low Dose”
[0.5 mg estradiol + 0.25 mg norethisterone acetate (NETA)]
Batch No.: PBBA095

All subjects received a single oral dose of 2 tablets each containing 0.5 mg 17ß-estradiol/0.1 mg NETA (Treatment A), a single oral dose of 2 tablets each containing 0.5 mg 17ß-estradiol/0.25 mg NETA (Treatment B) under fasting conditions with a wash-out phase of 3 weeks between consecutive dosing.

DURATION OF TREATMENT

each subject was randomised to a single dose of 1.0 mg estradiol + 0.2 mg norethisterone acetate (NETA) (Treatment A) or to a single dose of 1.0 mg estradiol + 0.5 mg norethisterone acetate (NETA) (Treatment B and C) in each of the three periods. The periods were separated by a three-week wash-out period.

The total duration of the trial for the individual subject will be up to 13 weeks (incl. Screening and Follow-up visit).

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

C 1 tablet of “Activelle”
[1.0 mg estradiol + 0.5 mg norethisterone acetate (NETA)].
Batch No.: PB50156

All subjects received also a single oral dose of 1 tablet containing 1 mg 17ß-estradiol/0.5 mg NETA (Treatment C – Activelle®) under fasting conditions with a wash-out phase of 3 weeks between consecutive dosing.

CRITERIA FOR EVALUATION – PHARMACOKINETICS

Monitored were norethindrone (NET) and estradiol (E2), together with estrone (E1) and estrone-3- sulfate (E1S)
• Primary endpoints were: area under the curve (AUC(0-∞)), maximal concentration (Cmax) of E2 and E1 after baseline correction, time of maximum (tmax) of E2 and E1 (measured), AUC(0-∞), Cmax and tmax of NET.
• Secondary endpoints were: AUC(0-∞), Cmax of E1S after baseline correction; tmax of E1S; AUC(0-t), terminal half-life (t½), terminal rate constant (λz) of E2, E1 and E1S after baseline correction; AUC(0-t), Cmax of E2 and E1 (measured); AUC(0-t), t½, and λz of NET.

For the primary analysis AUC(0-∞) had to be replaced by AUC(0-t) for E2 and E1.

CRITERIA FOR EVALUATION – SAFETY

The safety endpoints are the adverse event incidence during the whole study and vital signs, ECG, physical examination, and laboratory investigations during pre-study and end-of-trial visit.

STATISTICAL METHODS

Data analysis was performed for the ‘full-analysis set’, i.e. including data of all subjects with at least one intake of study drug and at least one post-dose measurement. AUC and Cmax of E2, E1 or E1S plasma levels were calculated with and without baseline correction, using the mean of three baseline samples separate for each period. Pharmacokinetic parameters were calculated by non-compartmental methods, e.g. linear trapezoidal rule for AUC and log-linear regression for λz. The comparisons between treatments in respect to AUC(0-t), AUC(0-∞), Cmax and t½ were performed using a mixed effects analysis of variance based on the logarithmic transformed values. The model included sequence, period and treatment as fixed effects and subjects as random effects. Based on the statistical model, least squares treatment means were derived with 95% confidence intervals and, furthermore, ratios between the three treatments were estimated with 90% confidence intervals.

Bioequivalence could be declared if the confidence intervals for the ratios of AUC(0-t) and Cmax were fully contained within the limits (0.80, 1.25).

tmax was compared between treatments by the corresponding nonparametric methods.
PHARMACOKINETIC RESULTS

<table>
<thead>
<tr>
<th>Substance</th>
<th>Parameter - Method</th>
<th>Ratio A / C with 90% Confidence Interval</th>
<th>Ratio B / C with 90% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (E2)</td>
<td>AUC(0-t) - Mixed (ln)</td>
<td>95.75% (89.39%, 102.56%)</td>
<td>98.01% (91.49%, 104.97%)</td>
</tr>
<tr>
<td></td>
<td>C_max - Mixed (ln)</td>
<td>101.18% (90.12%, 113.59%)</td>
<td>95.59% (85.14%, 107.31%)</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>AUC(0-t) - Mixed (ln)</td>
<td>97.70% (90.14%, 105.87%)</td>
<td>100.06% (92.32%, 108.43%)</td>
</tr>
<tr>
<td></td>
<td>C_max - Mixed (ln)</td>
<td>98.34% (90.80%, 106.49%)</td>
<td>99.50% (91.88%, 107.75%)</td>
</tr>
<tr>
<td>Norethindrone (NET)</td>
<td>AUC(0-∞) - Mixed (ln)</td>
<td>102.60% (94.68%, 111.17%)</td>
<td>96.70% (89.39%, 104.59%)</td>
</tr>
<tr>
<td></td>
<td>C_max - Mixed (ln)</td>
<td>113.12% (106.23%, 120.45%)</td>
<td>98.30% (92.31%, 104.67%)</td>
</tr>
</tbody>
</table>

* concentration dependant endpoints corrected for dose

- Relative bioavailability of estradiol was found to be comparable between all three treatments. As the same dose was administered, this was evident from almost superimposable plasma profiles of estradiol and its biotransformation products as estrone or estrone sulfate.
- T_max of E2 (median and range) was similar for all treatment groups.
- Bioequivalence with regard to E2 between the two Activelle Low Dose preparations (A or B) and Activelle® (C) can be stated formally: all confidence intervals for the pre-specified concentration dependent endpoints of the pharmacokinetic analysis were within the commonly used acceptance range for bioequivalence (0.8 to 1.25).
- Relative bioavailability of norethisterone acetate was found to be comparable between all three treatments. This was evident from almost superimposable plasma profiles of NET, if the administered dose was accounted for.
- T_max of NET (median and range) was similar for all treatment groups.
- Bioequivalence with regard to NETA between the two Activelle Low Dose preparations (A or B) and Activelle® (C) can be stated formally: all confidence intervals for the pre-specified concentration dependent endpoints of the pharmacokinetic analysis were within the commonly used acceptance range for bioequivalence (0.8 to 1.25).
- The pharmacokinetic behaviour of estradiol as observed was found to be fully compatible with all characteristics typical for substances with a substantial first pass effect.
- The pharmacokinetic behaviour of estrone as observed after administration of Activelle Low Dose preparations and Activelle® was typical for a substance generated by metabolism.
- Consistent t_max and t½ between treatments together with the dose proportional increase in the characteristics AUC and C_max provide evidence for dose proportional pharmacokinetics of NET within the dose range of 0.2 mg to 0.5 mg norethisterone acetate as administered in this study.
- There is evidence for dose proportional pharmacokinetics of NET within the dose administered in this study.
- Pharmacokinetics of estradiol and estrone were not influenced by the dose of norethisterone acetate within the dose range administered in this study.
- Bioequivalence between the two Activelle Low Dose preparations (Treatment A or B) and Activelle® (Treatment C) fulfilled all formal criteria used in a confirmatory analysis, since for AUC(0-t) and C_max of estradiol and estrone and for AUC(0-∞) and C_max of NET, the minimal lower and the maximal upper bounds of the 90% confidence intervals were 85.14% and 120.45%. This conclusion was supported by every supplementary analysis.

SAFETY RESULTS

- As to adverse events, all three treatments were safe and well tolerated. No serious adverse events were reported and none of the subjects withdrew due to an adverse event.
- In total, 69 treatment-emergent adverse events were reported, 22 events after Treatment A (2 tablets of ALD 0.1), 30 events after Treatment B (2 tablets of ALD 0.25) and 17 adverse events after Treatment C (1 tablet of Activelle®). The majority of the reported adverse events, i.e. 47 of 69 events were mild in intensity, 14 events were moderate and 8 were severe. The investigator considered a possible causal drug-event relationship for 22 adverse events and an unlikely causal drug-event relationship for 47 adverse events. For drug related adverse events there
was no trend with respect to treatment groups.

- The following possibly drug-related adverse events were reported: Disgeusia, buttock pain, diarrhoea, abdominal pain, back pain, pain in extremity, sensation of heaviness, arthralgia, rosacea, rash.
- There were no clinically relevant changes in any of the laboratory parameters, vital sign parameters (systolic and diastolic blood pressure and pulse), physical examination findings and in ECG between Screening and Follow-up.

CONCLUSIONS
- Based on all criteria of AUC, adjusted for dose, it can be concluded that the two Activelle Low Dose preparations (Treatment A or B) and Activelle® (Treatment C) are bioequivalent with respect to extent of bioavailability of both active ingredients. Confidence intervals for the pre-specified parameters of pharmacokinetic analysis were within the commonly used acceptance range for bioequivalence 0.8 to 1.25.
- Based on the criteria of Cmax and tmax it can be concluded that the two Activelle Low Dose preparations (Treatment A or B) and Activelle® (Treatment C) are bioequivalent with respect to rate and extent of absorption of both active ingredients.
- There is evidence for dose proportional pharmacokinetics of NET. Pharmacokinetics of estradiol and estrone were not influenced by the dose of norethisterone acetate within the dose range as administered in this study.
- With respect to the safety endpoints (adverse event incidence during the whole study as well as vital signs, ECG, physical examination, and laboratory investigations during pre-study and follow-up visit) there were no indications of any clinically relevant differences regarding safety and tolerability between single doses of 2 tablets each of the two Activelle Low Dose treatments and 1 tablet of Activelle®.

The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.