# Bioequivalence comparison between two different formulations of alverine citrate 120mg capsules: an open label, balanced, randomized-sequence, single-dose, two-period crossover study in healthy male volunteers

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## Abstract

An open-labeled, balanced, single-dose, 2-treatment, 2-period, 2-sequence, randomized crossover study was designed and conducted to determine the pharmacokinetic, bioavailability and bioequivalence of alverine citrate 120 mg capsules in comparison with Spasmonal Forte® 120 mg capsules after single dose administration under fasting conditions in 12 healthy adult male subjects. Each volunteer received a 120 mg capsule of the reference(or)test drug respectively. On the day of dosing, blood samples were collected before dosing & at various time points up to 4 days after dosing. Analysis of alverine and its metabolite 4-hydroxy alverine concentrations was performed using a validated LC-MS/MS method. The pharmacokinetic parameters were analyzed using the non-compartmental model. The primary pharmacokinetic parameters at 90% CI were within the 80 to 125% interval required for bioequivalence as stipulated in the current regulations of the EU acceptance criteria. The geometric mean ratios (Test/Reference) between the two products of alverine capsule under fasting condition were 111.15% (98.67%-122.4%) and 113.41% (94.68%-118.74%) for $C_{max}$ ratios, 112.72% (91.12-114.35%) and 104.54% (94.73%-103.85%) for $AUC_{0-t}$ ratios and 103.73% (94.45%-111.5%) and 104.56% (103.24%-107.58%) for $AUC_{0-inf}$ ratios of aiverine and its metabolite respectively. 12 volunteers had completed both treatments. There was no significant difference of the $T_{max}$ parameter between the two formulations ($p > 0.05$). Drug safety and tolerability were assessed. No serious adverse events related to the study drugs were found. This single dose study found that the test formulation alverine citrate capsules is bioequivalent to the reference formulation Spasmonal Forte® capsules of 120 mg under fasting condition in healthy adult male volunteers according to the EU regulatory guidance.

## Materials and Methods

***Study drugs:***

Alverine citrate capsules and Spasmonal Forte® capsules from Norgine Ltd, UK, were used as the test and the reference products respectively. Both products were prepared as Alverine citrate equivalent to Alverine 120 mg. Both the products were stored at controlled room temperature 25˚C (77 ˚F).

***Study population:***

The study was carried out at Actimus Biosciences Private Limited, India. The study protocol was approved by the Ethics Committee. In addition, the protocol was performed in accordance with the Declaration of Helsinki Principles as outlined in the ICH-E6 Guidelines for Good Clinical Practice (GCP). All subjects were given a detailed description of the study and written informed consent was obtained prior to the enrollment. The sample size was estimated based on, Coefficient of variation (C.V.) of the drug, sufficient statistical power to detect 20% difference with the power of 0.8 in Cmax and AUC between the test and reference product, Regulatory requirements. Sample size was based on estimates obtained from reported literature and previous studies. Assuming a formulation ratio (T/R) ranging from 0.95-1.05 a sample of 12 subjects including dropouts would be sufficient to show bioequivalence between the two formulations with a power of at least 80%. Hence sample size of 12 subjects was enrolled in the study. 12 healthy male volunteers between the ages of 18-45 years with a body mass index between 18.5 kg/m2 and 24.9 kg/m2, with body weight equal to or not less than 50 kg were assessed to be in good physical condition by a complete medical screening including a medical history, physical examination, chest radiography, electro radiography, laboratory screening test for hematologic and blood biochemistry parameters and nonsmoker status. Subjects with a history of hypersensitivity to any ingredients in the alverine products and/or related drugs or its constituents or who were taking any medication or alcohol for a 21-day period prior to the study were excluded. Subjects who had a history of cardiovascular, hepatic, renal, gastrointestinal or hematologic disease were excluded from the study.

***Study design:***

The study was an open-labeled, single-dose, two-treatment, two-period, two-sequence randomized two way crossover with at least one week washout period. Subjects were randomly allocated to two groups by the sequence of product administered [Test-Reference (TR) and Reference-Test (RT) group]. In each period, 1x120mg capsule of alverine citrate of the test or reference product was administered in the morning. Subjects were housed 12 hours prior to dosing in the clinical facility and allowed to leave the facility after 24.00 hours post-dose sample in each period. The subjects received a standard meal at about 4.0, 9.0 and 13.0 hours after dosing in each period. During housing, all meal plans were identical for all the periods. Drinking water was not allowed from one hour before dosing till one hour post-dose (except for 240 ± 02 mL of drinking water given for dosing). Before and after that, drinking water was allowed at ad libitum. After a minimum of 1 week washout period, the subjects were crossed over to the next treatment following the same procedure as conducted in the 1st period.

***Sample collection:***

During dosing day in each period, 22 blood samples (6 mL each) was collected as per the following schedule: Pre dose sample(0.00 hr) within 02 hrs prior to drug administration and the others at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 18.00, 24.00, 48.00, 72.00 and 96.00 hours post dose. The total volume collected per study participant in this study was not exceed approximately 321 mL including up to 9 mL for screening, and 7-9 mL for post clinical assessment of lab parameters and 18 mL for discarded blood sample resulting from use of intravenous cannula for 12 hours and 2-9 mL was collected for repeat/additional lab tests, if required. For separating plasma, all blood samples were centrifuged at 3800 RPM for 10 minutes at 4°C ± 2°C. Centrifugation of all samples was done as early as possible after each sample draw time point. After centrifugation, plasma samples were aliquoted into two sets in properly labeled polypropylene tubes and immediately stored at about -60°C or colder.

## Results

***Study population:***

12 healthy male adults eligible for the study enrollment were randomly divided into 2 groups [Test-Reference (TR) and Reference-Test (RT)] according to the sequence of drug administration. All the subjects had completed both the periods. Thus, this study was balanced in each sequence and the results from 12 volunteers were used for pharmacokinetic and statistical analysis. Table 1 demonstrates the demographic characteristics of the volunteers.

***Bio analysis and pharmacokinetics:***

The LC/MS/MS system consisted of four pumps for gradient solvent delivery, and a divert valve to direct LC effluent to the mass spectrometer in the analyte elution window. The analytical column effluent is directed through the divert valve to a thermo electron TSQ quantum discovery mass spectrometer.

The instrument is operated in the positive ion mode. The precursor ions at m/z 282.057, 297.553 and 263.353 for alverine, PHA and ticlopidine are selected by the first quadrupole (Q1), respectively. After collision-induced fragmentation in Q2, the product ions at m/z 91.036, 106.906 and 124.824 for alverine, PHA and ticlopidine are monitored in Q3, respectively. A resolution of one unit (at half peak height) is used for both Q1 and Q3.

The method was fully validated using these Q1 and Q3 masses for both compounds with satisfactory results. Linear calibration curves were obtained with a coefficient of correlation (r2) usually higher than 0.995 in range of 20 to 5000 pg/mL. For each calibration standard level, the concentration was back calculated from the linear regression curve equation. No significant difference was observed in any of the analyzed pharmacokinetic parameters for Alverine citrate and its metabolite p-hydroxy alverine was shown in Table 2.

***Bioequivalence analysis:***

Ninety percent confidence interval of geometric mean ratios of bioavailability parameters between the test and reference formulation are presented in Table 3. The statistical analysis obtained from this study showed that the point estimate (90% CI) of the geometric mean ratio (GMR) (T/R) of Cmax, AUC0-t and AUC0-inf was entirely within the equivalence criteria (80.00-125.00%) which was 111.15% (98.67%-122.4%) and 113.41% (94.68%-118.74%) for Cmax ratios, 112.72% (91.12-114.35%) and 104.54% (94.73%-103.85%) for AUC0-t ratios and 103.73% (94.45%-111.5%) and 104.56% (103.24%-107.58%) for AUC0-inf ratios of alverine and its metabolite p-Hydroxy alverine respectively. In addition, no significant difference of the Tmax parameter between the two studied formulations was observed ($p > 0.05$). Therefore, it was concluded that the two capsule formulations of alverine were bioequivalent in terms of rate and extent of absorption for the drug alverine and the metabolite data has been given as supportive evidence. The mean plasma concentration vs time profiles were given in Fig 1.

| Category | | Treatment | | Total |
|---|---|---|---|---|
| | | Test (T) | Reference (R) | |
| Age (years) | Mean ± SD | 23.42 ± 4.73 | 28.34 ± 3.80 | 25.88 ± 3.47 |
| | Range | 18.0 – 35.0 | 18.0 – 35.0 | 18.0 – 35.0 |
| | Median | 23 | 23 | 23 |
| Age Groups | N | 12 | 12 | 24 |
| | < 18 | 0 | 0 | 0 |
| | 18 – 40 | 12 | 12 | 24 |
| | 41 – 64 | 0 | 0 | 0 |
| | 65 – 75 | 0 | 0 | 0 |
| | > 75 | 0 | 0 | 0 |
| Gender | Female | 0 | 0 | 0 |
| | Male | 12 | 12 | 24 |
| Race | American | 0 | 0 | 0 |
| | Hispanic | 0 | 0 | 0 |
| | Caucasian | 0 | 0 | 0 |
| | Asian | 12 | 12 | 24 |
| Height (cm) | Mean ± SD | 164.84 ± 3.89 | 161.42 ± 5.65 | 163.13 ± 2.41 |
| | Range | 159.0 – 176.0 | 155.0 – 175.0 | 155.0 – 176.0 |
| | Median | 168 | 162 | 165 |
| | N | 12 | 12 | 24 |
| Weight (kg) | Mean ± SD | 66.54 ± 6.73 | 58.74 ± 5.24 | 62.64 ± 5.51 |
| | Range | 52.0 – 77.0 | 52.0 – 70.0 | 52.0 – 77.0 |
| | Median | 59 | 58 | 59 |
| | N | 12 | 12 | 24 |
| BMI (kg/m²) | Mean ± SD | 22.20 ± 1.79 | 23.01 ± 1.26 | 22.61 ± 0.58 |
| | Range | 20.0 – 24.9 | 20.1 – 24.8 | 20.0 – 24.9 |
| | Median | 21.6 | 22 | 21.8 |
| | N | 12 | 12 | 24 |

**Table No 1: Demographic characteristics**

| PK Parameters | Formulation (Alverine) | |
|---|---|---|
| | Test | Reference |
| $C_{max}$ (pg/mL) | 1198.443 | 1289.446 |
| $AUC_{0-t}$ (pg.h/mL) | 3750.396 | 4245.393 |
| $AUC_{0-inf}$ (pg.h/mL) | 4575.626 | 4955.522 |
| $T_{max}$ (H) | 1.045 | 1.049 |
| $K_{el}$ ($H^{-1}$) | 0.256 | 0.258 |
| $T_{1/2}$ (H) | 5.877 | 5.742 |
| PK Parameters | Formulation (P-hydroxy alverine) | |
| | Test | Reference |
| $C_{max}$ (pg/mL) | 2342.084 | 3393.422 |
| $AUC_{0-t}$ (pg.h/mL) | 9643.564 | 11768.975 |
| $AUC_{0-inf}$ (pg.h/mL) | 12314.978 | 14378.541 |
| $T_{max}$ (H) | 1.544 | 1.634 |
| $K_{el}$ ($H^{-1}$) | 0.124 | 0.173 |
| $T_{1/2}$ (H) | 11.415 | 13.676 |

**Table No 2: Pharmacokinetic Parameters of Alverine and P-Hydroxy Alverine for Both Formulations**

| Parameter | Alverine | | | P-Hydroxy Alverine | | |
|---|---|---|---|---|---|---|
| | $C_{max}$ | $AUC_t$ | $AUC_{inf}$ | $C_{max}$ | $AUC_t$ | $AUC_{inf}$ |
| 90% CI Lower Limit | 98.67 | 91.12 | 94.45 | 94.68 | 94.73 | 103.24 |
| 90% CI Upper Limit | 122.4 | 114.35 | 111.5 | 118.74 | 103.85 | 107.58 |
| T/R Ratio (%) | 111.15 | 112.72 | 103.73 | 113.41 | 104.54 | 104.56 |
| Power | 0.96 | 0.94 | 0.92 | 0.95 | 0.98 | 0.97 |
| Intra Subject Variability | 6.65 | 4.08 | 7.82 | 10.34 | 5.7 | 5.1 |
| Inter Subject Variability | 29.74 | 31.01 | 29.91 | 24.48 | 53.04 | 49.49 |
| ANOVA (p-Value) | | | | | | |
| Sequence | 0.714 | 0.1696 | 0.7122 | 0.1874 | 0.1177 | 0.6186 |
| Period | 0.004 | 0.534 | 0.5133 | 0.218 | 0.1477 | 0.425 |
| Treatment | 0.516 | 0.2905 | 0.5341 | 0.9244 | 0.1157 | 0.269 |

**Table No 3: Bioequivalence Parameters for Alverine and P-Hydroxy Alverine.**

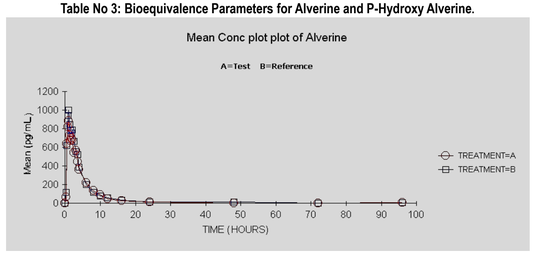


**Fig. 1: Time vs. Mean Plasma Concentration Graph of Alverine and P-Hydroxy Alverine.**

***Tolerability:***

Almost all volunteers taking both alverine formulations were noted for mild adverse events. Most common events were drowsiness, nausea and loss of appetite. However, no subject had any severe adverse event or withdrew from the study because of an adverse event.

## Conclusion

This single dose study found that the test formulation alverine citrate 120 mg Capsules is bioequivalent to the reference formulation Spasmonal Forte® capsules the extent and the rate of absorption of 120mg in healthy adult male volunteers according to the EU regulatory guidance.

## Literature Cited

1. Talley NJ. Irritable bowel syndrome: definition, diagnosis and epidemiology. Baillieres Best Pract Res Clin Gastroenterol 1999;**13**:371-384.
2. Kondapanaidu Y, Koteswara Rao D, Kalyan Reddy MP, Sarath Chandiran I and Jayaveera KN. Simultaneous quantification of Alverine and its metabolite p-hydroxy alverine in human plasma with robotic liquid-liquid extraction by using fully validated LC-MS/MS and its application to a bioequivalence study, Journal Of Pharmacy Research, 2010;**3**:1407-1411.
3. Sarath Chandiran I, Jayaveera KN, Raghunadha Reddy S. Development and validation of high-throughput liquid chromatography-tandem mass spectrometric method for quantification of Itraconazole and its metabolite in human plasma. Scholars Research Library, Der Pharmacia Lettre, 2011;**3**:316-328.
4. Sarath Chandiran I, Jayaveera KN, Raghunadha Reddy S, High-throughput liquid chromatography – tandem mass spectrometric method for simultaneous quantification of Carvedilol and its metabolite 4-hydroxyphenyl carvedilol in human plasma and its application to bioequivalence study. Journal of Chemical and Pharmaceutical Research, 2011;**3**:341-353.
5. Jayaveera KN, Koteswara Rao D, Sarath Chandiran I, Raghunadhareddy S. Quantification of Artemether in human plasma with liquid- liquid extraction by using fully validated high performance liquid chromatography–tandem mass spectrometric method, Journal of Pharmacy Research, 2010;**3(8)**:1943-1946.
6. Kalyan Reddy MP, Jaya Chandra Reddy P, Raghunadhareddy S, Jayaveera KN. Development and validation of bio-analytical method for Quantification of Pantoprazole in human plasma using LC-MS/MS, Journal of Pharmacy and Chemistry. 2010;**4**:80-86.
7. Chandiran IS, Jayaveera KN, Pharmacokinetic and bioequivalence comparison between extended release capsules of Venlafaxine hydrochloride 150mg: an open label, balanced, randomized-sequence, single-dose, two-period crossover study in healthy Indian male volunteers. International Research Journal of Pharmacy, 2011;2(3):262-269.
8. Seelam RR, Jayaveera KN, Koteswara Rao D. Quantification of ibuprofen in human plasma by using high throughput liquid chromatography–tandem mass spectrometric method and its applications in pharmacokinetics. Scholars research library. Archives of applied research. 2010;2(3):101-111.
9. Sarath Chandiran I, Koteswara Rao D, Jayaveera KN, Development and validation of high performance liquid chromatography-tandem mass spectrometric method for simultaneous quantification of Telmisartan in human plasma, International journal of pharmaceutical sciences and Drug Research, 2010;2(3):188-192.
10. Raghunadhareddy S, Sarath Chandiran I, Jayaveera KN and Koteswara Rao D. Quantification of Ursodeoxy cholic acid in human plasma by using high performance liquid chromatography–tandem mass spectrometric method and its applications in pharmacokinetics, Journal of Chemical and Pharmaceutical Research, 2010;2(3):59-69.
11. Kondapa Naidu Y, Raghunadha reddy S, Koteswara Rao D, Kalyan Reddy MP, Sarath Chandiran I and Jayaveera KN. Quantification of Levetiracetam in human plasma with precipitation extraction by using fully validated LC-MS/MS and its application to a bioequivalence study, Research J. Pharm. and Tech., 2010;3(3):847-853.

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