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Bioequivalence Study of a Newly Developed Ibuprofen Arginine 600 Mg Tablet Formulation versus Ibuprofen Arginine 600 Mg Granules in Healthy Volunteers

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Abstract

Background and Objectives: A novel ibuprofen L-arginine film-coated tablet formulation has recently been developed. The study objective was to assess bioequivalence of the newly developed formulation and a marketed granules for oral solution (reference), in terms of rate and extent of absorption of S(+)-ibuprofen, R(-)-ibuprofen and their sum.

Methods: In this randomized, two-way cross-over study, 24 healthy men and women received a single 600 mg oral dose of each product in 2 subsequent periods, with a washout of at least 3 days. Plasma S(+)- and R(-)-ibuprofen concentrations were determined with a chiral bioanalytical method up to 12 h post-dose, and total ibuprofen was calculated at each time-point as the sum of the two enantiomers' concentrations. Pharmacokinetic parameters were determined. The primary study endpoints for bioequivalence evaluation were plasma peak concentration (C_{max}) and area under the curve up to the last time-point (AUC_{0-t}).

Results: The bioequivalence test was fully satisfied for the two enantiomers and their sum, with the confidence intervals for C_{max} and AUC_{0-t} geometric means test/reference ratio within the 80.00-125.00% acceptance limits. Time to reach C_{max} (T_{max}) for both enantiomers occurred at two adjacent time-points (i.e. 0.5 h and 0.33 h) for the two products. Half-life of S(+)-, R(-)- and total ibuprofen was very similar for the two formulations.

Conclusions: The two products can be claimed bioequivalent in terms of both rate and extent of absorption of S(+)-ibuprofen, R(-)-ibuprofen and their sum, indicating that the novel ibuprofen L-arginine 600 mg tablet could be an effective alternative to the marketed ibuprofen L-arginine 600 mg sachet, when a rapid onset of action is needed.

Keywords: Ibuprofen L-Arginine; Pharmacokinetics; Bioequivalence

List of Abbreviations: ANOVA: Analysis of Variance; AUC_{0-t}: Area under the concentration-time curve from administration to the last observed concentration time t; $AUC_{0-\infty}$: Area under the concentration-time curve extrapolated to infinity; BMI: Body Mass Index; C: Celsius; CI: Confidence Interval; C_{max}: Maximum plasma concentration; ECG: electrocardiogram; h: hour(s); IU: International Units; LC: Liquid Chromatography; LC-MS/MS: Liquid Chromatography Mass Spectrometry; LD: Lowest diluted; LLOQ: Lower Limit Of Quantification; mg: milligrams; min: minute(s); mL: millilitres; ng: nanograms; PE: Point Estimate, i.e. Geometric Mean Ratio; PK: Pharmacokinetics; QC: Quality Control; SD: Standard Deviation; $t_{1/2}$: Half-life; T_{max} Time to achieve C_{max}; ULOQ: Upper Limit Of Quantification

Introduction

Ibuprofen, a chiral non-steroidal anti-inflammatory drug (NSAID), is a non-selective cyclooxigenase-1 and -2 (COX-1 and COX-2) inhibitor, used worldwide for its analgesic and anti-inflammatory effects [1].

Ibuprofen preparations contain equal amounts of S(+)-ibuprofen and R(-)-ibuprofen, with S(+)-ibuprofen (eutomer) possessing the majority of the beneficial anti-inflammatory activity [2]. After administration of the racemate to humans, a percentage of the R(-) enantiomer (distomer) is converted to S(+)-ibuprofen, whereas the opposite inversion is unlikely to occur or is negligible [3,4].

Ibuprofen is usually marketed as free acid, but different ibuprofen salts, esters and other derivatives are also used, among which ibuprofen L-arginine [5-8]. Since L-arginine increases solubility of the active ingredient without affecting its chemical stability, ibuprofen L-arginine has the advantage of delivering oral racemic ibuprofen in a more efficient way than the standard ibuprofen formulations with an associated quicker onset of action [8], particularly favourable in those conditions in which a very rapid analgesic effect is required [8-12].

When orally administered, ibuprofen L-arginine reaches maximum plasma levels at approximately 15-30 min post-dose, as compared to approximately 1-3 h for ibuprofen free base. In addition, ibuprofen L-arginine is characterised by higher concentration peaks (C_{max}) than ibuprofen, with, however, similar extent of absorption and terminal half-life (approximately 1.8-2 h), confirming that its administration increases ibuprofen absorption rate without affecting its bioavailability [8].

The absolute bioavailability of ibuprofen after oral administration is about 100% and 80% for the S(+)- and R(-) enantiomer, respectively [13]. Ibuprofen undergoes an extensive hepatic metabolism, with less than 1% of the dose excreted unchanged by the kidney. More than 90% is excreted in urine as pharmacologically inactive metabolites, the remaining is possibly excreted in the bile and eliminated via faeces. Excretion is essentially complete within 24 hours after the last dose [14].

Bioequivalence studies were performed comparing different ibuprofen formulations, using either chiral or achiral assays [13, 15-28]. In particular, in a randomised, single dose, 3-way crossover study conducted in 36 healthy male and female volunteers, ibuprofen acid 200 mg orodispersible tablets and ibuprofen acid 200 mg tablets were bioequivalent for both the rate and extent of ibuprofen absorption. Post-hoc analysis on the same samples for ibuprofen S(+) and R(-) enantiomers mirrored the findings for total ibuprofen [24]. Notably, based on a stereospecific method and following single dose administration of two ibuprofen 600-mg film coated tablets to healthy subjects, Matji et al. [25] concluded bioequivalence between the two formulations with respect to both rate and extent of ibuprofen absorption for the two enantiomers separately and for the sum of them. On the other hand, Torrado et al. [20] concluded that after single dose administration of two ibuprofen 2% oral suspension formulations, Application of the chiral method showed differences in rate and extent of ibuprofen absorption that resulted in non-bioequivalence of the individual enantiomers, whereas the achiral method and the sum of the concentrations of the two enantiomers gave a similar outcome, i.e. bioequivalence between the two formulations with respect to both rate and extent of exposure.

Several formulations containing ibuprofen L-arginine at different strengths are presently on the market in many countries worldwide. To our knowledge no bioequivalence studies for ibuprofen L-arginine formulations have been published to date. The present randomised, cross-over, two-stage study was conducted to evaluate the bioequivalence of a new ibuprofen L-arginine 600 mg film-coated tablet formulation (test product) versus the marketed ibuprofen L-arginine 600 mg sachet (Espidifen®; reference product; [29]) in terms of rate (C_{max}) and extent (AUC_{0-t}) of absorption of both S(+)-ibuprofen and R(-)-ibuprofen after single dose administration to healthy men and women. Bioanalysis was performed based on a chiral LC-MS/MS method. In addition, the bioequivalence of total ibuprofen evaluated on the pharmacokinetic parameters obtained from the sum of S(+)- and R(-)-ibuprofen concentrations was assessed to simulate the results that would have been obtained with an achiral method.

Methods

Study Design and Procedures

The study was open-label, randomized, two-way two-stage, cross-over and was designed according to the EMA Guidance on investigation of bioequivalence [30] and the EMA Ibuprofen oral use immediate release formulations 200-800 mg product-specific bioequivalence guidance [31]. The study (ISRCTN registration No. ISRCTN46595731) was approved by the Canton Ticino Ethics Committee, Switzerland, and the Swiss Federal Health Authority, and was performed in accordance with the Helsinki Declaration and Good Clinical Practice at CROSS Research S.A., Clinical Phase I Unit, Arzo (Switzerland), between September and October 2022. All subjects received a detailed study description and gave their written informed consent before enrolment.

The test product was Ibuprofen arginine 600 mg film-coated tablet (Zambon S.p.A., Italy) and the reference product was Espidifen® 600 mg granules for oral solution (Zambon S.A.U., Spain).

Subjects received a single 600 mg oral dose of the test and reference products under fasting conditions in two periods, according to a randomised 2-way cross-over design, with a wash-out interval of at least 3 days between the two administrations. The randomization list was computer-generated using SAS® version 9.3 PLAN procedure.

For the administration of the test product, one film-coated tablet was swallowed by the subjects with 150 mL of still mineral water. Before administration of the reference product, the entire content of one sachet of ibuprofen L-arginine 600 mg granules for oral solution was completely dissolved in 100 mL of still mineral water. The solution was taken by the subject, the glass was rinsed with a further 50 mL of still mineral water and the rinse drunk.

Subjects

Healthy men and women aged 18-55 years and with a body mass index of 18.5-30.0 kg/m^2 were enrolled.

All subjects were in good physical health, as assessed by a full physical examination, electrocardiograms (ECG), vital signs and clinical laboratory assays. Female participants were either post-menopausal or used reliable contraceptives. No subjects were on abnormal diets or had a history of drug, alcohol, caffeine or tobacco abuse. Exclusion criteria included: history or presence of any disease that could interfere with the study aims or put the subject to any safety risk; history of hypersensitivity or allergic reactions to the active principle and/or formulations' ingredients. No medications were allowed for 2 weeks before the study. Subjects were not enrolled if they had participated in other clinical trials or donated blood in the past 3 months.

Blood Sampling

Blood samples for the determination of ibuprofen S(+) and R(-) enantiomers' concentrations were collected at pre-dose (0), 5, 10, 20, 30, 40, 50 min and 1, 1.5, 2, 3, 4, 6, 9, 12 h post-dose using an indwelling catheter with switch valve. After each sampling, the cannula was rinsed with about 1 mL of sterile saline solution containing 20 I.U./mL Na-heparin. The first 2 mL of blood were discarded at each collection time to avoid contamination of the sample with heparin. The remaining 8 mL were collected from the catheter and transferred with a syringe into EDTA K_2 tubes. The samples were centrifuged at 1900g (± 38g) for at least 10 min at 4 °C (± 4 °C) to obtain plasma. Plasma samples were transferred into pre-labelled polypropylene tubes and stored frozen at ≤-20 °C until analyses.

Bioanalytical Assay

Plasma S(+)-ibuprofen and R(-)-ibuprofen concentrations were determined at Anapharm Europe, S.L.U., Spain, using a chiral LC-MS/MS method, developed and validated according to EMA guidance document requirements [32]. The chiral assay was chosen considering that work conducted by other authors [20,33] demonstrated that it enhances sensitivity to possible differences in bioequivalence results between enantiomers, making it possible to consider pharmacokinetic response separately for S(+)- and R(-)-ibuprofen. The method had a lower quantification limit of 199.92 ng/mL and 199.84 ng/mL for S(+)-ibuprofen and R(-)-ibuprofen, respectively, and adhered to the regulatory requirements for selectivity, sensitivity, precision, accuracy and stability. Calibration standards [199.92 (LLOQ), 399.84, 999.60, 7996.80, 15993.60, 23990.40, 31987.20 and 39984.00 (ULOQ) ng/mL for S(+)-ibuprofen; 199.84 (LLOQ), 399.68, 999.20, 7993.60, 15987.20, 23980.80, 31974.40 and 39968.00 (ULOQ) ng/mL for R(-)-ibuprofen] and low, medium, high and lowest diluted (LD) quality control (QC) samples [599.72, 19991.60, 29987.20 and 49978.50 ng/mL (LD) for S(+)-ibuprofen; 599.60, 19987.20, 29980.80 and 49968.00 ng/mL (LD) for R(-)-ibuprofen] were prepared in blank human EDTA K_2 plasma, as separate batches, using the reference standards S(+)-ibuprofen and R(-)-ibuprofen (Toronto Research Chemical). Aliquots were stored at ≤ -20°C and working solutions were prepared freshly for the analysis. Internal standard was racemic ibuprofen-d3 (Toronto Research Chemicals) and was separated to the two enantiomers during chiral separation. Before analysis, study samples, internal standard, calibrators and QC samples were processed by liquid-liquid extraction with tert-butyl methyl ether/n-Hexane (90/10). Processed samples were stored in polypropylene tubes at room temperature until analysis. The modular LC/MS system consisted of an HTC (CTC-PAL) Autosampler, a 1200 Series (Agilent) High Pressure LC Pump with a chiral column and isocratic elution with Methanol/water (90/10)/ 0.01% formic acid (flow rate 1 mL/min), and an API 4000 (Sciex) Mass Spectrometer. Data acquisition was performed using Analyst software, version 1.6.2 (Sciex). Following peak area integration, regression was also performed using Analyst. Study sample concentrations were obtained by interpolation from the calculated calibration curves.

Pharmacokinetic Parameters

The following pharmacokinetic parameters were measured and/or calculated with the validated software Phoenix WinNonLin® 6.3, Certara, Inc., using a Non-Compartmental analysis and the linear trapezoidal rule for plasma ibuprofen S(+)-enantiomer, for plasma ibuprofen R(-)-enantiomer and total ibuprofen, calculated as the sum of the plasma concentrations of the two enantiomers: C_{max} (maximum plasma concentration), T_{max} (time to achieve C_{max}), AUC_{0-t} (area under concentration-time curve from time 0 to the last concentration time t), $AUC_{0-\infty}$ (AUC extrapolated to infinity) and half-life ($t_{½}$).

The primary study outcome measures for the evaluation of bioequivalence were C_{max} and AUC_{0-t}.

Safety

Safety of the investigational products was assessed by physical examination, ECG, routine laboratory tests and vital sign check, performed at screening (before study enrolment) and final visit. Adverse events were recorded throughout the study.

Sample Size

The study was conducted according to a two-stage design. Twenty-four (24) men and women were enrolled in study stage 1. For this first stage, no formal sample size calculation was performed as planned, and no drop out replacement was foreseen. After the end of stage 1, pharmacokinetic parameters were calculated, and the ad interim bioequivalence test was performed on ibuprofen S(+)-enantiomer C_{max} and AUC_{0-t}, as planned according to the two-stage design. To safeguard the overall type I error, the one-sided α-level of the bioequivalence test was set to 0.0294 according to the Pocock spending function. Since the bioequivalence was proven with the results of the first stage, the primary objective of the study was satisfied. According to the study protocol and the EMA guideline on the investigation of bioequivalence [16], no further sample size calculation was necessary, and the second study stage did not take place.

Statistical Analyses

Study data were summarized by descriptive statistics. The statistical analyses were performed using SAS® version 9.3 (TS1M1) and Phoenix WinNonLin® 6.3.

According to the current EMA guideline on the investigation of bioequivalence [30], log-transformed AUC_{0-t} and C_{max} for ibuprofen S(+) enantiomer, ibuprofen R(-) enantiomer and total ibuprofen were analysed using analysis of variance (ANOVA) with treatment, period, sequence and subject as fixed effects. Acceptance criterion for bioequivalence was a two-sided 94.12% confidence interval of the test/reference ratio of the least-square geometric means of the pharmacokinetic parameters under consideration within the 80.00-125.00% range, according to the Pocock α spending function for the two-stage design. T_{max} was analysed using the non-parametric Wilcoxon signed-rank test.

Results

Subjects

Twenty-four (24) male and female healthy volunteers met all inclusion criteria, were randomised in the study, completed the study per protocol and were included in the safety and in the pharmacokinetic analyses. Demographic characteristics of the randomised subjects are presented in Table 1.

Table 1: Demographic data of study subjects. N=24

Demographic data	Safety and Pharmacokinetic data sets
Gender–n(%) Females Males	12 (50.0%) 12 (50.0%)
Ethnicity–n(%) White	24 (100.0%)
Age (Years) Mean ± SD Min, Max	42.0±9.6 21, 54
Body weight (kg) Mean ± SD Min, Max	68.34±12.39 51.1, 95.7
Height (cm) Mean ± SD Min, Max	167.8±9.2 151.0, 184.0
BMI (kg/m2) Mean ± SD Min, Max	24.09±2.65 18.7, 29.0

Abbreviations: BMI: Body mass Index; SD: standard deviation

Pharmacokinetics

The mean ± standard deviation (SD) plasma concentration-time profiles obtained after single oral dose of the test and reference products are shown in Figure 1 for S(+)-ibuprofen, in Figure 2 for R(-)-ibuprofen and in Figure 3 for total ibuprofen.

The main plasma pharmacokinetic parameters data (mean±SD) and the results of their statistical comparisons are presented in Table 2, Table 3 and Table 4 for S(+)-ibuprofen, R(-)-ibuprofen and their sum, respectively.

C_{max} and AUC_{0-t} of S(+)-ibuprofen and C_{max} of R(-)-ibuprofen were, on average, similar following administration of the two products, and their ratio of geometric means (PE%) close to 100%. In addition, R(-)-ibuprofen AUC_{0-t} values were only slightly higher for the test with respect to the reference formulation, as indicated by a PE% of approximately 108%. Consequently, the 94.12% confidence intervals (CIs) of the PE% for S(+)-ibuprofen and R(-)-ibuprofen plasma C_{max} and AUC_{0-t} were within the acceptance limits of 80.00 to 125.00%, demonstrating that the test and reference formulations are bioequivalent in terms of the two enantiomers rate and extent of exposure.

For total ibuprofen, PE% were in between those of the two enantiomers, and the 94.12% CIs of C_{max} and AUC_{0-t} PE% fell within the pre-specified 80.00-125.00% limits.

The time at which C_{max} was achieved (T_{max}) was only slightly later with the test than with the reference product (0.5 vs. 0.33 h) both for S(+)- and for R(-)-ibuprofen. This difference was, however, statistically significant for both enantiomers ($p<0.0001$).

Mean half-life ($t_{1/2}$) was also very similar for the two products corresponding to approximately 2.8-2.9 h, 1.9-2.0 h and 2.4-2.5 h for S(+)-ibuprofen, R(-)-ibuprofen and their sum, respectively.

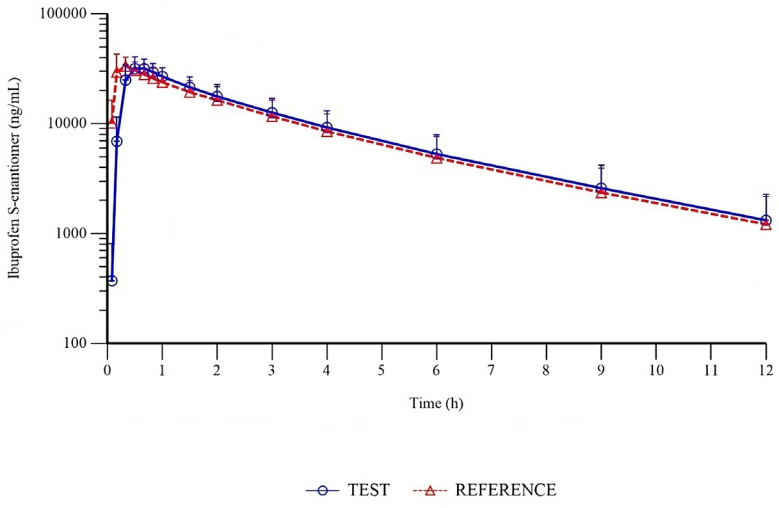

Figure 1: Mean (+ SD) plasma S(+)-ibuprofen concentration (ng/mL) vs. time profiles up to 12 h post-dose for the Test and Reference products. Logarithmic/linear scale. N=24

Test: Ibuprofen arginine 600 mg film-coated tablet; Reference: Espidifen® 600 mg granules for oral solution

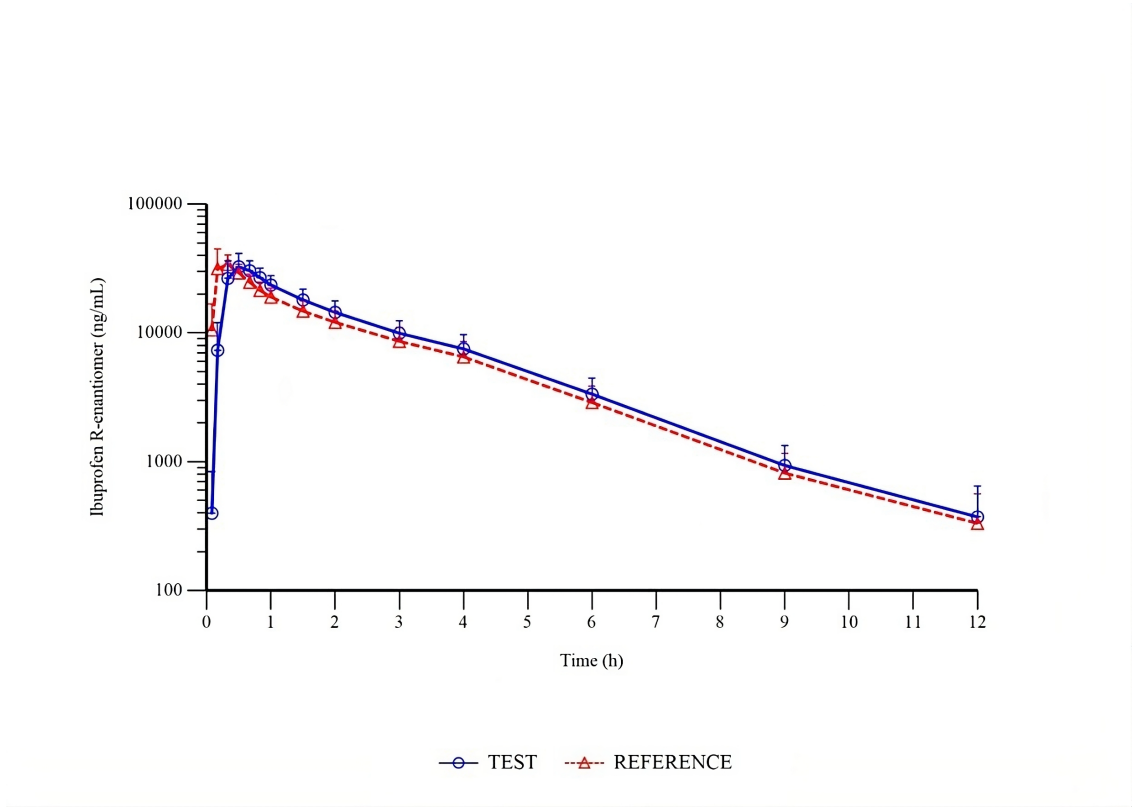

Figure 2: Mean (+ SD) plasma R(-)-ibuprofen concentration (ng/mL) vs. time profiles up to 12 h post-dose for the Test and Reference products. Logarithmic/linear scale. N=24

Test: Ibuprofen arginine 600 mg film-coated tablet; Reference: Espidifen® 600 mg granules for oral solution

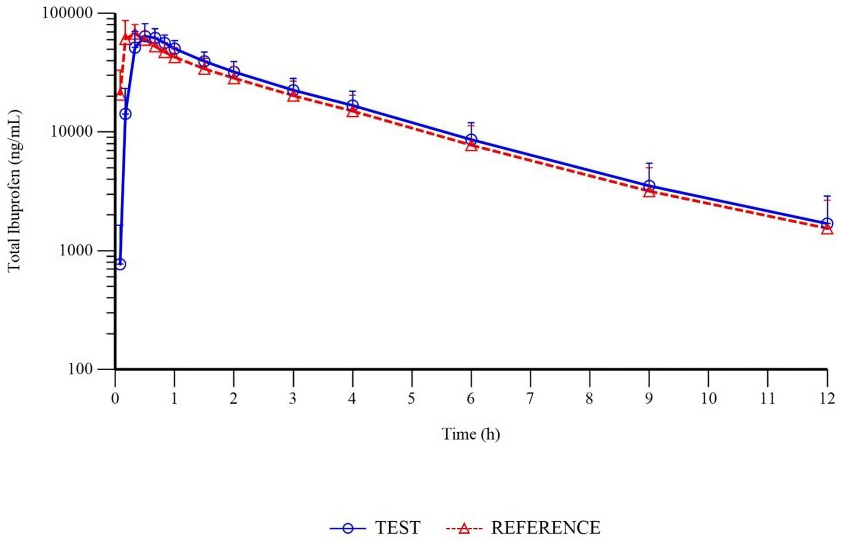

Figure 3: Mean (+ SD) plasma total ibuprofen concentration (ng/mL) vs. time profiles up to 12 h post-dose for the Test and Reference products. Logarithmic/linear scale. N=24

Table 2: Main S(+)-ibuprofen pharmacokinetic (PK) parameters and results of the bioequivalence test. N=24

PK parameter	Test	Reference	PE%*	94.12%CI
C_{max} (ng/mL)	34086.6±6716.2	35144.4±9775.4	98.16%	93.29-103.29%
AUC_{0-t} (ng/mL×h)	102842.3±33387.6	99622.6±36242.2	104.21%	101.07-107.45%
$AUC_{0-\infty}$ (ng/mL×h)	108850.5±38160.6	105193.6±40990.4	NA	NA
T_{max} (h)	0.5 (0.4–0.8)	0.3 (0.2–0.7)	NA	NA
$t_{½}$ (h)	2.9±0.5	2.8±0.6	NA	NA

Values are arithmetic means ± SD, except for T_{max}: median (range); *PE=Point estimate: ratio of geometric means; NA: Not Applicable; Test: Ibuprofen arginine 600 mg film-coated tablet; Reference: Espidifen® 600 mg granules for oral solution

Table 3: Main R(-)-ibuprofen pharmacokinetic (PK) parameters and results of the bioequivalence test. N=24

PK parameter	Test	Reference	PE%*	94.12%CI
C_{max} (ng/mL)	34835.4±7230.0	36966.4±9315.0	94.76%	87.77-102.31%
AUC_{0-t} (ng/mL×h)	80802.6±17567.6	74224.1±16260.4	108.73%	102.79-115.02%
$AUC_{0-\infty}$ (ng/mL×h)	82141.0±17879.6	75411.6±16530.0	NA	NA
T_{max} (h)	0.5 (0.3–0.8)	0.3 (0.2–0.5)	NA	NA
$t_{½}$ (h)	1.9±0.5	2.0±0.4	NA	NA

Values are arithmetic means ± SD, except for T_{max}: median (range); *PE=Point estimate: ratio of geometric means; NA: Not Applicable; Test: Ibuprofen arginine 600 mg film-coated tablet; Reference: Espidifen® 600 mg granules for oral solution

Table 4: Main total ibuprofen pharmacokinetic (PK) parameters and results of the bioequivalence test. N=24

PK parameter	Test	Reference	PE%*	94.12%CI
C_{max} (ng/mL)	68665.1±13087.7	71829.9±18967.3	96.60%	90.73 – 102.84%
AUC_{0-t} (ng/mL×h)	183738.3±45585.2	173953.5±49455.3	106.34%	102.68 – 110.13%
$AUC_{0-\infty}$ (ng/mL×h)	190407.8±50570.6	180080.5±54177.9	NA	NA
$_{Tmax}$ (h)	0.5 (0.4–0.8)	0.3 (0.2–0.5)	NA	NA
$t_{½}$ (h)	2.4±0.5	2.5±0.5	NA	NA

Values are arithmetic means ± SD, except for T_{max}: median (range); *PE=Point estimate: ratio of geometric means; NA: Not Applicable; Test: Ibuprofen arginine 600 mg film-coated tablet; Reference: Espidifen® 600 mg granules for oral solution

Safety

The investigational products orally administered as single dose were well tolerated. No subject withdrew from the study for an adverse event. An increase in blood creatinine, observed for one subject with the test and 2 subjects with the reference product, was the only adverse event considered related to the study product. The reported adverse events were mild, did not give rise to any safety concern, and resolved by study end. No significant effects on vital signs, body weight or ECG were observed.

Discussion

This study demonstrated that mean concentration-time profiles for S(+)-ibuprofen and R(-)-ibuprofen after single dose of a newly developed ibuprofen arginine 600 mg tablet formulation (test) and ibuprofen arginine 600 mg granules for oral solution (reference) were nearly superimposable. The bioequivalence test was fully satisfied for the two enantiomers and their sum, with the 94.12% CIs of the test/reference ratio of geometric means for C_{max} and AUC_{0-t} within the acceptance limits of 80.00–125.00%, in compliance with the European guideline on bioequivalence studies [30] and the statistics for two-stage studies. The trial was designed according to a two-stage design, considering the margin of uncertainty with respect to the sample size estimate to determine bioequivalence between the two study products, since ibuprofen rate of absorption from the test formulation was unknown.

For the bioanalysis a chiral method was used taking into consideration that the S(+) enantiomer is the active form of ibuprofen, predominant after oral administration of the racemic compound, and previous work demonstrating that chiral assays seem to be more sensitive to differences between formulations in either rate or extent of absorption of individual enantiomers. In a study comparing two ibuprofen 2% oral suspensions [20], the non-chiral method for racemate analysis and the achiral approach on the sum of the two enantiomers demonstrated bioequivalence of the two formulations, with a similar outcome. Contrarily the chiral method showed difference in AUC_{0-t} for S(+)-ibuprofen and in C_{max} for R(-)-ibuprofen, resulting in non-bioequivalence for the individual enantiomers. These findings support the fact that ibuprofen bioequivalence evaluations could be biased using non-chiral methods. However, if chiral methods are used, also achiral approaches with the evaluation of the sum of the two enantiomers should be performed to have a complete picture of the outcome.

In their study, Garcia-Arieta *et al.* [34] compared two ibuprofen suspensions with different rates of absorption. Plasma concentrations of S(+)- and R(-)-ibuprofen were determined using a chiral method, and bioequivalence for the two enantiomers was evaluated separately and again as the sum of both enantiomers as an approach for an achiral method.
Whereas for the S(+)-enantiomer bioequivalence was concluded, no bioequivalence was confirmed for the R(-) or the sum of S+R, further indicating that the results that would have been obtained with a non-chiral method cannot be generalized to the active enantiomer for which bioequivalence was confirmed, or the distomer for which a larger difference was observed.

In the present study, the bioequivalence test on the individual active ibuprofen S(+) enantiomer is in accordance with the EMA guideline on bioequivalence investigation [30] that reports "if one enantiomer is pharmacologically active and the other is inactive or has a low contribution to activity, it is sufficient to demonstrate bioequivalence for the active enantiomer". On the other hand, EMA guideline on the investigation of bioequivalence for ibuprofen products [31] reports that a non-chiral method should be used. For this reason, in the present study bioequivalence was also assessed for R(-)-ibuprofen and for the sum of the two enantiomers as a simulation of an achiral approach, as also previously done by other authors [13, 20, 25, 34].

In 1997, Mehvar and Jamali [35] already recommended the use of stereospecific assays in bioequivalence assessments for racemic drugs undergoing chiral inversion. This is advisable especially when the rate of ibuprofen absorption of different formulations is unknown [20].

In a very recent study based on a dataset composed of 11 Phase I clinical trials [33], physiologically based pharmacokinetic modelling was applied, incorporating stereoselectivity, non-linearity in plasma protein binding and metabolism, as well as ibuprofen R(-) to S(+) unidirectional inversion. The deterministic bioequivalence risk assessment confirmed that the R(-) enantiomer was the most sensitive analyte to detect differences, suggesting that achiral bioanalytical methods would increase Type II error in the statistical analysis thus declaring non-bioequivalence for formulations that are bioequivalent for the eutomer. This is in opposition to what required by the EMA guideline on the investigation of bioequivalence for ibuprofen products

[31], stating that achiral methods can be used to assess bioequivalence of ibuprofen formulations, as also discussed above.

Taking into account the points above, bioequivalence conclusion should anyway be based on both ibuprofen enantiomers, considering that ibuprofen distomer is not completely inert, in contrast to what is required in general for enantiomers in the EMA guideline on bioequivalence investigations [30]. In this study, conclusion with the chiral analysis of the two enantiomers and with their sum, simulating the use of the achiral method, is the same, confirming the bioequivalence of the two products in terms of both rate and extent of absorption both for the eutomer and the distomer. Results of this study are in line with the conclusions of Garcia-Arieta *et al.* [16], who showed that achiral and chiral methods provide similar results if the absorption rate is similar enough. Similarly, in the study by Matjii and co-workers [25] investigating the bioequivalence of two 600 mg tablet products based on a chiral method and an achiral approach, the two formulations were bioequivalent in terms of C_{max} and AUC_{0-t} for the two enantiomers separately and for the sum of them.

Median T_{max} for plasma S(+)-ibuprofen, R(-)-ibuprofen and total ibuprofen after single dose of the Test and Reference formulations in this study corresponded to 0.5 h (30 min) and 0.33 h (20 min), respectively, and T_{max} individual ranges overlapped. On the other hand, differences in T_{max} values were statistically significant for all comparisons.

The guidance on bioequivalence for ibuprofen products reports that T_{max} values should be similar between test and reference products but does not report a specific range for claiming similarity. It has been reported that if the difference between T_{max} values is larger than the time between two adjacent or consecutive sampling times or larger than 0.5 h [20], the difference is not acceptable. In the present study median T_{max} values occurred at adjacent/consecutive blood sampling time-points, with samples collected at 0.33 ad 0.5 h post-dose, and thus can be regarded as similar. In addition, it has been reported that C_{max} is more sensitive than T_{max} in detecting differences in absorption rate, and in the present study C_{max} was bioequivalent for both enantiomers confirming a similar rate of absorption of the two formulations.

Pharmacokinetic data for S(+)-ibuprofen, R(-)-ibuprofen and total ibuprofen for Espidifen®granules in the present study are in line with those obtained by Gonzales *et al.* [13] in 2 reported studies in which different bioequivalence evaluations were based on a chiral assay for the two enantiomers and an achiral approach for the sum of the two enantiomers. Elimination half-life ($t_{1/2}$) values for S(+)- and R(-)-ibuprofen were similar for the two investigational products in this study and also comparable to those of previously published data.

Notably, data of the present study confirmed that peak concentrations were achieved at 15-20 minutes post-dose with both investigational products, as also previously observed with other ibuprofen arginate formulations. In a randomised, single-dose, cross-over study performed in 36 healthy South Korean volunteers, rapid absorption and higher peak concentrations were observed with ibuprofen arginine and solubilised ibuprofen capsules as compared to standard ibuprofen [36]. Similar results were also obtained in previous work suggesting that ibuprofen formulations with early pharmacokinetic profiles achieved a faster analgesia [37].

The early T_{max}, in fact, is associated with a rapid onset of action of the analgesic effect of ibuprofen arginate, as demonstrated by different authors in some frequent acute pain conditions. In an open trial in patients with primary dysmenorrhoea, an initial oral dose of 600 mg ibuprofen arginine, followed by the same dose every 6 hours (maximum daily dose 2400 mg) resulted in a significant improvement of pain relief observed already at 15 min post-dose compared with baseline, with 82.2% and 97.6% of patients reporting a marked decrease in pain intensity at 15 and 30 min, respectively [38]. Results of a double-blind, randomised, placebo-controlled, parallel-group trial in 500 patients demonstrated that ibuprofen arginate was superior to conventional ibuprofen in both the amount and the time to onset of pain relief in postoperative dental pain [39]. Similarly, ibuprofen arginate was proven to be superior to standard ibuprofen or other NSAIDs in different pain-related measures in acute pain of dental origin and in dysmenorrhea [8-11].

A possible study limitation was its open-label design. However, the endpoints were based on objective assessments, i.e., the determination of ibuprofen enantiomers' levels in the plasma samples by blinded analysts, thus minimising any bias in the final study outcome.

The safety data collected during this study are in line with the known safety profile of ibuprofen L-arginine products and did not raise any safety concern.

To conclude, in healthy volunteers the test and reference products are bioequivalent in terms of both rate and extent of absorption of S(+)-ibuprofen, R(-)-ibuprofen and their sum, indicating that the novel ibuprofen L-arginine 600 mg tablet could be an effective clinical alternative to the already marketed ibuprofen L-arginine products, when a rapid onset of action is needed.

Declarations

Funding

The study was funded by Zambon S.p.A., Italy.

Conflicts of Interest

VDF, FS and MM are employees of Zambon S.p.A., Italy; MR and CL are employees of CROSS Research S.A., Switzerland, which was contracted by Zambon S.p.A. for the conduction of this study and received financial support for its services. The authors declare that they have no other relationships or activities that could appear to have influenced the submitted work.

Availability of Data and Material

The study is registered on ISRCTN (No. ISRCTN46595731). The data supporting the study fundings are available from VDF (Zambon S.p.A.) upon reasonable request.

Ethics Approval

The study was approved by the Canton Ticino Ethics Committee, Switzerland, and the Swiss Federal Health Authority, and was performed in accordance with the Helsinki Declaration and Good Clinical Practice.

Consent

All subjects received a detailed study description and gave their written informed consent before enrolment.

Authors' Contribution

All authors have made substantial contributions to the conception and design of the study, the acquisition of data, the analysis and interpretation of data, drafting of the article or critical revision for important intellectual content and final approval of the submitted version.

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