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An open-label, randomized, crossover bioequivalence study of mesalamine 400 mg tablets in Indian healthy volunteers under fasting conditions

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ABSTRACT

The aim of this study was to examine the bioequivalence of generic MESALAMINE 400 mg tablets (T) manufactured by APL Research Centre Pv.T., Ltd, India and a reference (R) manufactured by SUN Pharma Ltd., India (Mesacol®) in healthy volunteers. Thirty-four subjects (+4 reserve) were randomly selected. Each subject was randomly allocated to one of two treatment sequences (TR or RT). After an overnight fasting period of 10 h, the subjects were administered either the test or the reference formulation as per the randomization schedule. The two treatments were separated by a 7-day washout period. Twenty nine blood samples (4 mL each) were drawn at predose (0 h), 2, 3, 4, 5, 6, 7, 8,9,10,11,12.5,14,16, 18, 20,22, 24, 26, 28, 30, 32, 36, 40, 44, 48, 60, 72, 84 and 96 h post-dose. Concentrations of mesalamine in plasma were analyzed by a validated LC-MS/MS method, with LLOQ 2 ng/mL for Mesalamine and 10 ng/mL for N-Acetyl mesalamine . The pharmacokinetic parameters, C_{max}, $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, T_{max}, $t_{1/2}$, and k_{el}, were calculated using a non-compartment model. The log transformed geometric mean ratios (GMR) of C_{max}, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$ were tested for bioequivalence using a 2-way ANOVA. Thirty-eight subjects were enrolled and completed the study. The mean (test and reference) were: C_{max} (849.41 and 719.92 ng/mL); $AUC_{0\text{-}tlast}$ (8711.94 and 7352.55 ng.h/mL); $AUC_{0\text{-}\infty}$ (8728.24 and 7367.14ng.h/mL); and $t_{1/2}$ (9.40 and 9.41h) for mesalamine and , C_{max} (1170.33 and 1118.30ng/mL); $AUC_{0\text{-}tlast}$ (30021.90 and 26136.86ng.h/mL); $AUC_{0\text{-}\infty}$ (30694.39 and 26429.16ng.h/mL); and $t_{1/2}$ (15.20 and 12.54h) for N-Acetyl mesalamine respectively. The respective median T_{max} were 6 and 7 h for mesalamine and 9 h for N-Acetyl mesalamine respectively. The GMR (90% CI) of C_{max}, $AUC_{0\text{-}tlast}$, and $AUC_{0\text{-}\infty}$ were within the bioequivalence limit of 80-125%. Both formulations were well tolerated. The study demonstrated the bioequivalence of the two formulations of mesalamine 400 mg.

Keywords Mesalamine• N-Acetyl mesalamine • LC/MS/MS. bioequivalence • pharmacokinetics

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INTRODUCTION

Mesalazine (INN, BAN), also known as Mesalamine (USAN) or 5-aminosalicylic acid (5-ASA), is an anti-inflammatory drug used to treat inflammation of the digestive tract (Crohn's disease) and mild to moderate ulcerative colitis. Mesalazine is a bowel-specific aminosalicylate drug that is metabolized in the gut and has its predominant actions there, thereby having fewer systemic side effects. As a derivative of salicylic acid, 5-ASA is also an antioxidant that traps free radicals, which are potentially damaging by-products of metabolism. The major metabolite of mesalamine (5-aminosalicylic acid) is N-acetyl-5- aminosalicylic acid or N-acetyl mesalamine. Its formation is brought about by N-acetyltransferase activity in the liver and intestinal mucosa.The recommended dosage for the induction of remission in adult patients with active, mild to moderate ulcerative colitis is two to four 1.2g tablets to be taken once daily with meal for a total daily dose of 2.4g or 4.8g. Treatment duration in controlled

clinical trials was up to 8 weeks. The total absorption of mesalamine from Lialda 2.4g or 4.8g given once daily for 14 days to healthy volunteers was found to be approximately 21-22% of the administered dose. Mesalamine is approximately 43% bound to plasma proteins at the concentration of 2.5 µg/mL. Elimination of mesalamine is mainly via the renal route following metabolism to N-acetyl-5-aminosalicylic acid (acetylation). However, there is also limited excretion of the parent drug in urine. Of the approximately 21-22% of the dose absorbed, less than 8% of the dose was excreted unchanged in the urine, compared with greater than 13% for N-acetyl-5- aminosalicylic acid.

The apparent terminal half-lives for mesalamine and its major metabolite were, on average of 7-9 hours. About 80% of N-Ac-5-ASA is bound to plasma proteins, whereas 40% of mesalamine is protein bound. The mean elimination half-life was 5 hours for 5-ASA and six hours for N-acetyl-5-ASA following the initial dose. At steady state, the mean elimination half-life was seven hours for both 5-ASA and N-acetyl-5-ASA.Despite its effectiveness in Crohn's disease and mild to moderate ulcerative colitis the use of Mesacol®, The original product is limited as it is very expensive.

Availability of the cheaper locally made generic drug formulations will increase patient accessibility, but it requires bioequivalence data to establish that the generic drug product is therapeutically equivalent to and can be used interchangeably with the original product.

Indian FDA has recommended that a bioequivalence study in human is needed for registration of generic drug products. This study is therefore designed to determine the bioequivalence of enteric coated tablet formulations of mesalamine 400 mg in healthy Indian male volunteers under fasted condition.

Literature survey reveals that, only few methods were developed and validated for quantification of Mesalamine and N-Acetyl mesalamine by using LC-MS (12-16), HPLC(17-31), micellar electrokinetic capillary chromatography (32), differential pulse voltammetry(33), Voltammetric studies(34).Among all, LC-MS (12-16) methods are most accurate. These methods were developed in biological matrices by LC-MS (12-14), Pharmaceutical compounds by LC-MS (15,16). Gu GZ et.al., reported sulphasalazine and its main metabolite sulphapyridine and 5-aminosalicylic acid in human plasma by LC-MS/MS and established pharmacokinetic study.The reported method (12) have some drawbacks in terms of sensitivity, repeatability and matrix effect issues. The main aspect of the present study is to develop and validate simple extraction method,high sensitive,rugged and reproducible bioanalyticle method.At the same time suitable deuterated internal standard to be used for comparision of analyte.Finally, The develoed method could be usefulto determine the pharmacokinetic parameters of two brands of mesalamine 400mg tablets and then to compare these parameters statistically to evaluate the bioequivalence between the two brands i.e Mesacol®(SUN Pharma Ltd.,India) was used as Reference and Test Tablet was used as APL Research Centre Pv.T., Ltd, India.

MATERIALS AND METHODS

Test Product: Test 400mg(APLRC)
Batch No: 006,Expiry 09/2013
Manufacturer: APL Research Centre-Hyderabad,India.

Reference Product: Mesacol® 400 mg tablets
Batch No:7D0912
Manufacturer: SUN Pharma Ltd.,India, Baroda, India.
Each enteric coated tablet of both formulations contained mesalamine equivalent to 400 mg. The clinical study was conducted at Clinical and Pharmacological Research Unit,AXIS Life Sciences -Hyderabad,India., and it was sponsored by APL Research Centre, (A Division of Aurobindo pharma Pv.t. Ltd.,) India.

Study Subjects
The study was carried out in accordance with the current revision of the Declaration of Helsinki concerning medical research in humans. The study protocol was approved by the Ethics Committee.

Thirty eight healthy male subjects, including 4 subjects as standby to replace dropouts, were included in the study. All volunteers gave a written informed consent prior to participation, after they had been informed of the nature and details of the study which they thoroughly understood. Subject screening examinations were performed by a study Vijaya Hospital, Hyderabad, India. All clinical laboratory tests were performed by the ISO 15189 certified laboratories, Department of Pathology, Vijaya Hospital, Hyderabad,India. The daily results of the clinical laboratory tests including the quality control data were verified by its own independent quality assurance personnel before

reporting. Subject inclusion criteria included Indian male, aged between 18-45 years, no consumption of drugs or food supplements for 4 weeks prior to the study, and no participation in any bioavailability or bioequivalence study at least 30 days prior to the present study.

The exclusion criteria included history of hypersensitivity to mesalamine and/or related chemical structure and/or any of the components of the product, history or concurrent symptoms of cardiovascular, liver, kidney, gastrointestinal or hematological disorders and/or any disease that might affect the bioavailability of drug, subjects with malignancy, AIDs, allergy, vital sign abnormalities, or clinically significant abnormal values during pre-study screening, smoker (>10 cigarettes/day) or smoker of < 10 cigarettes/day who could not quit at least 7 days before study and throughout study (including washout period), regular alcohol consumption (more than 1 time/week) or alcohol consumption within 7 days prior to the study, coffee consumption within past 7 days, and drug addiction.

Study Design

The study was conducted as an open label, randomized two-period, two-sequence, single-dose crossover bioequivalence study under fasting condition, and a wash-out period of 7 days. All subjects arrived at the clinical research laboratory, at least 12 h prior to the start of the study. They were housed in an air-conditioned facility and were given a standard dinner, which was finished at least 10 h before dosing in each period of the study. On the day of drug dosing in period 1, volunteers were randomly assigned to one of two treatment sequences (TR (sequence 1) or RT (sequence 2)), as indicated in a pre-printed randomization scheme, which was generated using block randomization with the blocks of size 4 and 6, and the allocation ratio of 1:1. Subjects in sequence 1 received treatment T at the first dosing period and then crossed over to receive treatment R at the second dosing period (after the 7-day washout period). Subjects in sequence 2 received treatments in the order of R and T at the two dosing periods. The subjects were administered the assigned mesalamine formulation with 240 mL of plain drinking water. After the intake of the study formulations, the oral cavity was checked to ensure completion of the administration process. Subjects were required to refrain from lying down during the first 4 h after dosing. No meal was permitted until 4 h after dosing. Drinking water was restricted from 1 h before dosing till 2 h after dosing and *ad libitum* thereafter. Excess water intake (> 100 mL/h) was not permitted. Lunch, snacks, and dinner were served as per the scheduled time. All subjects abstained from any xanthine-containing food or beverages for at least 72 h and alcoholic products for at least 7 days prior to formulation administration and throughout the sampling schedule during each period. They were informed not to take any drug at least 30 days prior to the study, especially digoxin. Subjects abstained from the use of tobacco- or nicotine-containing products for 7 days prior to dosing and during confinement in the clinical research laboratory. No concomitant medication was permitted during the study period.

Blood Sampling

Blood samples (4 mL) were collected from an antecubital vein by an indwelling venous catheter and transferred into coded 5-mL polypropylene centrifuge tube containing K_2EDTA as an anticoagulant. The tubes were covered with aluminum foil to protect samples from exposure to light. Blood samples were obtained at pre-dosing (0 h), 2, 3, 4, 5, 6, 7, 8,9,10,11,12.5,14,16, 18, 20,22, 24, 26, 28, 30, 32, 36, 40, 44, 48, 60, 72, 84 and 96 h after dosing. Blood samples were centrifuged at 4000 g at approximately 4ºC for 10 min, within 15 min of the sample collection. The plasma was separated, transferred into 2 (1 and 1.5-mL) ria vial tubes and stored frozen at -80ºC until shipment on dry ice to Bioanalytical unit. During the sample collection, all subjects were under medical supervision. Vital signs were examined at scheduled time as described in the protocol.

Analytical procedure

Instrumentation and chromatographic conditions

A validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was used for determination of mesalamine/N-Acetyl mesalamine concentration in human plasma. Equipment used was a HPLC (1200 Series Agilent Technologies, Germany). MS/MS (ABI-SCIEX,Toronto, Canada) system. Column type used was a Thermo, HyPURITY C18, 150 x 4.6 mm, 5 µ, and the mobile phase used was 10mM Ammonium acetate:methanol (85:15 v/v) delivered at the flow rate of 0.6 mL/min, Injection volume was 10µL, Column temperature was 40°c with a isocretic elution system.

Calibration and Qualitycontrol Standards

Calibration curve of mesalamine/N-Acetyl mesalamine was prepared within the concentration range of 2-1500 ng/mL (2.00, 4.00, 10.00, 75.00, 150.00, 300.00, 600.00, 900.00,1200.00,1500.00 ng/mL) for mesalamine and 10.00-2000.00 ng/mL(10.00, 20.00, 50.00, 100.00, 200.00, 400.00, 800.00, 1200.00,1600.00,2000.00 ng/mL) for N-Acetyl mesalamine (r^2 > 0.998). The calibration curve consisted of one replicate of 10 non-zero standards. The concentrations of quality control (QC) samples were 2.00, 6.00, 450.00 and 1050.00 ng/mL for mesalamine and 10.00, 30.00, 600.00 and 1400.00 ng/mL for N-Acetyl mesalamine as low (LQC), middle (MQC) and high (HQC) concentrations, respectively.

Detection
Electro spray Positive mode was used to select the mass transitions as *m*/*z* 207.8 / 163.9, 194.0 / 149.9, and *m*/z 197.0 / 153.0, for quantification of mesalamine, N-Acetyl mesalamine, and N-Acetyl mesalamine-D3, respectively.

Sample preparation
Liquid-liquid extraction was used for extraction of drug and IS. For this purpose, An internal standard working solution (150.00 ng/mL of N-Acetyl mesalamine -D3) of 100 µL was added to 200µL aliquot of plasma sample (respective concentration) into riavial. To this, 25 µL of derivatisation solution(10%propionic anhydride in methanol) was added and vortexed briefly.After that, 100 µL of 0.5% formic acid was added into each tube and vortexed briefly .Then, 3 mL of methyl t-butyl ether was added and vortexed for 10 minutes. Samples were then centrifuged for 5 minutes at 4000 rpm at 20°C. Supernatant from each sample was transferred into ria vial and evaporated to dryness. This was followed by reconstitute the each sample with 800 µL of reconstitution solution(10 mM ammonium acetate : methanol 85:15 v/v). and vortex briefly. From this, 5 µL of sample was injected into the LC–MS/MS system through the autosampler. The lower limit of quantification was 2.00 ng/mL for mesalamine and 10.00 ng/mL for N-Acetyl mesalamine, respectively with *S/N* > 5.

Tolerability assessments
Throughout the study, subjects were monitored by a clinician, a clinical pharmacist, and 4 nurses. Tolerability was determined by monitoring of vital signs (sitting blood pressure, heart rate, and axillary body temperature), and physical examinations at baseline and at the end of each study period. Subject interviews were also conducted regarding the potential occurrence of adverse events (AEs) at each mesalamine study period.

Serious AEs (SAEs) were considered to be when the subject outcome was death, life threatening, requiring hospitalization, leading to disability, or requiring medical intervention to prevent permanent impairment or damage. All AEs and SAEs were recorded in the source data record and on the case-report form, and their relationship to the study drug was determined by the study physician who was blinded to the randomization schedule.

Pharmacokinetic Analyses
The primary pharmacokinetic parameters compared between treatments were maximum plasma concentration (C_{max}), the area under the concentration time curve (AUC) from time zero to 96 h or to the last quantifiable time point after dosing ($AUC_{0\text{-}tlast}$), and the AUC from time zero to infinity ($AUC_{0\text{-}\infty}$). Other pharmacokinetic parameters examined were time to C_{max} (T_{max}), apparent terminal half-life ($t_{1/2}$) and elimination rate constant (k_{el}). All pharmacokinetic parameters were determined by non-compartmental methods. Values below the quantification limit (< 2.00 ng/mL for mesalamine and 10.00 ng/mL for N-Acetyl mesalamine) were set to zero for calculation purposes. The C_{max} and the T_{max} were obtained by visual inspection of individual plasma concentration-time profiles. The $AUC_{0\text{-}tlast}$ was calculated using linear-log trapezoidal approach. The k_{el} was estimated by a slope of linear regression line of log-transformed data. The $t_{1/2}$ was obtained by dividing 0.693 by k_{el}. The $AUC_{0\text{-}\infty}$ was calculated from the sum of $AUC_{0\text{-}tlast}$ and C_{last}/k_{el}, where C_{last} is the last measurable concentration of mesalamine/N-Acetyl mesalamine in plasma. The pharmacokinetic parameters were calculated using WinNonlin® software version 5.3 (Pharsight®, North Carolina, USA).

The test and the reference formulations were considered to be equivalent if the calculated 90% confidence interval (CI) of geometric means for the log transformed ratios (test/reference) of the C_{max}, $AUC_{0\text{-}tlast}$, and $AUC_{0\text{-}\infty}$ were within the bioequivalence criteria range of 80.00-125.00 as established by the US FDA [35-37].

Statistical Analysis
WinNonlin® software version 5.3 (Pharsight®, North Carolina, USA) program was used for statistical evaluation of the pharmacokinetic parameters. The pharmacokinetic parameters were statistically analyzed by analysis of variance (ANOVA) test, and Schuermann's two one-sided *t*-test. Descriptive analyses including mean, standard deviation (SD), median, and range were performed for variables such as age, weight, height, and BMI. Pharmacokinetic parameters C_{max}, $AUC_{0\text{-}tlast}$, and $AUC_{0\text{-}\infty}$ were analyzed using a two-way ANOVA accounting for sequence, subjects, period and treatments, and the statistical significance was evaluated at 95% confidence level ($p < 0.05$). A non-parametric test, Wilcoxon signed rank test, was performed on T_{max} and considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The average accuracy 93.31-113.09% for mesalamine and 95.9 – 105.00 for N-Acetyl mesalamine respectively. The mean extraction recovery was 79.39% for mesalamine and 23.97 for N-Acetyl mesalamine respectively.

Thirty eight healthy male subjects, including 4 subjects as standby to replace dropouts, were included and completed the study. All 34 subjects were included in the pharmacokinetic and statistical analyses.

The mean±SD of age, weight, height, and BMI were 21.4 ± 1.9 years (range 18-25), 61.7±7.1 kg (range 50-76), 1.71±0.05 m (range 1.63-1.82), and 20.9±1.9 kg/m^2 (range 18.3-24.8). The mean plasma concentrations of mesalamine & N-Acetyl mesalamine versus time for the test and reference formulations were similar after administration of the 400 mg mesalamine enteric-coated tablet (Figure.1). Both formulations were well tolerated, No deaths or serious AEs occurred during the conduct of this study. The only AE reported was drowsiness in 1 (2.7%) of 38 subjects receiving the test formulation. The AE was assessed to be mild in intensity and was not related to the study drug.

The average pharmacokinetic parameters of 400 mg mesalamine enteric -coated tablets for test and reference products are presented in Table 1.

It was observed that absorption of both formulations were similar with the median (range) of the time to reach the T_{max} for the test formulation and for the reference formulation.

Table 2 presents the geometric mean ratios between the test and reference formulations (T/R) of C_{max}, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$ for log-transformed data, as well as the intrasubject variations. The 90% CI ratios of geometric mean C_{max}, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$ were within the range of 80-125%, which meet the regulatory criteria for bioequivalence. The study had sufficient power to detect bioequivalence between the two formulations i.e., 95% for C_{max} and 99% for both $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$. The T_{max} of the test and the reference products comparing by using Wilcoxon signed rank test revealed a significant difference (p = 0.0421).

The ANOVA results revealed that period, sequence and treatment had no statistically significant effects on C_{max}, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$. Since the sequence or carry-over effect was not significant, the ANOVA test was valid. The statistically significant subject within sequence effect on C_{max}, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$ were observed that are usually seen in small sample size study as in crossed over phase I and bioequivalence studies.

Bioequivalence between the 400-mg enteric-coated tablet formulations of mesalamine under fasting condition was demonstrated by the 90% CI of the geometric mean ratios of C_{max}, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$ lying within the acceptable criteria of 80-125%. The T_{max} of the test formulation was slightly shorter than that of the reference product for mesalamine and higher than that of reference product for N-Acetyl mesalamine. The test and reference formulations had very similar $t_{1/2}$ at approximately 9-9.5 h for mesalamine and 12-15.5 h for N-Acetyl mesalamine. Period, sequence and treatment had no significant effects on C_{max}, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$.

A bioequivalence study of 400 mg mesalamine tablets conducted among 34 healthy Indian males reported very similar C_{max}, $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and T_{max}, and $t_{1/2}$ of mesalamine and N-Acetyl mesalamine.

The intrasubject CVs obtained from the present study were quite smaller than the previously reported values, which were assumed in the sample size calculation. The tests, therefore, had 95-99% power to detect bioequivalence.
mesalamine 400 mg enteric-coated tablet was found to be well tolerated in the present study. This finding was consistent with previous reports where no adverse event was observed in subjects given mesalamine.

Tab. 1. Mean pharmacokinetic parameters for Mesalamine and N-Acetyl Mesalamine of the test and the reference formulations

Parameter	Mesalamine		N-Acetyl Mesalamine	
	Test mean	Reference mean	Test mean	Reference mean
C_{max} (ng/mL)	849.41	719.92	1170.33	1118.30
$AUC_{0\text{-}tlast}$ (ng.h/mL)	8711.94	7352.55	30021.90	26136.86
$AUC_{0\text{-}\infty}$ (ng.h/mL)	8728.24	7367.14	30694.39	26429.16
T_{max} (h)	6	7	9	9
k_{el} (h^{-1})	0.07373	0.07369	0.04560	0.05526
$t_{1/2}$ (h)	9.40	9.41	15.20	12.54

Tab. 2. Ratios (Test/Reference) of C_{max}, $AUC_{0\text{-}tlast}$, and $AUC_{0\text{-}\infty}$ for Mesalamine and N-Acetyl Mesalamine following the administration of 400 mg mesalamine film-coated tablet formulations

Test/Reference	Mesalamine	N-Acetyl Mesalamine
C_{max}	117.99	104.65
$AUC_{0\text{-}tlast}$	118.49	114.86
$AUC_{0\text{-}\infty}$	118.48	118.48

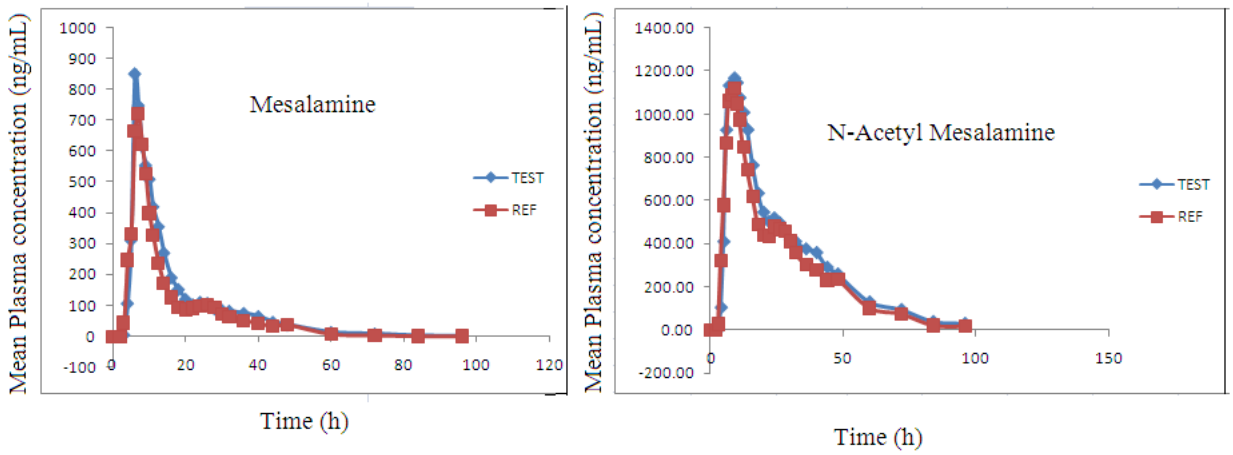

Fig. 1. Mean plasma concentration versus time curves for mesalamine and N-acetyl mesalamine of the test and reference of 400 mg mesalamine enteric-coated tablets (n = 34).

CONCLUSION

This study has demonstrated the bioequivalence of the 400 mg mesalamine enteric-coated tablet formulation manufactured by APL Research Pvt., Ltd., India and the reference product Mesacol® manufactured by SUN Pharma Lt.d.India. It thus can be concluded that the two formulations can be used interchangeably.

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REFERENCES

[1] Pharmacotherapy. **1994** Jul-Aug;14(4):385-98. Chemistry, pharmacology, pharmacokinetics, and clinical applications of mesalamine for the treatment of inflammatory bowel disease. Small RE, Schraa CC. Medical College of Virginia, Virginia Commonwealth University, Richmond 23298-0533.PMID:7937276
[2] Aliment Pharmacol Ther. **1994** Oct;8(5):535-40. Bioavailability of single and multiple doses of a new oral formulation of 5-ASA in patients with inflammatory bowel disease and healthy volunteers. Gionchetti P, Campieri M, Belluzzi A, Boschi S, Brignola C, Miglioli M, Barbara L. Clinica Medica e Gastroenterologia, Policlinico S. Orsola, Università di Bologna, Italy. PMID:7865646 Adv Ther. **2009** Aug;26(8):749-61.
[3] Pharmacokinetics and safety of single and multiple doses of Asacol tablets in Japanese healthy volunteers. Ito H, Furuta S, Sasaki H, Yoshida T, Takano Y, Hibi T. PMID:19730804
[4] Rasmussen SN, Bondesen S, Hvidberg EF, Hansen SH, Binder V, Halskov S, Flachs H. *Gastroenterology*. **1982** Nov;83(5):1062-70.PMID:7117789
[5] Kunes M, Kvetina J, Kholova D, Bures J, Tlaskalova-Hogenova H, Pavlik M. *Neuro Endocrinol Lett*. 2011;32 Suppl 1:46-52. PMID:22167206
[6] Aliment Pharmacol Ther. **2004** May 15;19(10):1089-98. Comparative pharmacokinetics of equimolar doses of 5-aminosalicylate administered as oral mesalamine (Asacol) and balsalazide: a randomized, single-dose, rossover study in healthy volunteers. Sandborn WJ, Hanauer SB, Buch A. Source Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. sandborn.william@mayo.edu PMID:15142198
[7] Aliment Pharmacol Ther. **1994** Jun;8(3):289-94. Comparative bioavailability of 5-aminosalicylic acid from a controlled release preparation and an azo-bond preparation. Christensen LA, Fallingborg J, Jacobsen BA, Abildgaard K, Rasmussen HH, Hansen SH, Rasmussen SN. Source Department of Medical Gastroenterology, Aalborg Hospital, Denmark. PMID:7918923
[8] Norlander B, Gotthard R, Ström M. *Aliment Pharmacol Ther*. **1990** Oct;4(5):497-505. PMID:2129637
[9] Vree TB, Dammers E, Exler PS, Maes RA. J *Pharm Pharmacol*. **2000** Jun;52(6):645-52. PMID:10875540
[10] Kvetina J, Svoboda Z, Nobilis M, Pastera J, Anzenbacher P. *Gen Physiol Biophys*. **1999** Oct;18 Spec No:80-5. PMID:10703724
[11] Corey AE, Rose GM, Conklin JD. *J Int Med Res*. **1990** Nov-Dec;18(6):441-53. PMID:1981363
[12] Gu GZ, Xia HM, Pang ZQ, Liu ZY, Jiang XG, Chen J. *J Chromatogr B Analyt Technol Biomed Life Sci*. **2011** Feb 15;879(5-6):449-56. Epub 2011 Jan 4. PMID:21251889

[13] Pastorini E, Locatelli M, Simoni P, Roda G, Roda E, Roda A. *J Chromatogr B Analyt Technol Biomed Life Sci.* **2008** Sep 1;872(1-2):99-106. Epub 2008 Jul 29. PMID:18691952
[14] Nobilis M, Vybíralová Z, Sládková K, Lísa M, Holcapek M, Kvetina J. *J Chromatogr A*. 2006 Jun 30;1119(1-2):299-308. Epub **2006** Feb 8. *Erratum in: J Chromatogr A.* **2006** Dec 15;1136(2):248. PMID:16466733
[15] Novak P, Tepes P, Fistrić I, Bratos I, Gabelica V. *J Pharm Biomed Anal*. 2006 Mar 18;40(5):1268-72. Epub **2005** Oct 25. PMID:16253464
[16] Orioli M, Marinello C, Cozzi R, Piodi LP, Carini M. *J Pharm Biomed Anal.* **2004** Sep 3;35(5):1263-72. PMID:15336371
[17] Palumbo G, Bacchi S, Primavera L, Palumbo P, Carlucci G. *Biomed Chromatogr*. **2005** Jun;19(5):350-4. PMID:15586374
[18] Hansen SH. *J Chromatogr*. **1981** Dec 11;226(2):504-9. No abstract available. PMID:6274891
[19] Simplified high-performance liquid chromatographic method for 5-aminosalicylic acid in plasma and urine. Fischer C, Maier K, Klotz U. J Chromatogr. **1981** Oct 9;225(2):498-503. No abstract available. PMID:6117565
[20] Bystrowska B, Nowak J, Brandys J. *J Pharm Biomed Anal.* **2000** Mar;22(2):341-7. PMID:10719917
[21] Hussain FN, Ajjan RA, Moustafa M, Anderson JC, Riley SA. *J Chromatogr B Biomed Sci Appl*. **1998** Sep 25;716(1-2):257-66. PMID:9824239
[22] Palumbo G, Carlucci G, Mazzeo P, Frieri G, Pimpo MT, Fanini D. *J Pharm Biomed Anal*. **1995** Dec;14(1-2):175-80. PMID:8833980
[23] De Vos M, Verdievel H, Schoonjans R, Beke R, De Weerdt GA, Barbier F. *J Chromatogr*. **1991** Mar 8;564(1):296-302. PMID:1860925
[24] Nagy E, Csipo I, Degrell I, Szabo G. *J Chromatogr*. **1988** Mar 4;425(1):214-9. No abstract available. PMID:3360872
[25] Lee EJ, Ang SB. *J Chromatogr*. **1987** Jan 23;413:300-4. No abstract available. PMID:2881937
[26] Simultaneous determination of 5-aminosalicylic acid and 5-acetylaminosalicylic acid by high-performance liquid chromatography. Brendel E, Meineke I, Witsch D, Zschunke M. J Chromatogr. **1987** Jan 9;385:299-304. PMID:3558586
[27] Cendrowska I, Drewnowska M, Grzeszkiewicz A, Butkiewicz K. *J Chromatogr*. **1990** Jun 15;509(1):195-9. No abstract available. PMID:2398126
[28] Kersten BS, Catalano T, Rozenman Y. *J Chromatogr*. **1991** Dec 27;588(1-2):187-93. PMID:1818083
[29] Liu H, Zhang S, Yuan Z. Se Pu. **1998** Jul;16(4):321-3. *Chinese*. PMID:11367763
[30] Larsen J, Staerk D, Cornett C, Hansen SH, Jaroszewski JW. *J Pharm Biomed Anal*. **2009** Apr 5;49(3):839-42. Epub 2008 Dec 27. PMID:19185440
[31] Elmasry MS, Blagbrough IS, Rowan MG, Saleh HM, Kheir AA, Rogers PJ. *J Pharm Biomed Anal*. **2011** Mar 25;54(4):646-52. Epub 2010 Oct 14. PMID:21106317
[32] Allen L, Weinberger J, Weinberger R. *J Chromatogr* A. **2004** Oct 22;1053(1-2):217-26. PMID:15543986
[33] Nigović B, Simunić B. *J Pharm Biomed Anal*. **2003** Feb 5;31(1):169-74. PMID:12560061
[34] Nigović B, Mandić Z, Simunić B, Fistrić I. *J Pharm Biomed Anal*. **2001** Dec;26(5-6):987-94. PMID:11600311
[35] Guidance for industry: bioanalytical method validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), May **2001**.
[36] Guidance for industry Food- effect bio availability and Fed Bio equivalence studies. U.S Department of Health and Human services Food and Drug Administration Centre for Drug Evaluation and research (CDER) December **2002**.
[37] Guidance for industry Bio availability and Fed Bio equivalence Studies for Orally Administered Drug Products-General considerations U.S.Department of Health and Human services Food and Drug Administration Centre for Drug Evaluation and research (CDER) March **2003**.