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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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A B S T R A C T

This present bioequivalence study was designed 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 healthy adult male subjects. An open-labeled, balanced, single-dose, 2-treatment, 2-period, 2-sequence, randomized crossover study was conducted in 12 healthy male volunteers. 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. Drug safety and tolerability were assessed. 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 ($p > 0.05$). 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 the extent and the rate of absorption, of 120 mg under fasting condition in healthy adult male volunteers according to the EU regulatory guidance.

Keywords: Alverine, 4-Hydroxy Alverine, Bioavailability, Bioequivalence, Intrasubject Variability.

A R T I C L E I N F O

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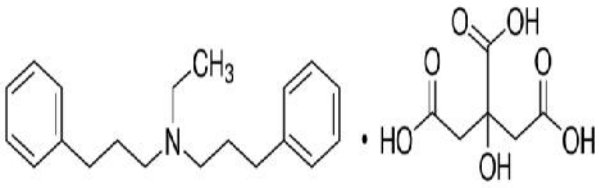
1. Introduction

Irritable bowel syndrome is a functional bowel disorder of the gastrointestinal tract characterized by alterations in the pattern of defecation and accompanied by pain or discomfort [1]. Whilst irritable bowel syndrome is not a life-threatening disease, for most patients it represents a chronic condition with significant physical and/or psychosexual morbidity [2]. Due to its high prevalence, irritable bowel syndrome has a considerable economic impact [3, 4]. There is no single pathological marker of irritable bowel syndrome, although it is generally accepted that some irritable bowel syndrome sufferers have a disturbance of intestinal motility [5-7] and/or enhanced visceral sensitivity [8,9]. In the absence of a unifying cause of irritable bowel syndrome, a wide variety of therapeutic agents have been used. None have proven efficacy, although smooth muscle relaxants may be beneficial, especially when abdominal pain is the predominant symptom [10, 11].

Drugs aimed at relieving abdominal pain (anti-spasmodics) [11] and disturbed bowel habit (anti-diarrhoeal and bulking agents)[12], drugs treating affective disorders (anti-depressants)[13] and, more recently, drugs targeted at components of the brain–gut axis and visceral sensation [5-hydroxytryptamine-3 (5-HT_3) antagonists, 5-HT_4 agonists, κ receptor agonists, somatostatin analogues][14-17] have all been investigated in irritable bowel syndrome. Alverine citrate is a spasmolytic, which has a specific action on the smooth muscle of the alimentary tract and the uterus, without affecting the heart, blood vessels or tracheal muscles at therapeutic doses. It has been used in the treatment of irritable bowel syndrome for many years and indeed is available over the counter (Relaxyl) for the treatment of irritable bowel syndrome symptoms [18-20]. The structure for Alverine citrate is as follows.



The mode of action of alverine is different of tricyclic antidepressants and specific or non-specific inhibitors of the recapture of serotonin, since alverine interacts marginally with serotonin or noradrenaline recapture systems. However, the exact mechanisms of alverine's inhibitory action are still not clear, due to the lack of information of its effects on isolated smooth muscle *in vitro*. After oral administration, alverine is rapidly converted to its primary active metabolite, which is then further converted to two secondary metabolites. There is a high renal clearance of all metabolites indicating that they are eliminated by active renal secretion. The peak plasma level of the most active metabolite occurs between 1 and 1.5 hours after oral dosing. The plasma half-life averages 0.8 hours for alverine and 5.7 hours for the active primary metabolite [21-24]. Alverine has a very low toxicity and side effects which are highly limited, as compared to the classic antidepressants.

The rationale of this present bioequivalence study for two formulations of 120 mg alverine citrate release capsule was examined between generic drug alverine citrate 120 mg capsules as the test product and Spasmonal Forte® alverine citrate 120 mg capsules as the reference product. This bioequivalence study could give assurance when prescribing less expensive generic drugs as alternatives with similar efficacy and safety. The study objectives of this present study are to assess the single dose bioequivalence of alverine citrate 120 mg capsules with Spasmonal Forte® in healthy, adult, human study participants under fasting conditions and to monitor the clinical status, adverse events and laboratory investigations and assess relative safety and tolerance of alverine formulations under fasting conditions.

2. 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

[25] as outlined in the ICH-E6 Guidelines for Good Clinical Practice (GCP) [26]. 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 C_{max} 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/m^2 and 24.9 kg/m^2, 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) were 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.

Alverine and its metabolite analysis by LC-MS/MS [27-40]:

Retrieved the frozen CC, QC samples and subject samples from the deep freezer, thawed in water bath maintained at room temperature and vortexed to mix. Removed the caps from the polypropylene tubes. Aliquoted 0.500 mL (500 µL) of CC, QC samples and subject samples into pre-labelled polypropylene tubes. Added 50 µL of ISTD dilution (about 100 ng/mL), and vortexed to mix. Added 2.5 mL of Extraction solvent (diethyl ether: dichloro methane 70:30) and vortexed for 20 min. Centrifuged the polypropylene tubes at 4,000 rpm and 10°C for 10 min and transferred approximately 2.0 mL of supernatant to prelabelled glass tubes and evaporated till dryness. After completion of evaporation, reconstituted by using 0.250 mL of mobile phase, vortexed for 30 seconds and transferred to prelabelled HPLC vials, then to the auto sampler. The samples were analyzed by using a validated LC/MS/MS detection method [28].

HPLC was carried isocratically at room temperature using a Thermo BDS Hypersil C18 (4.6 X 50 mm) column. The mobile phase consisted of acetonitrile: 10 mM ammonium formate buffer (90:10 v/v). The flow-rate was 1 mL/min by using 40:60 splitter. The duration of the analytical time was 3 min. The analytical column effluent is directed through the divert valve to a thermo electron TSQ quantum discovery mass spectrometer. Source/gas parameters such as spray voltage is operated at 4500, sheath gas pressure, auxiliary gas and capillary temperature settings are maintained at 60 psi, 35 psi and 300°C, respectively.

Chromatograms were acquired on a TSQ tandem mass spectrometry (Thermo Finnegan, Sanjose, CA, USA) equipped with Electrospray ionization (ESI) and connected to a PC runs with the standard software Xcalibur 2.0.7 and LC Quan 2.5.6. Mass spectroscopic detection was performed on a Triple quadrapole instrument (Thermo, TSQ Quantum Discovery Max). Robotic liquid handling system is operated using the software package supplied from the cohesive technologies Aria^{TM}. The calibration curve is constructed by weighted $1/x^2$ least-square linear regression analysis of the peak area ratio (drug/ISTD) *vs.* the concentration of drug and peak area ratio (metabolite/ISTD) *vs.* the concentration of metabolite. Representative chromatograms from an extract of human blank plasma spiked with internal standard and from an extract of human blank plasma spiked with drug, metabolite and internal standard are shown in Fig.1A and B.

Pharmacokinetic and statistical analysis [41-43]

For the purpose of Average Bioequivalence analysis C_{max}, AUC_{0-t} and AUC_{0-inf} were considered as the primary variables and T_{max}, $t_{1/2}$ and K_{el} were considered as the secondary variables. General Linear Model for analysis of variance (ANOVA) for crossover design was performed for log-transformed data and used to assess the effect of formulations, periods, sequences and subjects nested in sequence on these parameters. The difference between two related parameters was considered statistically significant for a *p*-value equal to or less than 0.05. 90% confidence interval (CI) for the ratios of geometric mean Test/Reference (T/R) for C_{max}, AUC_{0-t} and AUC_{0-inf} was calculated based on least squares means from the ANOVA of log-transformed data. The 90% geometric CI of the ratio (T/R) of least squares means from the ANOVA of the log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf} should be within 80.00% to 125.00%.

Tolerability assessment

Physical examination and measurement of vital signs (Blood Pressure, Pulse Rate and Oral Temperature) were examined at the time of Check-in, prior to administration of the each study drug (0.00 hr), 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 and during the entire study period. Adverse events were monitored throughout the study and recorded by physicians.

3. Results and Discussion

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.

Bioanalysis 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 [28] 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 (r^2) 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 C_{max}, AUC_{0-t} and AUC_{0-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 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 alverine and its metabolite p-Hydroxy alverine respectively. In addition, no significant difference of the T_{max} 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 2.

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.

Table 1: Demographic characteristics

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
	N	12	12	24
Age Groups	< 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/m^2)	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 2: Pharmacokinetic Parameters of Alverine and P-Hydroxy Alverine for Both Formulations

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 3: Bioequivalence Parameters for Alverine and P-Hydroxy Alverine

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

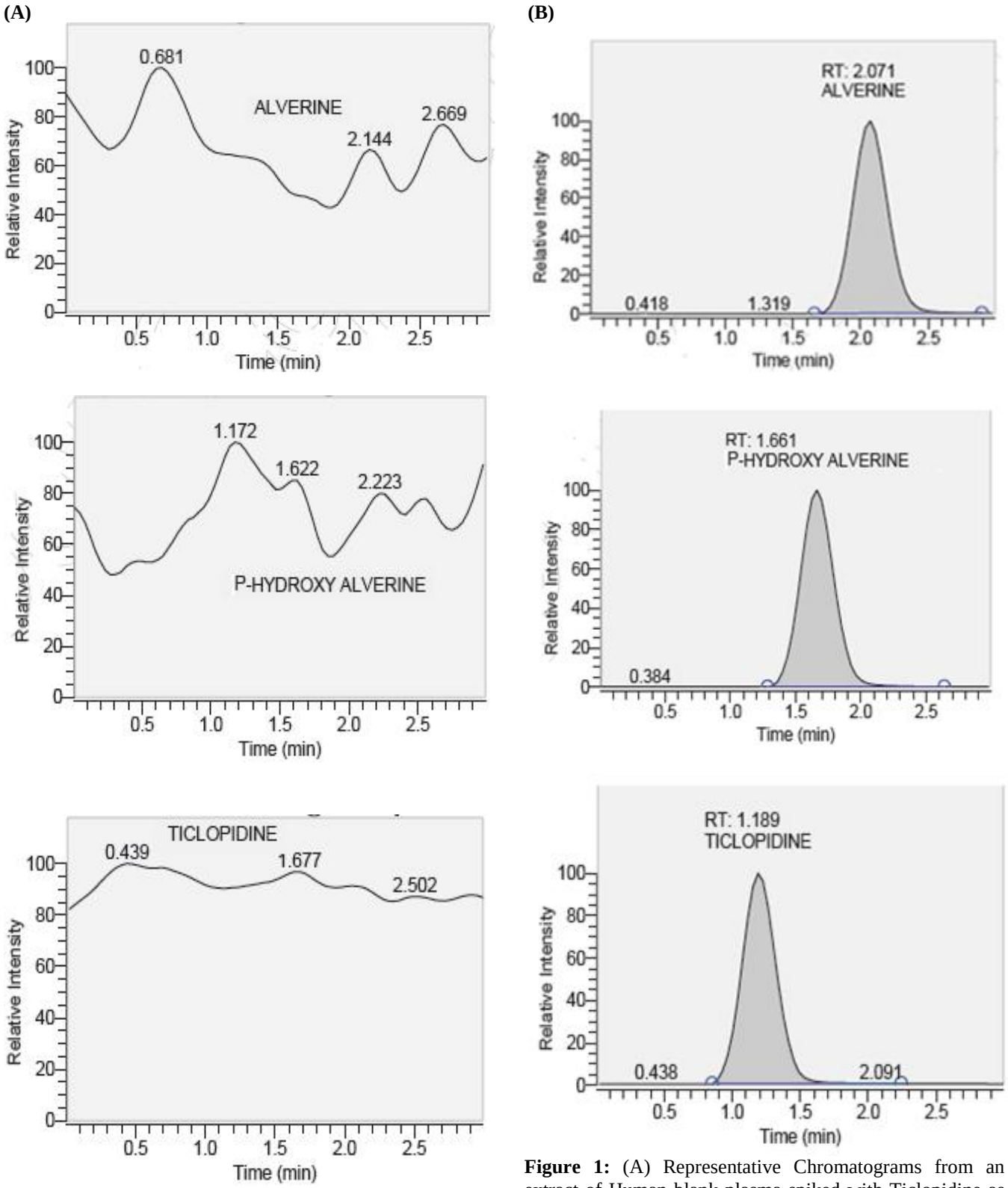


Figure 1: (A) Representative Chromatograms from an extract of Human blank plasma spiked with Ticlopidine as

IS. (B) Representative Chromatograms from an extract human blank plasma spiked with Alverine, P-Hydroxy Alverine and Ticlopidine (as IS).

Discussion

An open-labeled, single-dose, two-treatment, two-period, two-sequence randomized two way crossover design in 12 healthy adult volunteers was considered appropriate and standard for bioequivalence evaluation of the generic and the reference products. The study simulates real life conditions as well as circadian effects on the performance of the product. In general, the pharmacokinetic parameters for both formulations were similar to the pharmacokinetic parameters of alverine in previous published data. This study demonstrated that 90% CI of the logarithmic transformed of parameters C_{max}, AUC_{0-t} and AUC_{0-inf} were contained in 80.00-125.00%. In addition, no significant differences of the T_{max} values between the two formulations were observed ($p>0.05$). Therefore, the two capsule formulations of alverine are considered bioequivalent in terms of the rate and extent of absorption. Moreover, both formulations were well tolerated. Hence, the test (alverine) and reference (Spasmonal Forte®) formulations of alverine 120 mg are bioequivalent.

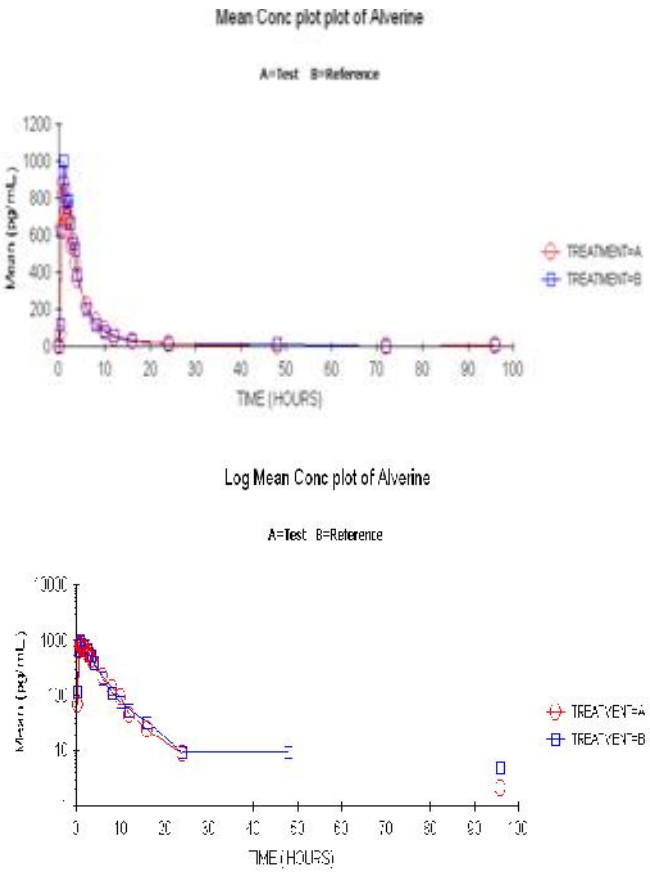


Figure 2: Time vs. Mean Plasma Concentration Graph of Alverine and P-Hydroxy Alverine

4. 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.

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