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Pharmacokinetics of a new fixed-dose combination of candesartan cilexetil, hydrochlorothiazide, and rosuvastatin in healthy adult subjects

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Key words
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Abstract. Background: A fixed-dose combination (FDC) of candesartan cilexetil, hydrochlorothiazide and rosuvastatin (CC/HCTZ/RSV) has been developed to enhance patient compliance in the primary prevention of cardiovascular diseases. Objective: To evaluate if the combination of the product components in the new FDC capsule formulation affects their respective pharmacokinetic and in vitro dissolution patterns. Materials and methods: In vitro dissolution profiles were compared in USP-43 and in biorelevant dissolution media. In vivo comparisons were obtained in a randomized, open-label, single-dose, two-treatment, two-way crossover study in 24 healthy subjects. During each treatment period, subjects received the test formulation (FDC hard capsule containing CC/HCTZ/RSV) or the reference formulation (co-administration of a FDC CC/HCTZ tablet and a RSV tablet). Plasma samples were collected periodically over 48 hours post-dose. Safety and tolerability were assessed. Results: Dissolution profiles of all active drugs in the Test (capsule) and Reference Products (as tablets) were within the tolerance dissolution criteria of USP-43 conditions. HCTZ dissolution profiles were closely similar whereas those for RSV and CC did not match at specific pHs. In the pharmacokinetic study, the 90% confidence intervals (CIs) for the geometric least-square mean ratios of C_{max}, AUC_{0-last}, and AUC_{0-inf} were 0.95 – 1.18, 0.95 – 1.15 and 0.95 – 1.13 (CC); 0.91 – 1.10, 0.96 – 1.08, and 0.96 – 1.09 (HCTZ) and 0.82 – 1.23, 0.81 – 1.13, and 0.82 – 1.12 (RSV), respectively. All adverse events were mild. Conclusion: The new FDC product (Sinlip Prevent), a stable FDC hard capsule, was bioequivalent (similar pharmacokinetics) when compared to the co-administration of the components and may be considered as a suitable and simplified medication for cardiovascular disease management.

What is known about this subject

- Recent studies indicate that patients with hypertension should be started on combined therapy with antihypertensive agents and statins as soon as possible to prevent the development of cardiovascular diseases. Up-to-date guidelines recommend that patients with hypertension in the moderate risk category be treated with statins.
- Fixed-dose combination products (FDC) often contain drugs with different mechanism of action to produce additive and, or synergistic effects in the treatment of the disease, provided there is evidence of a positive benefit-risk input for the combination in the targeted indication.

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What this study adds

- A new fixed-dose formulation combining an immediate release fixed-dose combination tablet of candesartan cilexetil/hydrochlorothiazide 16/12.5 mg and an immediate release tablet of rosuvastatin 10 mg, both contained in a hard capsule has been developed in Argentina.
- This study constitutes the first report describing both in-vitro dissolution characteristics and bioavailability and bioequivalence of a new FDC oral hard gelatine capsule formulation containing CC 16 mg, HCTZ 12.5 and RSV 10 mg compared to the co-administration of single doses of the innovator products in healthy adult subjects.

Introduction

Cardiovascular disease is the primary cause of death globally based on data from the World Health Organization, with an estimated 18 million deaths each year, constituting 31% of all deaths [1]. Moreover, in spite of current recommendations and availability of effective medication, more than 23 million people are predicted to die annually from cardiovascular diseases by 2030 [2]. Dyslipidemia and hypertension are well recognized risk factors for heart disease and stroke [3]. In surveys, more than 60% of patients with hypertension also have hypercholesterolemia [4]. The association of these two risk factors promotes the coronary endothelial impairment generated by each risk factor alone and the effects on endothelial function are accompanied by a shift in oxidative status to a pro-oxidant condition. This interaction may therefore increase the incidence of coronary heart disease and cardiac events when hypercholesterolemia and hypertension coexist [5].

Recent studies show that patients with hypertension should be started on combined therapy with antihypertensives and statins at an early stage to prevent the development of cardiovascular diseases [6]. A meta-analysis showed that cardiovascular events were lowered by 45% if the blood pressure and blood lipid levels were both decreased by 10% [7]. An international, multicenter, double-blind, randomized, placebo controlled trial (Heart Outcomes Prevention Evaluation-3 study: HOPE Study) conducted in 12,705 patients with a mean follow-up of 5.6 years, demonstrated that the combination of rosuvastatin (RSV) (10 mg/day), candesartan (16 mg/day), and hydrochlorothiazide (HCTZ) (12.5 mg/day) was associated with a significantly lower rate of cardiovascular events than dual placebo in patients at intermediate risk without cardiovascular disease. There was a 29% reduction in the primary outcomes i.e., cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke; and by 28% reduction in secondary outcomes i.e., heart failure, cardiac arrest, and revascularization. These benefits were clearly observed in the group of compliers. Adverse events associated with combined therapy comprised muscle weakness and dizziness [8].

Up-to-date guidelines recommend that patients with hypertension in the moderate risk category be treated with statins [9]. Thiazide-type diuretics and angiotensin receptor blockers are included among the first-line therapies in patients with hypertension, and two or more drugs may be used simultaneously if blood pressure is not controlled [10]. Candesartan cilexetil (CC)/HCTZ, in a fixed-dose combination (FDC) tablet (16/12.5 mg), was approved by the FDA in 2000 for the treatment of hypertension [11]. RSV (tablets; 5 – 40 mg) was approved by the FDA in 2003 to treat patients with primary hyperlipidemia and mixed dyslipidemia and also as primary prevention of cardiovascular diseases in patients with several risk factors but without clinically evident disease [11, 12].

CC is rapidly and completely bioactivated to candesartan, a selective angiotensin II type 1 receptor antagonist, by ester hydrolysis during absorption from the gastrointestinal tract [13]. Candesartan belongs to class IV (low solubility, low permeability) according to the biopharmaceutical classification system [14, 15]. Candesartan shows linear pharmacokinetics for oral doses up to 32 mg. After tablet ingestion, oral bioavailability is ~ 15% due to incomplete absorption, and peak concentrations are reached after 3 – 4 hours. It undergoes minor hepatic metabolism by O-deethylation to an inactive metabolite and is mostly excreted unchanged in urine and feces with an elimination half-life of ~ 9 hours [13, 16]. HCTZ acts on renal tubular electrolyte reabsorption, directly raising the excretion of sodium

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and chloride by similar amounts. [13]. HTCZ is considered to be a BCS-class II drug (low solubility, high permeability) although it has also been classified as class III [17, 18, 19]. Following oral dosing, the bioavailability is nearly 70% with a biphasic decline in plasma concentration and a terminal half-life of ~ 10 hours (range 5.6 – 14.8 hours) [20]. HTCZ is not metabolized and is excreted primarily unaltered in the urine within 24 hours [13]. RSV calcium is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, causing suppression of the synthesis of low-density lipoprotein cholesterol in the liver [21]. RSV is usually categorized as a BCS-class II drug. The bioavailability after oral dosing is ~ 20% and C_{max} is achieved at 3 – 5 hours. Both the maximum plasma concentration and the AUC for the plasma concentration-time curve increase proportionally to dose. RSV is not extensively metabolized and is primarily eliminated by excretion in the feces with an elimination half-life of almost 19 hours [22].

Because of the higher patient compliance and adherence, FDC products are being increasingly preferred for diseases requiring long-term treatment such as hypertension and hypercholesterolemia. In addition, enhanced patient convenience, simplification of disease management, cost reduction to the patient and possibly improved clinical effectiveness are accompanied in some patients by improved blood pressure lowering effects [23, 24, 25]. Fixed-dose combination products often incorporate drugs with different mechanism of action to produce additive and, or synergistic effects often representing a positive benefit-risk input for the targeted indication [23, 26, 27].

We describe here a new fixed-dose formulation, developed in our facilities in Argentina, combining an immediate release FDC tablet of CC/HCTZ 16/12.5 mg and an immediate release tablet of RSV 10 mg, in a single hard gelatine capsule. The new formulation is obtained by filling a hard gelatin capsule with a single rosuvastatin film coated tablet (10 mg) and a single FDC tablet of CC/HCTZ tablet (16/12.5 mg). It is anticipated that the new formulation will be effective in the primary prevention of cardiovascular disease in adult patients over 60 years who are at intermediate risk and who will benefit from a cholesterol and a blood pressure-lowering agent.

The objective of the present study was to determine the dissolution characteristics of CC/HCTZ/RSV, in the new FDC formulation and to confirm the absence of any interactions affecting the pharmacokinetic (PK) profile of the active agents. Both in vitro dissolution characteristics and in vivo PK profiles were determined and compared to those obtained using the innovator products.

Materials and methods

In vitro dissolution tests

In vitro dissolution studies were conducted at Gador S.A. Pharmaceutical Development Unit (Buenos Aires, Argentina). The dissolution rates for CC, HCTZ, and RSV were measured using n = 12 tablets of the new fixed-dose formulation (considered as test product: Sinlip Prevent), as well as the innovator products (considered as reference products: Atacand D and Crestor). Dissolution profiles were obtained for all drug products in USP-43 media in concordance with the recommendations of the United States Pharmacopeia (USP) [28, 29]. Dissolution tests were carried out in 3 bio-relevant media using conditions for dissolution testing of immediate release (IR) products as specified in the FDA guidance [30]. USP-43 dissolution profiles for CC, HCTZ and RSV were obtained using USP II apparatus, paddle type at 50 rpm in 900 mL, phosphate buffer at pH 6.5 for CC and HCTZ and citrate buffer at pH 6.6 for RSV. Dissolution conditions for the bio-relevant multi-pH media were: USP II apparatus, paddle type at 75 rpm in 900 mL water, multi-pHs adjusted to pH 1.2 (0.1N hydrochloric acid), and pH 4.5 (acetate) and pH 6.8 (phosphate) buffers at 37 °C. Samples were withdrawn through a syringe filter at the following predetermined time intervals: 0, 10, 15, 20, 30, and 45 minutes for CC/HCTZ; and 0, 10, 15, 20, and 30 minutes for RSV. The volume withdrawn was replaced by the same volume of fresh dissolution media. Blank, standard, and sample preparations of equal volume were injected separately in a HPLC UV chromatograph and chromatograms recorded. Drug content was determined spectrophotometrically at 264 nm for CC/HCTZ and at 242 nm for RSV.

The similarity factor (f2) was calculated to compare the dissolution profile of the

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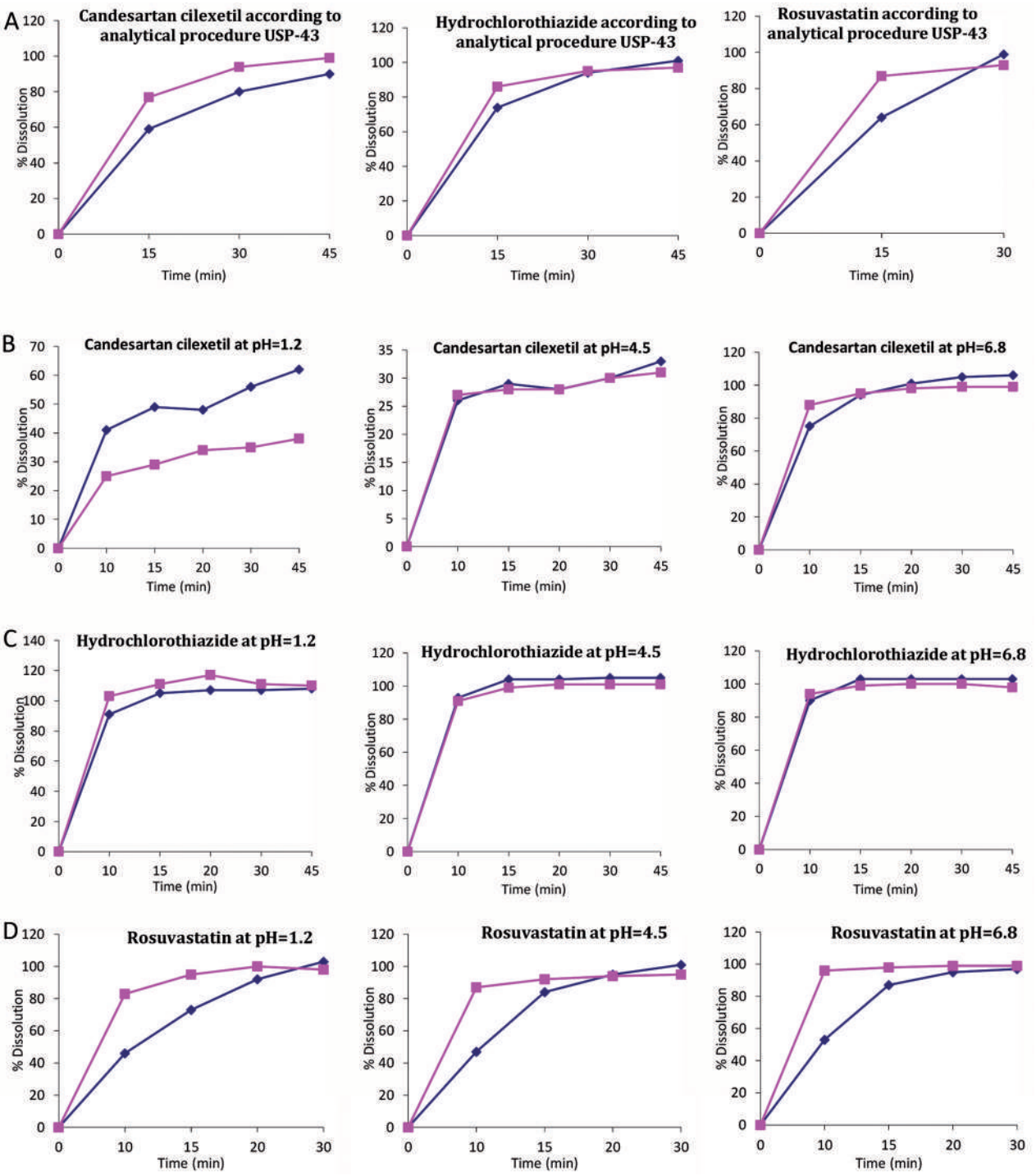

Figure 1. A: In vitro dissolution profiles of candesartan cilexetil, hydrochlorothiazide and rosuvastatin from test and reference formulations in USP-43 media, test (rhombus) and reference (square). B: In vitro dissolution profiles of candesartan cilexetil from test and reference formulations in biorelevant multi-pH media, test (rhombus) and reference (square). C: In vitro dissolution profiles of hydrochlorothiazide from test and reference formulations. In biorelevant multi-pH media, test (rhombus) and reference (square). D: In vitro dissolution profiles of rosuvastatin from test and reference formulations test in biorelevant multi-pH-media, test (rhombus) and reference (square).

test and reference products and to assess pharmaceutical equivalence. When dissolution was more than 85% or more of the labeled amount of the drug substance within 15 minutes, f2 was not determined since the drug entity was assumed to be a very rapidly dissolving drug [31].

Stability tests

Stability tests were conducted according to ICH guidelines (International Conference on Harmonization (of Technical Requirements for Registration of Pharmaceuticals for Human Use)) to assure compatibility of product components (active ingredients, tablets excipients and capsules) and as a consequence, to guarantee that the formulation remains within acceptance release criteria during its shelf life. Stability assays of the test formulation, packaged in OPA/AL/PVC – AL blisters, were conducted at Gador S.A. according to ICH Q1AR2 guideline conditions at three temperatures (25, 30, and 40 °C) and under controlled relative humidity (60, 75, and 75%, respectively) [32]. Assay, dissolution under compendial conditions, and released substances – both known and unknown – were measured by HPLC and used as stability marker parameters for establishing the shelf-life of the new product. For this purpose, samples were collected at predefined time points (3, 6, 9, 12, 18, and 24 months) in concordance with validated analytical procedures, properly adjusted and verified for the pharmaceutical form based on compendial acceptance criteria (USP 43) for each active pharmaceutical ingredient.

Clinical study

A PK in vivo study was carried out as an open label, single-dose, single-center, randomized, two-treatment, two-period, balanced, crossover trial in healthy adult subjects under fasting conditions at the F.P. Clinical Pharma Pharmacokinetic Unit, Buenos Aires, Argentina, from June to July 2018. All clinical procedures were executed pursuant to Local Regulations, ICH Good Clinical Practice Guideline and rules of the Ethical Principles for Medical Research Involving Human Subjects stated in the latest version of the Declaration of Helsinki [33, 34, 35]. The study protocol and the Informed Consent Form were examined and approved by an Independent Ethic Committee (Comité de Ética en Investigación Clínica "CEIC", Buenos Aires, Argentina, revision number 1394/19/2017) and the Local Regulatory Agency (ANMAT-MoH- Provision DI-2017-11739-APN-ANMAT#MS) prior to study commencement. All subjects who agreed to take part in the study signed the approved informed consent form prior to the start of the study.

Interventions

The study subjects were randomly assigned to receive two separate single doses of CC 16 mg, HCTZ 12.5 mg and RSV 10 mg, each in one of two treatment sequences (Test-reference or reference-test). The test product consisted of a single hard gelatin capsule containing a FDC film-coated tablet of CC/HCTZ 16/12.5 mg and a tablet of RSV 10 mg (Sinlip Prevent, Batch No. 0970318), manufactured by Gador S.A. (Argentina). Reference product comprised the co-administration of a CC/HCTZ 16/12.5 mg FDC film-coated tablet (Atacand D, batch No. 0823), manufactured by AstraZeneca (Gaithersburg, CA, USA) and RSV 10 mg (Crestor, batch No. 17060213) also manufactured by AstraZeneca (Gothenburg, Sweden). Both reference products were purchased at a local pharmacy.

The single doses were administered with 240 mL of non-sparkling mineral water after an overnight fast of at least 10 hours. The treatments were administered in two distinct dosing periods according to a predetermined randomized sequence of treatments. A 7-day wash-out period was carried out between treatments. Subjects were not permitted to chew or crush the study medication and water ingestion was not allowed in the period 1-hour pre-dosing and until 2-hour after dosing. A "mouth check" was carried out immediately after dosing. Subjects remained in the fasting state until after the 4-hour PK blood sample time point. A standard diet was given to subjects during confinement.

Study population

The study was carried out in 24 subjects. Since it was the first bioavailability assessment of the newly developed FDC formulation, sample size was considered suitable.

Inclusion criteria

Healthy male and female adult subjects (non-pregnant, non-lactating) aged 21 – 55

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years with a body mass index (BMI) of 19 – 27 kg/m^2. A negative pregnancy test was required for female subjects of childbearing potential who agreed to use non-hormonal contraception methods. Health status was verified using medical history, physical checks, vital signs determinations, clinical laboratory tests, 12-lead ECGs and results were required to be within normal ranges. Negative serological tests for virus detection and drug abuse were also requirements for inclusion in the study. Subjects were excluded if they had a history or current evidence of clinically significant disease affecting any body system. Subjects were not allowed to take any type of medication, including herbal preparations, within the preceding two weeks and throughout the study. Smokers were asked to refrain from using any type of tobacco whilst in the study. Other standard exclusion criteria for bioequivalence (BE) studies were implemented for subject enrollment [36]. Subjects were requested not to consume foods and beverages containing alcohol or xanthines and to avoid exposure to sunlight, strenuous exercise and sports in the period 72-hour preceding the administration of the study medication and during the confinement at the PK unit.

Sample collection and bioanalytical methods

Serial blood samples for PK analysis were collected by venipuncture over a 48-hour period at the following time points: 0 (predose), 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 9, 12, 24, 36 and 48 hours after oral ingestion of each treatment. A volume of ~ 8 mL of blood for each sample was collected into polypropylene tubes containing K2 EDTA as anticoagulant. Plasma was separated by centrifugation and frozen at –20 °C until analysis.

An analytical method was developed and validated for measuring CC, HCTZ and RSV concentrations in K2 EDTA human plasma using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS, MA-RSV-CND-HDR-01, Mass Spectrometry Laboratory of Hospital Italiano de Buenos Aires, Buenos Aires, Argentina). Plasma sample preparation with the respective internal standards involved liquid-liquid extraction with ethyl ether, followed by evaporation of the organic extract to dryness under a stream of nitrogen. The residue was reconstituted in 0.5% acetic acid in water and injected into the UPLC-MS/MS system. Quantification was performed using a plasma calibration curve that represented the relationship between the response of the instrument (area of the analyte/area of its internal standard) at known concentrations of each analyte under study. Electrospray ionization (ESI) was conducted in the negative ion mode. The multiple-reaction monitoring mode (MRM) using specific precursor/product ion transition was used for quantification. Detection of the ions was performed by monitoring the m/z transitions of 439.3 > 309.2 for CS; 296.1 > 269.1 for HCTZ and 480.2 > 418.1 for RSV. Instrument identifier was XEVO-TQS#WAA828. Data were acquired by the analyst software Waters MassLynx 4.1 SCN876. Stability of samples was sufficient to cover the time between drug sampling and concentration measurement for all samples.

A calibration curve was constructed for peak area ratios using a least-squares weighted (l/concentration^2) linear regression for CS and RSV and a weighted (l/concentration) cubical model for HCTZ. The lowest limit of quantification (LLOQ) and upper limit of quantification (ULOQ) for CS were 3.0 ng/mL and 300.0 ng/mL, respectively. The LLOQ and ULOQ for HCTZ were 2.0 ng/mL and 200.0 ng/mL, respectively. The LLOQ and ULOQ for RSV were 0.15 ng/mL and 30.0 ng/mL, respectively. The calibration curves were linear over the calibration concentration range (r >0.98) for CS and RSV and cubic fit (R^2 > 0.98) for HCTZ.

The accuracy and precision of the validated method was assessed using 3 separate analytical runs, each containing three quality control (QC) levels for CS (LQC: 9 ng/mL; MQC: 150 ng/mL, HQC: 250 ng/mL), HCTZ (LQC: 6 ng/mL; MQC: 100 ng/mL, HQC: 160 ng/mL) and RSV (LQC: 0.45 ng/mL; MQC: 15 ng/mL, HQC: 25 ng/mL) covering the linear range of quantification in replicates of 5 conducted on different days. Inter-and intra assay accuracy showed mean BIAS values within ± 15% of the nominal values and within ± 20% at the LLOQ. Inter- and intra-assay precision measurements gave coefficients of variations (CVs) of ± 15% of nominal concentrations; except ± 20% at LLOQ. The methodological validation was carried

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out in concordance with local regulatory requirements and with the FDA guidance for bioanalytical method validation [37].

Pharmacokinetic evaluation

The plasma concentration-time data after single dose administration of test and reference formulations were obtained and PK parameters were calculated using a non-compartmental model (WinNonlin, version 8.0; Certara, US Inc). The maximum plasma concentration and the time of occurrence, C_{max} and t_{max}, were recorded.. The gradient of the log-linear regression function (λ) was the first order rate constant associated with the terminal segment of the linear regression curve for time vs. log-concentration. The area under the plasma concentration-time curve (AUC) from the time of dosing (zero) to the last measurable concentration ($AUC_{0–last}$) was estimated using the trapezoidal rule (linear trapezoidal with linear interpolation), The AUC, from dosing time extrapolated to infinity, based on the last observed concentration ($AUC_{0–inf}$) was computed using the equation $AUC_{0–inf}$ = AUC + (Cn/λ) where Cn is the last quantifiable concentration and λ is the slope of the log-linear regression function. The elimination half-life ($T_{1/2}$) was ascertained as ln2/λ. Subjects were eliminated from the PK analysis set of all CC, HCTZ, and RSV if emesis occurred at or before a time point equal to twice the median t_{max} [36].

Safety assessment

Physical examination, hematology, serum chemistry, urinalysis, 12-lead ECG and drug abuse tests were undertaken at the screening visit (day –21 to –1) for safety purposes. A urine pregnancy test was carried out at the screening visit and prior to each dosing period in the case of females with childbearing potential. An abbreviated physical examination before drug administration was also carried out in the morning. Vital signs (heart rate, systolic and diastolic blood pressure and axillary temperature) were registered at the screening visit, 30 min before dosing and at time-points 2.0, 4.0, 9.0, 12, and 24-hours after drug intake. Adverse events (AEs) were recorded either following spontaneous reporting or after direct questioning of study participants, regarding their general health, before the dosing period and during the study follow-up visit. Severity, intensity and relationship of the AEs to the study medications were evaluated by the investigators.

Statistical analysis

Demographic data included age, gender, race, height, weight and BMI. PK endpoints comprised C_{max}, t_{max}, $AUC_{0–last}$, $AUC_{0–inf}$, Ke, Half-life (T1/2β). The PK parameters: C_{max}, $AUC_{0–last}$, $AUC_{0–inf}$, for CC, HCTZ and RSV were analyzed using natural log-transformed data. These PK variables were compared by means of ANOVA to obtain the variances for a 2-treatment crossover design. The model addressed the fixed effects of period, sequence and treatment and the random effect of subjects within-sequence. The average bioavailability of CC, HCTZ and RSV in the test formulation relative to the reference formulations was expressed as the ratio of respective estimated mean exposure and 90% confidence intervals (CIs) in terms of C_{max}, $AUC_{0–last}$, and $AUC_{0–inf}$. In concordance with scientific standards and international guidelines for BE studies, BE was established if the 90% CIs for the ratio of the geometric least-squares means (test treatment/reference treatment) was inside the boundaries of 80 – 125% for the primary PK parameters. Differences at the 5% level were regarded as significant for all statistical tests [36].

Results

In vitro dissolution profiles

Comparative in vitro dissolution parameters of CC, HCTZ, and RSV from test and reference formulations tested in four different media are summarized in Table 1. All three active agents from test and reference products had dissolution characteristics within tolerance criteria specified in the USP-43. In-vitro dissolution data obtained in USP-43 media are shown in Figure 1A and for the three bio-relevant media in Figure 1B, C, and D.

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Table 1. Comparative in vitro dissolution parameters of candesartan cilexetil, hydrochlorothiazide, and rosuvastatin from test and reference products in different standard media.

Active principle	USP-43	pH = 1.2	pH = 4.5	pH = 6.8
Candesartan cilexetil	> 80% at 45 min	f 2= 39.6	f 2 = 97.0	> 85% at 15 min
Hydrochlorothiazide	> 80% at 45 min	> 85% at 15 min	> 85% at 15 min	> 85% at 15 min
Rosuvastatin	> 75% at 30 min	f 2 = 32.6	f 2 = 34.2	> 85% at 15 min

Table 2. Demographic characteristics of subjects.

Demographic characteristics	Results (n = 24)
Ethnicity (Caucasian/Non-Caucasian), n (%)	23 (95.8)/1 (4.2)
Age (yrs), mean ± SD	35.00 ± 9.00
Gender (male/female), n (%)	17 (70.8)/7 (29.2)
Height (cm), mean ± SD	170.00 ± 9.32
Weight (kg), mean ± SD	71.43 ± 9.49
BMI (kg/m^2), mean ± SD	24.63 ± 2.12

Stability

Stability results showed no significant changes or trends in dissolution and assay parameters (conforms at S1/S2 dissolution stage with no greater than a 5% decrease in assay values for each agent). However, after storage at high heat/humidity levels, the gelatin capsules showed cross-linking. Since cross-linking is not expected to occur in vivo because of the acid conditions maintained under fasting, enzymes (papain, protease activity range pH 4 – 6.8) were added to the dissolution medium to resolve this in vitro phenomenon in accordance with USP recommendations. Liberation of degradation products, other than the expected slight increase in some known compounds (RSV lactone, benzothiadiazine and related compounds Cathelicidin-BF-30 (BF-30), from RSV, HCTZ, and C, respectively) remained within acceptance criteria using 25°C/60% relative humidity (RH) and 30 °C/75%RH storage conditions. Increases occurred at high temperature and humidity. At 40 °C/75% RH the changes were above specification levels in the case of benzothiadiazine.

Subject population

32 healthy subjects were initially enrolled in the present study but 8 subjects were considered screening failures and did not receive the study medication. Therefore 24 subjects were randomized to the sequence groups. All subjects completed the study in accordance with the protocol. Demographic characteristics of study subjects are shown in Table 2.

Pharmacokinetics

The PK analysis involved 24 subjects. Figures 2 and show mean plasma concentration-time curves after oral dose administration of CC, HCTZ, and RSV as test and reference treatments respectively. The three treatment curves were similar and reflected typical profiles for conventional immediate release formulations with short half-lives. After reaching C_{max}, CC, HCTZ, and RSV concentrations declined in a biphasic manner for both, test and reference treatments. Table 3 summarize plasma PK parameters for CC, HCTZ, and RSV.

The analysis of variance revealed no statistically significant differences between test and reference formulations ($p > 0.05$) in respect of fixed effects of period, sequence, treatment and subjects within sequence as random effect for the main PK parameters (C_{max}, $AUC_{0–last}$, and $AUC_{0–inf}$) analyzed.

Statistical analysis of CC, HCTZ and RSV PK log-transformed parameters and their geometric least squares mean (GeoLSM) ratios for the test and reference treatment are shown in Tables 4, Table 5, and Table 6. Test-reference ratio for the geometric means (%) for all main PK parameters (C_{max}, $AUC_{0–t}$, $AUC_{0–inf}$) and the corresponding two-sided 90% CIs were within the predefined limits of 80 – 125%.

Safety and tolerability

Safety and tolerability were evaluated in the 24 subjects who received the study medication. The CC, HCTZ/RSV formulations were well-tolerated in all subjects. No clinically significant changes in vital signs were seen. A total of 3 non-serious adverse events including headache (MedDRA v21.0)

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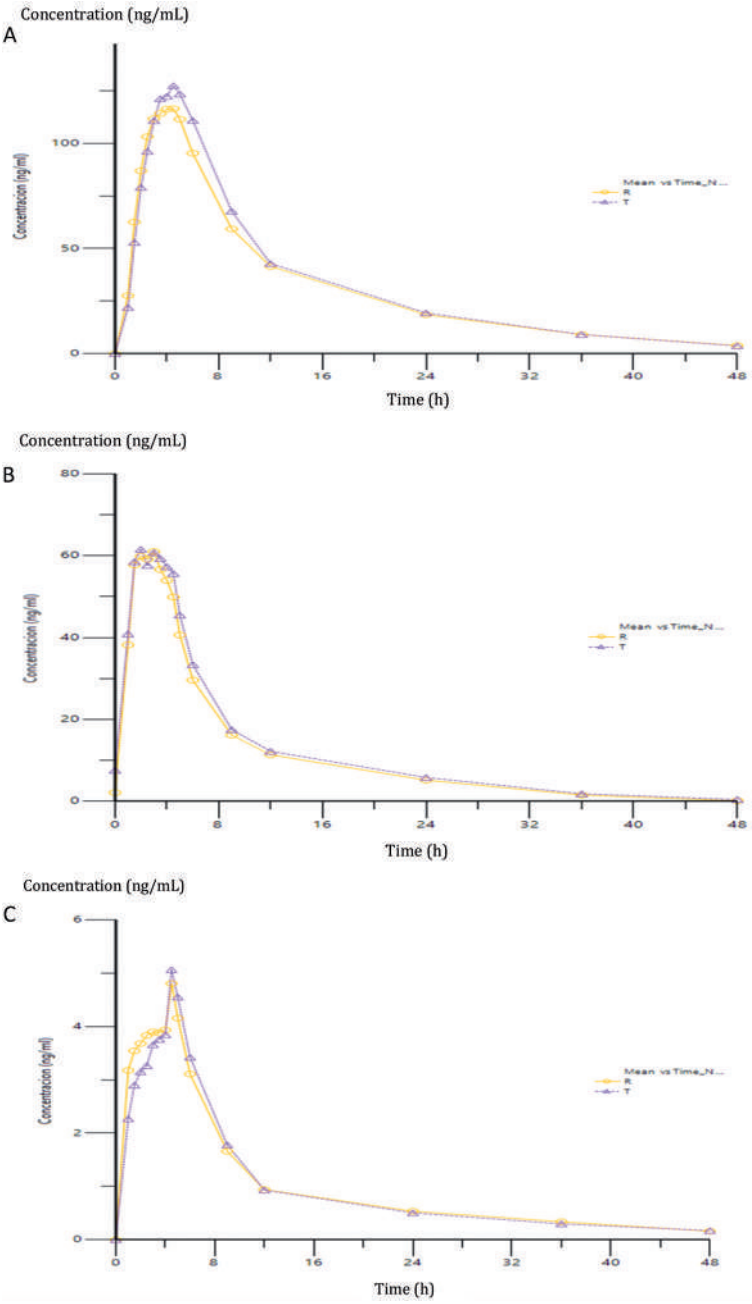

Figure 2. A: Mean plasma concentration-time curves for candesartan cilexetil following administration of single oral doses of test (triangle) and reference (circles) products (n = 24). B: Mean plasma concentration-time curves of hydrochlorothiazide following administration single oral doses of either test (triangle) and reference (circles) products (n = 24). C: Mean plasma concentration-time curves of rosuvastatin following administration of either single oral doses of test (triangle) and reference (circles) products (n = 24).

were reported in 3 subjects. All events were judged as possibly related to the investigational product. No serious adverse events were reported. Only one adverse reaction of moderate intensity required medication treatment and all were completely resolved. No unexpected adverse events were recorded. The safety profiles observed were in accord with the product description information [13, 22].

Discussion

The objective of the present study was to characterize the biopharmaceutical characteristics of the new combined fixed-dose formulation of CC, HCTZ, and RSV. Therefore, both in vitro dissolution and in vivo PK profiles were assessed and compared with those of the innovator products.

In vitro dissolution profiles of the active agents in the test product compared to the innovator products fulfilled the dissolution criteria specified in USP-43. The comparison analysis between test and reference formulations showed that f2 values were below 50% for RSV at pH 1.2 and pH 4.5, and below 50% for CC at pH 1.2, whereas HCTZ was greater than 85% at 15 minutes in all biorelevant pH media, suggesting that test and reference formulations were not pharmaceutically equivalent. However, the in vivo PK study demonstrated that no significance differences existed between test and reference products in terms of rate and extent of absorption, according to C_{max} and AUC and the similarity of plasma CC, HCTZ, and RSV concentration-time curves. Since the 90% CIs of the ratios of µT/µR for the PK parameters (C_{max} and AUCs) were within the predetermined range (80 – 125%), the null hypothesis, that the estimated parameters exceeded limits of acceptance, was rejected, The two formulations tested were considered to be bioequivalent.

This study constitutes the first report presenting both in-vitro dissolution comparisons and in vivo single-dose comparative bioavailability and BE data for the new FDC oral hard capsule formulation containing CC 16 mg, HCTZ 12.5, and RSV 10 mg and the innovator products used to formulate the new FDC product co-administered.

The FDC formulation test product in a hard capsule contained three active agents with low solubility (BCS-class IV for CC and II for HCTZ and RSV). Formulation factors involving these active drugs can be expected to influence their dissolution and PK behavior and hence the BE results [30]. These drugs have characteristically low bioavailability

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Table 3. Pharmacokinetic parameters of candesartan cilexetil, hydrochlorothiazide and rosuvastatin following oral administration of single doses of the test and reference treatments (n = 24).

Pharmacokinetic parameters	Test treatment (n = 24)			Reference treatment (n = 24)		
	CC	HCTZ	RSV	CC	HCTZ	RSV
C_{max} (ng/mL), mean ± SD; geometric mean; median (range); (CV%)	150.64 ± 60.89; 138.77; 143.71 (59.12 – 292.59); (40.40)	78.35 ± 30.40: 73.59; 73.46 (36.34 – 182.55); (38.80)	5.67 ± 4.02; 4.50; 4.17; (0.75 – 16.35); (70.90)	142.80 ± 71.73; 130.71; 127.78; (66.36 – 402.42); (50.20)	75.04 ± 26.06; 70.94; 73.17; (39.34 – 134.00); (34.70)	5.27 ± 3.47; 4.45; 4.55 (1.64 – 15.69); (65.90)
t_{max} (hours), mean ± SD; geometric mean; median (range); (CV%)	4.40 ± 1.87; 4.04; 4.25 (1.50 – 9.00); (42.40)	2.81 ± 1.27; 2.54; 2.50 (1.00 – 5.00); (45.00)	4.35 ± 1.14; 4.11; 4.50 (1.00 – 6.00); (26.10)	4.02 ± 1.08; 3.37; 4.25 (2.00 – -6.00); (26.80)	2.67 ± 1.13; 2.44; 2.50 (1.00 – 4.50); (42.30)	3.79 ± 1.23; 3.49; 4.50 (1.00 – 5.00) (32.50)
AUC_{0-last} (ng×h/mL), mean ± SD; geometric mean; median (range); (CV%)	1,563.95 ± 572.90; 1,473.92; 1,442.99 (723.78 – 3,288.34); (36.60)	560.25 ± 310.62; 507.60; 505.90 (273 – 1,759.43); (55.40)	47.34 ± 27.87; 39.67; 39.63; (8.79 – 113.86); (58.90)	1,482.58 ± 528.45; 1,398.54; 1,361.05 (571.20 – 2,871.72); (35.60)	513.86 ± 191.21; 486.20; 458.90 (329.75 – 1,038.61); (37.20)	48.25 ± 29.89; 41.30; 38.29 (13.59 – 123.93); (62.00)
Ke (1/h), mean ± SD; geometric mean; median (range); (CV%)	0.07 ± 0.02; 0.07; 0.07 (0.03 – 0.09); (23.20)	0.07 ± 0.02; 0.07; 0.07 (0.09 – 1.12); (20.60)	0.06 ± 0.05; 0.05; 0.04 (0.02 – 0.29); (23.20)	0.06 ± 0.02; 0.06, 0.07 (0.03 – 0.09); (25.40)	0.08 ± 0.02: 0.07; 0.07 (0.05 – 0.12); (21.90)	0.05 ± 0.02; 0.04; 0.05 (0.02 – 0.09); (41.30)
Half-life(hours), mean ± SD; geometric mean; median (range); (CV%)	11.6 ± 3.26; 10.68; 10.12 (7.45 – 21.02); (29.40)	9.73 ± 1.86; 9.56; 9.86 (5.97 – 14.33); (19.10);	17.36 ± 9.61; 14.93; 15.52 (82.37 – 38.73); (55.30)	11.59 ± 3.45; 11.15; 10.66 (7.32 – 20.33); (29.80)	9.59 ± 2.04; 9.38; 9.26 (5.87 – 14.32); (21.30)	17.40 ± 8.71; 15.74; 14.63 (8.06 – 43.19); (50.10)
AUC_{0-inf}* (ng×h/mL), mean ± SD; geometric mean; median (range); (CV%)	1,655.99 ± 610.64; 1,560.55; 1,545.36 (784.09 – 1,549.57); (36.90)	609.16 ± 323.08; 556.20; 550.97 (299.81 – 1851.82); (53.00)	53.60 ± 29.70; 46.50; 43.80 (16.2 – 132.8); (55.40)	1,587 ± 583.12; 1,494.84; 1,445.98 (624.98 – 3,602.86) (36.70)	557.69 ± 194.20; 530.94; 499.32 (376.05 – 1,081.50) (34.80)	55.40 ± 32.00; 48.20; 44.30 (15.40 – 131.1); (57.80)

CV% = Coefficient of variation; SD = standard deviation. *There were 4 subjects with an extrapolated part of AUC-inf higher than 20% regarding RSV and no subjects with an extrapolated part of AUCinf of 20% regarding CC and HCTZ.

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Table 4. Bioequivalence analysis for candesartan cilexetil following single-oral dose administration of either a test or reference treatments (n = 24).

Pharmacokinetic parameters	Ref GeoLSM[1]	Test GeoLSM[2]	Ratio (% Ref)	90% CI Classical
C_{max}, ng/mL	130.71	138.77	106.17	95.01 – 118.62
AUC_{0-last}, ng×h/mL	1,398.54	1473.91	105.39	95.51 – 115.09
AUC_{0-inf}, ng×h/mL	1,494.84	1560.54	104.40	95.72 – 113.85

[1]Ref GeoLSM= reference geometric least squares mean; [2]Test GeoLSM = test geometric least squares mean.

Table 5. Bioequivalence analysis for hydrochlorothiazide following single-oral dose administration of either test or reference treatments (n = 24).

Pharmacokinetic parameters	Ref GeoLSM[1]	Test GeoLSM[2]	Ratio (% Ref)	90% CI Classical
C_{max}, ng/mL	70.00	70.39	100.55	91.88 – 110.05
AUC_{0-last}, ng×h/mL	467.79	478.64	102.32	96.22 – 108.80
AUC_{0-inf}, ng×h/mL	512.18	525.54	102.61	96.56 – 109.04

[1]Ref GeoLSM = reference geometric least squares mean; [2]Test GeoLSM = test geometric least squares mean.

Table 6. Bioequivalence analysis for rosuvastatin following single-oral dose administration of either test or reference treatments (n = 24).

Pharmacokinetic parameters	Ref GeoLSM[1]	Test GeoLSM[2]	Ratio (% Ref)	90% CI Classical
C_{max}, ng/mL	4.45	4.50	101.07	82.43 – 123.92
AUC_{0-last}, ng×h/mL	41.29	39.66	96.05	81.05 – 113.83
AUC_{0-inf}, ng×h/mL	48.80	46.49	96.46	82.54 – 112.72

[1]Ref GeoLSM = reference geometric least squares mean; [2]Test GeoLSM = test geometric least squares mean.

when dispensed as immediate-release dosage forms. The dissolution rate is lower for BCS-class II drugs due to low solubility in aqueous solutions. Although they are able to permeate membranes easily, the low solubility results in a low concentration gradient in the intestine thus limiting absorption [31]. BCS-class IV drugs also exhibit low solubility but permeability through membranes constitutes an additional impairment to absorption [38, 39]. In order to match the different dissolution profiles of the three active substances in a fixed-dose formulation with respect to the innovator products was an important issue prior to the clinical PK study (Figure 1). The dissolution profiles of the three active drugs for test and reference formulations were in agreement with previous in vitro results reported. CC and RSV are poorly soluble at low pH values, whereas solubility values for HCTZ are variable at low pH values and decrease slightly with increasing pH-values [40, 41]. As shown in Figure 1A, the dissolution profiles from the three active drugs of test and reference formulation in USP-43 media were similar and within dissolution tolerance ranges. However, in vitro dissolution profiles for CC from test and reference formulations differed in hydrochloric acid media and matched in pH 4.5 and pH 6.8 buffers as shown in Figure 1B. The HCTZ dissolution profiles for test and reference products did not differ (Figure 1C). RSV profiles for test and reference formulations were dissimilar in low pH standard media (pH 1.2 and pH 4.5) at early sampling times (Figure 1D). These differing dissolution profiles in test and reference products for CC and RSV can be partially explained by differences in composition (hard capsule containing tablets as test formulation versus tablets (non-encapsulated) as reference formulation) that could have influenced the percentage of each drug released. However, these differences in the in vitro dissolution profiles regarding CC and RSV were not reflected in the PK comparison in the in vivo study since similar PK profiles for all active substances were observed for test and reference formulations.

Stability under recommended storage conditions is guaranteed for the three ac-

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tive ingredients in the newly developed FDC capsule. Long-term stability results meet the specifications for all tests carried out under climatic zone IVb conditions (30 °C /76% RH), whereas at 6 months, under accelerated conditions (40 °C/75% RH), a single known impurity identified as benzothiadiazine, a HCTZ related compound A, was, as expected, slightly above the acceptable limit. However, it is emphasized that this result is not observed under the storage conditions stated in the product description leaflet.

Bioavailability parameters for CC and HCTZ were in good agreement with a previous report describing a single-dose, open label, crossover BE study in healthy adult subjects submitted to the FDA by the marketing authorization holder (MAH) for U.S. registration of the innovator product (CC/HCTZ FDC tablet). The study compared the bioavailability between the fixed combination of CC/HCTZ 16/12.5 mg tablet and co-administration of a CC 16 mg tablet and a HCTZ 12.5 mg tablet [20]. Thus, after a single oral dose of CC/HCTZ 16 mg/12.5 mg under fasting condition, the mean C_{max}, and mean $AUC_{0–inf}$ values observed for CC (CC/HCTZ FDC tablet) were 111.3 ± 38.7 ng/mL and 1,187.8 ± 351.5 ng×h/mL, respectively and for HCTZ: 69.8 ± 21.2 ng/mL and 446.3 ± 100.4 ng×h/mL, respectively. These values for CC and HCTZ are slightly lower than those observed in the present study. However, half-life data for CC and HCTZ in the previous study for both formulations were also comparable to the results observed in the present study. The PK data reported for CC in this study correlates well with data from an open-label, single-dose, crossover BE study carried out in healthy subjects where plasma concentrations of CC were also determined by LC-MS/MS. C_{max} in the reference and test formulation (142.6 ± 41.0 and 139 ± 41.4 ng/mL) were quite similar to our report [42]. These minor differences in PK parameters can be attributed to variability in C_{max} (expressed as CV%) of CC for test and reference C_{max} (40.4% and 50.2%) and $AUC_{0–inf}$ (36.9% and 36.7%) and C_{max} for HCTZ for test and reference (38.8%) and $AUC_{0–inf}$ (53.0%) in the present study. The PK parameters for CC and HCTZ reported in the present study were also closely similar to the PK values for the first dose of CC 16 mg and HCTZ 12.5 mg obtained in a subgroup of patients with moderate essential hypertension stated in the comparative PK study referred to above. In that study no statistically significant differences in CC and HCTZ C_{max} or AUC could be observed when the drugs were used in combination or administered separately [20]. Plasma PK parameters for RSV 10 mg from both test and reference formulations obtained in the present study did not differ greatly from those values in a previous PK report where the mean C_{max} and $AUC_{0–inf}$ values were 6.3 (range 2.6 – 12.7) ng/mL and 47.6 (range 23.9 – 85.5) ng×h/mL, respectively [16, 22]. These results must be interpreted with caution because of the relatively high variability in the PK parameters of RSV with (C_{max} CV: 70.9% and $AUC_{0–inf}$ CV: 55.4%). The half-life of RSV reported in this study is in agreement with literature reports [16].

PK profiles in regard to the three active ingredients examined showed no drug-drug interactions in both PK and pharmacodynamic (PD) data when the active agents were combined in an FDC formulation. Moreover, the occurrence of non-linear PKs which could affect the BE results have also not been observed [23]. Food effects can increase the absorption of BCS-class II compounds such as RSV and HCTZ, although there is no clear trend for BCS-class IV such as CC [23]. As a precaution, to minimize the impact of a food-effect on bioavailability, the study was conducted under fasting condition.

In accordance with international guidelines, monotherapy lowers blood pressure in some patients, but most patients require treatment with two or more active agents. In patients with grade 1 hypertension, treatment with antihypertensive drugs should be considered in those at risk of cardiovascular disease, or if there is a risk of cardiovascular disease, or when there is a chronic rise in blood pressure. If the target blood pressure is not reached, combination therapy is recommended with more than one active agent [43]. The fixed combination product containing CC/HCTZ/RSV is intended for use in patients with hypertension and hypercholesterolemia where there is an intermediate risk of CVD. Treatment can be administered either as initial combination treatment or as substitution therapy when switching patients already stabilized on the same drug entities administered single at the same dose interval and time of day [26]. Switching (or substitution) from a variable-dose, multiple-pill angiotensin II receptor blocker (ARB)

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together with a thiazide to therapy with a fixed-dose, single-pill formulation has been effective and safe in decreasing blood pressure in clinical practice [24]. In a systematic review of nine randomized controlled trials with more than 7,000 patients, FDC therapy, by simplifying disease management, improved adherence by 44% (26 – 65%) compared to a multidrug strategy compared to the commonly used in treatment schedules (4 trials, 3,835 participants, quality of evidence stated to be moderate) [44].

Conclusion

1. Despite differences in in vitro dissolution performance of the active agents and components in the formulation impinging on the pharmaceutical equivalence, this study has shown that the new FDC of CC, HCTZ and RSV 16 mg/12.5 mg/10 mg in a hard capsule formulation is bioequivalent to the co-administration of the component reference formulations.

2. In the in vivo PK profiles, both the point estimates and 90% CIs for the C_{max}, $AUC_{0–last}$, and $AUC_{0–inf}$ were in the range 80 – 125% and no statistically significant difference were found for fixed effects using ANOVA for ln C_{max}, ln $AUC_{0–last}$, and ln $AUC_{0–inf}$.

3. Since the safety profiles were also satisfactory, the triple combination is pharmacokinetically comparable to similar formulations investigated in the HOPE study on the primary prevention of cardiovascular disease.

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Authors' contributions

All authors have contributed to the composition of the manuscript.

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Conflict of interest

The authors GA Yerino, EC Feleder, EK Halabe state no conflict of interest in relation to the present manuscript. The study has been financed by Gador SA.

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