Section C: Pharmaceutics and Pharmaceutical Manufacturing.

Research Article

Formulation and pharmacokinetic evaluation of a single tablet formula containing montelukast sodium and fexofenadine hydrochloride

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Abstract

The approval of leukotriene antagonists like montelukast and antihistamines such as fexofenadine has opened up new possibilities for the treatment and prevention of asthma and allergic rhinitis. It has been observed that combining both drugs concurrently yields more favorable results compared to using them individually. In light of this, the aim of this study was to develop, evaluate, and perform a pharmacokinetic analysis of a single-dose tablet containing both montelukast sodium (a leukotriene antagonist) and fexofenadine hydrochloride (an antihistamine). The goal was to enhance patient compliance and reduce treatment expenses. Four tablet formulations were prepared, and the impact of different diluents and super disintegrants was examined. Based on in vitro dissolution testing, the formulation containing Avicel PH 101 as the diluent and Crospovidone as the super disintegrant was chosen as the test formulation for pharmacokinetic study. This study involved human volunteers, and the bioequivalence of the test formulation with the commercial products Telfast® and Singular® was assessed. The results demonstrated that the test formulation exhibited comparable rates and extents of absorption to the reference products. To analyze montelukast sodium and fexofenadine hydrochloride in a single-dose film-coated tablet in both dissolution medium and human plasma, new HPLC and LC-MS/MS analytical methods were successfully developed and validated.

Introduction

Numerous studies have been undertaken to enhance our comprehension of the pathophysiology of asthma and the crucial role played by the underlying inflammatory process. [1]. The pharmacological management of asthma primarily relies on the administration of corticosteroids as the initial choice for preventive anti-inflammatory treatment. Additionally, long-acting inhaled β2 agonists, theophyllines, and more recently, anti-leukotrienes are employed as second-line controller treatments [2].

The disadvantage of inhaled corticosteroid is the high probability of dose related systemic adverse effects e.g. adrenal suppression, growth suppression, osteoporosis, ocular hypertension and cataracts [3]. To mitigate the potential local and systemic adverse effects linked to inhaled corticosteroids, the substitution of low-dose inhaled corticosteroids with leukotriene antagonists has been proposed as an alternative for individuals with mild to moderate asthma [4]. Leukotriene antagonists, including montelukast, possess anti-inflammatory and bronchodilator properties without inducing tolerance. Additionally, their oral administration provides an

advantage by eliminating potential compliance issues associated with the inhalation route of administration [1]. The compliance aspect of leukotriene antagonists may be further supported by the fact that they demonstrate effectiveness within the initial 24 hours of use, whereas inhaled corticosteroids require a longer duration to reach their maximum response. [5]. In addition, regular treatment with montelukast produces a sustained high protection against exercise induced bronchoconstriction [6] and are effective in treating coexistent allergic rhinitis [7].

Fexofenadine, a type of antihistamine that doesn't cause drowsiness, works by preventing bronchospasm triggered by antigens and blocking the release of histamine from peritoneal mast cells. Clinical trials have demonstrated that fexofenadine can alleviate symptoms related to allergic conditions, such as seasonal allergic rhinitis [8], [9].

Research has been carried out to assess the enhanced effectiveness of combining leukotriene antagonists with antihistamines. One such study examined the effectiveness of montelukast, either administered on its own or simultaneously with loratadine, an H1-receptor antagonist, in treating seasonal allergic rhinitis. The findings indicated that the combined use of montelukast and loratadine was an effective treatment for seasonal allergic rhinitis and related eye symptoms, with a safety profile similar to that of a placebo [10]. Preliminary data suggest that antihistamines and leukotriene antagonists may show additive effects on control in asthma and allergic rhinitis [7, 11].

The aim of this recent study was to develop and assess a single dose formulation that combines montelukast and fexofenadine. This approach was intended to enhance patient adherence by reducing the number of tablets required from two to one, potentially lowering costs. Additionally, a pharmacokinetic study was carried out on the chosen formula using human volunteers to investigate the impact of combining both drugs into a single tablet on the pharmacokinetic parameters of each drug. The study also compared the bioequivalence of the newly formulated single dose tablet with commercially available tablets containing montelukast (Telfast®) and fexofenadine (Singular®).

Materials and methods

Materials

Montelukast sodium, fexofenadine hydrochloride and levocetirizine were purchased from Matrix Lab. Ltd, India, Hetero Drugs Ltd, India and Proctor and Gamble, Egypt, respectively. Crospovidone; magnesium stearate; lactose monohydrate; sodium starch glycolate; colloidal silicon dioxide; talc powder purified were supplied from CID company, Egypt. The other materials used in the study were Avicel PH 101 (FMC corp., USA), methocel E-5, PEG 6000 and titanium dioxide (chemSwiss ag, switzerland), sodium lauryl sulphate (SLS) (El Nasr Pharmaceutical Chemicals Co., Egypt) and hydrochloric acid (ADWIC, Egypt). The solvents (HPLC grade) used in the study were acetonitrile and methanol (Sigma – Aldrich, USA), triethanolamine (Alpha Chemika, India) and orthophosphoric acid (ADWIC, Egypt).

Methods

Compatibility tests

A variety of excipients were used to prepare physical mixtures of montelukast sodium and fexofenadine hydrochloride. These excipients included Avicel PH 101, Crospovidone, magnesium stearate, lactose monohydrate, sodium starch glycolate, colloidal silicon dioxide, and purified talc powder. The potential interactions of these prepared mixtures were then evaluated through several methods, including visual examination, differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FTIR).

Visual examination

Mixtures of montelukast sodium and fexofenadine hydrochloride, along with the specified excipients, were visually inspected both immediately and after being stored for four weeks at a temperature of 50°C. During the first week, samples were taken and examined daily, and then on a weekly basis for the remaining period. These samples were visually assessed for any changes in appearance, such as discoloration, caking, liquefaction, or the formation of clumps.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry studies (Schimadzu DSC 50, Kyoto, Japan) were performed for montelukast sodium, fexofenadine hydrochloride, the drugs mixture, the aforementioned excipients, and for mixtures of each drug with each excipient powder. Samples (1-2 mg) were placed in aluminum pan and heated at a scan rate of 10°C/min from 25 °C to 250 °C, with indium in the reference pan, in an atmosphere of nitrogen [12].

Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectra (FT-IR spectrophotometer; Bruker 22, UK) in the range of 4000 and 500 cm^{-1} for montelukast sodium, fexofenadine hydrochloride, the drugs mixture, the aforementioned excipients, and for each drug-excipient powder mixtures were determined using the KBr disc technique [13].

Formulation of tablets

Factorial design experiments were built up to study the effect of the diluent type (Avicel PH 101 and lactose monohydrate) and the superdisintegrant type (Crospovidone and sodium starch glycolate) (Table 1 & 2).

Table 1: The formulae of montelukast sodium-fexofenadine hydrochloride tablets for factorial experimental design.

Diluent	Superdisintegrant	
	Crospovidone	Sodium starch glycolate
Avicel PH 101	F1	F2
Lactose monohydrate	F3	F4

Table 2: Factors and Levels for the Factorial Design of Montelukast Sodium –Fexofenadine HCl Tablet.

Factor	Level
Diluent	Avicel 101
	Lactose monohydrate
Superdisintegrant	Crospovidone
	Sodium starch glycolate

Four tablet formulae were prepared containing drugs, diluent, superdisintegrant, lubricants (magnesium stearate and talc powder purified) and glidant (colloidal silicon dioxide). In all formulae the concentrations of drugs were held constant at 120 mg fexofenadine hydrochloride and 10.5 mg montelukast sodium (equivalent to 10 mg montelukast) per 450 mg of formulation (the tablet weight). The superdisintegrant, lubricant and glidant concentrations were also kept constant at 2.2 %, 3 % and 0.2 % respectively.

The blends were weighed, blended using the wet method [14]. Fexofenadine, montelukast, diluent (Avicel PH 101 or lactose monohydrate) and superdisintegrant

(Crospovidone or colloidal silicon dioxide) were sieved and mixed well. The mixture was granulated using ethyl alcohol 70 % and the resulting wet mass was then dried using clean stainless-steel trays in a circulating-air oven

at 40°C till moisture content was not more than 1 %. Sodium starch glycolate, magnesium stearate and talc powder were added and mixed and then compressed. The blends were compressed on 12 mm punch and die set (Single punch compression machine; Royal Artist, India) with a single punch machine at 450 mg theoretical weight and at approximately equal hardness (14-16 kg).

Since both drugs are optically active [15, 16] all tablet formulae were film coated. Tablets were coated in Manesty 150 coating pan (Liverpool, UK). A solution of methocel E-5 in isopropyl alcohol and a dispersion of PEG 6000, talc purified powder and titanium dioxide in purified water were mixed and transferred to the coating vessel. Tablets cores were added into the coating pan and the coat was sprayed till the average weight of the tablets reached 465 mg at pan speed 5-8 rpm, product temperature 40 – 45°C and air volume 2000 m^3/hr. The inlet and outlet temperatures were 50 -55°C and 30 – 35°C, respectively.

Characterization of the blends to be compressed

Random samples of the blends were examined to ensure that the mixing was sufficiently uniform. The flow properties of the prepared blends were evaluated by determining the bulk density, Carr's index, and Hausner ratio. Each blend contained 25 grams and was placed in a 25 ml graduated cylinder to measure the volume it occupied (referred to as the bulk volume, V_b). The cylinder was then tapped until a consistent volume was achieved, and the resulting volume of the powder (known as the true or tapped volume, V_t) was recorded. The parameters were calculated as outlined below:

Bulk density = weight of the powder under test / V_t [17].

Carr's index (%) = $(1 - V_t / V_b) \times 100$ [18].

Hausner ratio = V_b / V_t [19].

ANOVA test was performed at 95 % confidence limit using StatView program (version 4.5) to test the significance of changing diluent and disintegrant on the flow properties of the blend.

Evaluation of the prepared tablets

Weight variation

The test was carried out according to the British Pharmacopoeia (BP) [20]. From each formula, a total of twenty tablets were weighed individually. The mean weight of the tablets was calculated, and based on this, the weight variation was determined. In order to pass the test, the tablets should have no more than two tablets that deviate from the average weight by more than 5% (for uncoated and film-coated tablets weighing 250 mg or more), and none should deviate by more than twice that percentage.

Thickness and diameter

The thickness and diameter of ten tablets from each formula were measured using a micrometer and the mean values were calculated.

Content uniformity

For each formula, a single tablet was crushed and placed in a 100 ml volumetric flask. Then, 70 ml of the mobile phase, which consisted of a mixture of acetonitrile, triethylamine buffer solution, and methanol in a ratio of 400:350:250 by volume, was added to the flask. The mixture was shaken for 15 minutes and sonicated for an additional 15 minutes. The volume in the flask was completed with the mobile phase and then filtered.

According to the British Pharmacopoeia (BP) 20, the determination of the active ingredient's content in film-coated tablets is required only when the amount of the active ingredient is either less than 25 mg or less than 25% of the total tablet weight. Consequently, in this case, only the amount of montelukast sodium (10.5 mg) was determined using the high-performance liquid chromatography (HPLC) method described in section 2.6. The test was conducted on a total of 10 tablets for each formula.

Hardness

Ten tablets from each formula were tested for their hardness (Hardness Tester, Campbell Electronics, Type C-DHT 200, Bombay, India). The mean hardness was determined in kg.

Disintegration

The evaluation of film-coated tablets was carried out according to the BP 20 guidelines. To determine the disintegration time, six tablets from each formula were utilized. Each tablet was placed into the disintegration apparatus, which was filled with distilled water maintained at a temperature of 37 ± 2 degrees Celsius. The apparatus was operated for a duration of 30 minutes. Subsequently, the tablets were inspected to check for any remaining clumps. To pass the test, all six tablets must have undergone complete disintegration.

In-vitro dissolution study

The dissolution of both drugs present in each tablet formulation was conducted in 900 ml of 0.5% SLS (sodium lauryl sulfate) simulated gastric fluid, which had a pH of 1.2 and was prepared using 0.1 N HCl. The USP paddle dissolution test apparatus (Dr. Schleuninger Pharmatron, Thun, Switzerland) was utilized for this purpose. The dissolution test was carried out at a rotation speed of 100 rpm for a duration of 90 minutes at a temperature of $37°C \pm 0.5°C$.

At specific time intervals (5, 10, 15, 30, 45, 60, and 90 minutes), aliquots of 5 ml were withdrawn from the dissolution medium. These aliquots were then filtered and subjected to analysis using high-performance liquid chromatography (HPLC). The peak response for each drug in each sample was recorded, and the percentage of drug released was calculated based on these measurements.

HPLC analysis

Reverse phase chromatography was performed on Hypersil BDS C18 columns (150×3.9 mm, 5µm particle size) from Jones Chromatography Inc., Lakewood, CO.

The chromatography was carried out under isocratic reverse phase conditions at a temperature of $25 \pm 1°C$.

The mobile phase used in the analysis consisted of a mixture of acetonitrile, triethylamine buffer solution, and methanol in a ratio of 400:350:250 by volume. The pH of the mobile phase was adjusted to 4.5 using orthophosphoric acid and triethylamine. The mobile phase was delivered to the analytical columns at a flow rate of 0.5 ml min^{-1}.

For the detection of peaks, a Diode Array Detector (DAD) was employed, operating at a wavelength of 254 nm. Twenty micrograms of sample solutions were injected into the chromatographic system.

Due to the sensitivity of montelukast and fexofenadine to light, precautions were taken to protect the stock solutions, calibration standards, quality control samples, and dissolution samples. These samples were either stored in amber glass containers or shielded from light by wrapping the tubes in aluminum foil.

Validation of the HPLC method

Linearity

To assess linearity, five distinct concentrations of montelukast sodium and fexofenadine hydrochloride standard solutions were prepared. Calibration curves were constructed to establish the correlation between the measured areas and the corresponding concentrations. Regression equations were then calculated based on these calibration curves. Linearity was determined by evaluating the squared correlation coefficient, which is expected to be equal to or ≥ 0.99.

Precision

To assess repeatability (intraday precision) and intermediate precision (interday precision), nine determinations were performed across three concentration levels that spanned the specified range. Each concentration level consisted of three replicates. The intraday precision was evaluated by calculating the relative standard deviation (RSD) based on the results obtained within the same day. Similarly, the interday precision was determined by calculating the RSD using the results obtained over different days.

Accuracy and recovery

The placebo matrix was enriched with specific amounts of fexofenadine hydrochloride and montelukast sodium from a stock standard solution. The concentration of each drug in every sample was determined using the respective regression equation. The accuracy of this process was evaluated by measuring the percentage recovery of each drug.

Selectivity and specificity

The selectivity of this new analytical method was tested by examining the response of fexofenadine hydrochloride and montelukast sodium in the presence of known concentrations of excipients. The criteria for acceptance are met if there is no overlap between the peaks of the drug in the test sample and the peaks associated with the

excipients or impurities, and if the resolution factor between the sample peak and solvent peak is at least 1.5.

Pharmacokinetic and bioequivalence study

The research was carried out at the Drug Research Centre (DRC) in Cairo, Egypt, under the study code no.: MON-FEX-RES-S-0511/0001 and was supervised by Dr. Nagwa Sabri. The study adhered to the guidelines of the International Conference on Harmonization (ICH) and Good Clinical Practice (GCP), as adopted by The European Agency for the evaluation of medicines for human use (EMEA). All procedures in this study were in accordance with standard operating procedures (SOPs). The DRC's Institutional Review Board (IRB) was responsible for ensuring the study's ethical conduct and approving the protocol, operating in line with the principles of the Declaration of Helsinki.

The study was done to investigate the effect of the combination of both drugs in single tablet (montelukast/fexofenadine 10/120 mg) on their pharmacokinetic parameters and to study its bioequivalence versus Singular® 10 mg tablets and Telfast® 120 mg film coated tablets (reference products).

Subjects

Eight healthy non-smoking adult volunteers participated in this study; the mean age and body weight were 37 years (18-56 years) and 70 kg (60-80 kg), respectively. All subjects read and signed informed consent forms before participating. Physical assessments and clinical laboratory tests for all subjects were within normal limits. Subjects were required to abstain from alcohol and any food or drink containing methylxanthines for 48 hours before the study until the final blood sample was collected for that study period. Furthermore, the intake of medications and any food or drink containing grapefruit was not allowed one week prior to the study and continued to be prohibited throughout the entire duration of the study.

Study design

The study was a single-center, open-label, randomized, single-dose study with a two-way crossover design. Subjects were divided into two groups A and B, each consisting of 4 subjects. Subjects of group A received one tablet of montelukast/fexofenadine 10/120 mg (test tablet) and group B received one tablet of Singular® 10 mg and one film coated tablet of Telfast® 120 mg (reference tablets). The subjects administered the tablets with 300 ml of water under fasting condition. All volunteers fasted 4 h after the drug administration, and then they received a snack. Standardized meals (lunch, afternoon snack, dinner and breakfast) were provided to volunteers. After a 7 days washout interval, each group received the other treatment.

Sampling

For the purpose of determining drug levels in plasma, a volume of 5 ml of blood was drawn per sample. Blood samples were taken at the following times: immediately before dosing (0), and then at 0.25, 0.5, 0.75, 1, 1.25, 2, 2.5, 3, 4, 5, 6, 8, 10, 12-, 24-, 48-, and 72-hours post-

administration. The blood was collected into tubes containing disodium EDTA as an anticoagulant, gently mixed, and then centrifuged at roughly 4000 rpm for 10 minutes. Following centrifugation, the plasma samples were immediately transferred to a 5 ml plastic tube and stored in a freezer at an approximate temperature of -20°C. They were later moved to a deep freezer for storage at -80°C until analysis. The samples were analyzed using liquid chromatography–mass spectrometry (LC-MS/MS).

Pharmacokinetic calculations

The study determined the pharmacokinetic parameters of montelukast/fexofenadine, which included the maximum plasma concentration (C_{max}), the time at which the maximum plasma concentration is reached (t_{max}), the half-life of drug elimination during the terminal phase ($t_{1/2e}$), the rate constant of elimination (K_e), the area under the plasma concentration-time curve from zero to the last measurable concentration (AUC_{0-t}), the area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$), and the mean residence time (MRT). Additionally, the study estimated the amount of drug absorbed and calculated the percentage of the area measured by AUC_{0-t} relative to the extrapolated total $AUC_{0-\infty}$.

The computation of all pharmacokinetic parameters was performed as follows: The maximum plasma concentration (C_{max}, ng/ml) and the time to reach it (t_{max}, hr) were directly determined from the individual plasma level curve of each volunteer. The area under the plasma concentration-time curve from time zero to t hrs (AUC_{0-t}, ng.hr/ml) was calculated using the linear trapezoidal rule. The terminal elimination rate constant (K_e, hr^{-1}) was calculated from the negative slope of the linear regression of the log-transformed plasma concentrations versus time in the terminal period of the plasma curve. The terminal half-life ($t_{1/2e}$, hr) was calculated by dividing 0.693 by K_e. The amount of drug absorbed was calculated by dividing C_{max} by $AUC_{0-\infty}$. The area under the plasma concentration-time curve was extrapolated to infinity and $AUC_{0-\infty}$ (ng.hr/ml) was calculated by dividing the last detectable plasma concentration (C_t, ng) by the corresponding elimination-rate constant (K_e, hr^{-1}) and adding the result to the respective AUC_{0-t} value, i.e., $AUC_{0-\infty} = AUC_{0-t} + (C_t/K_e)$. The relative bioavailability (F_{rel}) of the tested formulations was then calculated:

$$F_{rel} = \frac{AUC_{0-\infty} \text{ (tested formula)}}{AUC_{0-\infty} \text{ (reference formula)}} \times 100$$

All calculations were conducted using the Winnonlin Pharmacokinetic Program. The statistical analysis was carried out using a one-way analysis of variance (ANOVA) test. Any differences were deemed statistically significant at a certain level $p < 0.05$.

Table 3: General mass spectroscopy parameters

Name	Scan	Precursor ion	Product ion	Delta EMV*	Dwell time (ms)	Fragmentor	Collision energy (J)
Montelukast	MRM⁺	586.2	568.2	400	200	140	15
Fexofenadine	MRM	502	466.4	400	200	150	32
Levocetirizine	MRM	389.9	166.1	400	400	80	70

*EMV: electron multiplier voltage

⁺MRM: multiple reaction monitoring

Bioanalytical drug determination methodology

The determination of montelukast and fexofenadine in human plasma was carried out using a high-performance liquid chromatography system, which was paired with a Triple Quad Mass Detector LC-MS/MS.

Chromatographic conditions

The LC-MS/MS system (Agilent 1200 series, UK) with a data system (Mass Hunter, Agilent) consisted of a degasser (Agilent 1200 series, UK), a mass detector (Agilent 1200 series Triple Quad, UK) equipped with an autosampler injector (Agilent 1200 series, UK). The analytical column employed was C18 Hypersil Gold, 50 x 4.6 mm, 5 µm particle size (Agilent Technologies, Inc. UK). The mobile phase, which was a mixture of acetonitrile and 20 mM formic acid in an 85:15 v/v ratio, was prepared on a weekly basis and filtered prior to use.

The separations were conducted at a flow rate of 0.6 ml/min, with the column condition kept at room temperature.

Mass spectrometric conditions (MS)

The Agilent 6410 operated in positive electrospray ionization mode. General MS parameters are shown in Table 3. The Chromquest software automatically processed all the chromatograms in the same batch, using identical processing parameters such as integration type, smoothness, peak-to-peak amplitude, and peak detection.

Calibration standards and quality controls

Montelukast and fexofenadine, due to their sensitivity to light, were stored in amber glass containers or shielded from light by wrapping the tube in aluminum foil. The stock solutions of montelukast and fexofenadine, each with a concentration of 100 µg/ml, were prepared separately in a methanol-water mixture (80:20, v/v).

For montelukast, six calibration standards were prepared with concentrations of 10, 25, 50, 100, 200, and 800 ng/ml. For fexofenadine, eight calibration standards were prepared with concentrations of 10, 50, 100, 200, 400, 1200, 1500, and 2000 ng/ml. These standards were prepared by diluting portions of the stock solution with human plasma. Quality control (QC) solutions were

prepared at three different concentrations: low (15 ng/ml; LQC), medium (75 ng/ml; MQC), and high (750 ng/ml; HQC). These were prepared in a manner similar to the calibration standards. Additionally, a working solution of the internal standard, levocetirizine, was prepared in a methanol/water mixture (12:88) to achieve a concentration of 15 µg/ml. A solution of 0.72 µl/ml of formic acid in water was also prepared. All stock solutions were stored at 4 °C and all prepared plasma samples were stored at -40°C until analysis.

Sample preparation

All plasma samples were allowed to thaw at room temperature. To 500 µl of the thawed plasma, 50 µl of the internal standard solution (15 µg/ml levocetirizine working solution) and 1 ml of acetonitrile were added in a screw-cap glass tube. This mixture was then vortex-mixed for 60 seconds and centrifuged at 4000 rpm for 5 minutes at 4 °C. The supernatant was carefully transferred to an HPLC vial, and an 8 µl aliquot was injected into the LC-MS/MS system.

Validation of analytical method

This analysis was conducted in conformity with the study protocol and FDA guidance for analytical methods validation.

Specificity

The proposed extraction procedure and chromatographic or spectroscopic conditions were used to test blank samples for interference. These results were then compared with those obtained from an aqueous solution of the analyte, which had a concentration close to the limit of quantification (LOQ).

Linearity

Three calibration curves, prepared independently from different master solutions, of montelukast and fexofenadine were constructed by plotting the peak response (area) (y) versus concentration in plasma (x), and then the regression equations were computed.

Accuracy and precision

To evaluate the precision and accuracy of the analytical method that was developed, three unique concentrations within the anticipated range were tested. Over the course

Table 4: Characterization of the blends used in preparation of different formulae.

Formula	Composition	Mean bulk density (g/ml)	Mean Hausner ratio	Mean Carr's index
F1	Fex+Mon+A101+Cros	0.79 ± 0.001	1.12 ± 0.00	11.06 ± 0.05
F2	Fex+Mon+A101+Na starch	0.77 ± 0.001	1.15 ± 0.00	13.2 ± 0.2
F3	Fex+Mon+Lac+Cros	0.78 ±0.001	1.19 ± 0.00	15.67 ± 0.03
F4	Fex+Mon+Lac+Na starch	0.76 ± 0.002	1.18　0.00	15.28 ± 0.32

Fex (fexofenadine hydrochloride, Mon (montelukast sodium), A101 (Avicel PH 101), Cros (Crospovidone), Lac (lactose monohydrate), Na starch (sodium starch glycolate)

of five different days, each concentration underwent eight determinations. This process was used to establish both the intra-day and inter-day accuracy and precision.

Determination of the limit of quantification (LOQ)

The limit of quantification was defined taking into account the sensitivity, precision and accuracy of the method. To evaluate precision and accuracy, specific quality control samples were included in the validation procedure. Measures were taken to guarantee a LOQ to 1-3 % of the anticipated C_{max}.

Results and discussion

Compatibility of montelukast-fexofenadine tablets with different pharmaceutical excipients

Montelukast sodium, fexofenadine hydrochloride, the drugs mixture, the used excipients, and mixtures of each

drug with each excipient were tested for compatibility by visual examination, DSC and FTIR. Throughout the storage period, both the fresh mixtures and the stored ones did not exhibit any changes in color or appearance. The DSC thermograms (Fig. 1 & 2) and infrared spectra (Fig. 3 & 4) maintained the characteristic peak features of both drugs and the excipients. However, minor shifts were consistently observed in the thermograms and IR spectra of montelukast. These shifts were attributed to the presence of a free carboxylic group in the montelukast salt, which can easily physically interact with other excipients. This interaction, however, is reversible.

Based on the information obtained from previous studies, it can be concluded that, both drugs are compatible with each other and with all tested excipients.

Characterization of the blends to be compressed

The evaluation of the flow characteristics of the blends of the formulated tablets are shown in Table 4. Knowledge of the bulk density of drug substance may indicate the size of final dosage form and powder flow properties. Table 4 shows that the effect of diluent or disintegrant type was not significant on bulk density of the tested tablets.

Carr's compressibility index is used to predict the flow properties based on density measurement. Compressibility index values between 12-18% usually give rise to good flow characteristics, while those above 23% indicate poor flow [17]. Regarding diluents used, it

is obvious from Table 4 that diluent type had a significant effect on Carr's index. Changing the diluent from Avicel PH 101 (F1, F2) to lactose monohydrate (F3, F4) increased Carr's index. This result was in accordance with Sinha et al., 2005 [21] who reported that pellets containing lactose had lower flow properties than those containing Avicel, which they believed to be attributed to the surface and shape irregularities of the former. Moreover, the presence of moisture within the porous structure of microcrystalline cellulose (Avicel) serves as an internal lubricant, promoting slippage and flow among the individual microcrystals [22].

For formulae F1 and F2 the type of superdisintegrant employed appeared to also have a significant effect on Carr's index value. The introduction of sodium starch glycolate instead of Crospovidone increased Carr's index, which may be due to the good flowing properties known for the highly porous popcorn like Crospovidone [23]. Hausner's ratio gives an idea about the flow properties and interparticle friction of the powder; the flow is better when the value of Hausner ratio is close to one. Powders with low interparticle friction have a ratio of 1.2, less free flowing powder have Hausner ratio greater than 1.6. Powder with Hausner ratio between 1.25 and 1.5 needs the addition of glidant to improve flowability [19]. From Table 2 it could be concluded that although the introduction of lactose monohydrate as diluent and Na starch glycolate as disintegrant raised the Hausner ratio values, all formulae had still a Hausner ratio below 1.2, which is an indication of good flowing properties. ANOVA test revealed a significance of difference of $p<0.05$ between the tested factors.

Evaluation of the prepared tablets

Hardness, weight variation, content uniformity, and disintegration time

The physical characterization of the tablets is represented in Table 5. The least hardness value was recorded for F1 (14.99 ± 0.53 kg), while the highest value was recorded for F2 (15.29 ± 0.58 kg), which were within the set-up range for tablet compression. All formulae fulfilled the pharmacopoeia requirements for weight variation, none of the tablets deviated from the average weight by more than 5%. All formulae revealed a drug content of 97% with RSD not more than 6%.

Table 5: Physical characterization of the prepared tablets (before coating)

Formula	Average weight (mg)	Average thickness (mm)	Average diameter (mm)	Average drug content of montelukast Na. (%)	Average disintegration time (min)
F1	449.5±3.5	2.99±0.000	12.99±0.00	97.52±1.01	5.52±.04
F2	450.7±1.89	2.88±0.077	12.89±0.083	97.29±2.65	6.15±.06
F3	450.7±4.11	2.63±0.072	12.99±0.00	97.44±2.24	17.8±.05
F4	448.9±3.48	2.98±0.004	12.99±0.004	97.22±1.43	18.6±.04

The disintegration test showed that all tablet formulations complied with the pharmacopoeia requirements, demonstrating the effectiveness of both disintegrants used in the tablets. Crosspovidone, with its densely crosslinked polymers and porous structure, quickly absorbs liquids into the particle, enhancing swelling and disintegration. Sodium starch glycolate, on the other hand, has a high swelling capacity and excellent water penetration, leading to rapid tablet disintegration.

From Table 5, it's clear that formulas containing Avicel PH 101 as a diluent (F1, F2) disintegrated faster than those containing lactose monohydrate (F3, F4). Avicel PH 101 is known to promote quick water penetration into the tablet matrix through capillary action, causing rapid disintegration by breaking the hydrogen bonds between the bundles of cellulose microcrystals. [23].

Table 6: Percentage of montelukast and fexofenadine released from different formulae in simulated gastric fluid (pH 1.2) after 15 minutes.

Formula	Montelukast Q 15min	Fexofenadine Q 15min
F1	67.82	40.15
F2	62.74	38.41
F3	50.88	37.34
F4	49.48	35.68

In vitro dissolution study

Dissolution study is represented graphically in Fig. 5 and 6. From the different dissolution profiles it could be concluded that formulae F1 and F2 containing Avicel showed better dissolution characteristics than formulae containing lactose (F3, F4). This may be attributed to the disintegration properties of Avicel PH 101 which consequently promoted drug dissolution [23]. Furthermore, the type of superdisintegrant influenced the dissolution rate of formulae containing the same diluent. Formulae containing sodium starch glycolate showed slightly poorer dissolution when compared to the dissolution of tablets containing Crospovidone. This may be due to the fact that starch becomes gel-like when wetted and the gelatinous layer formed would impede the penetration of water into the tablet, thus decreases its contact with the dissolution medium and reduces rate of drug release [24]. From all the above F1 containing both Avicel PH 101 and Crospovidone, which revealed the best dissolution rate (Table 6), was selected for further pharmacokinetic and bioequivalence study.

Validation of HPLC analysis method

A new HPLC analytical method for the determination of montelukast sodium and fexofenadine hydrochloride at a single dose film coated tablet was successfully developed and validated. A complete separation of the two drugs was noticed with sharp peaks, clear baseline separation (Fig. 7), which indicates specificity and selectivity of the method. Satisfactory results were obtained for linearity (Fig. 8), accuracy and precision of the proposed method (Table 7)
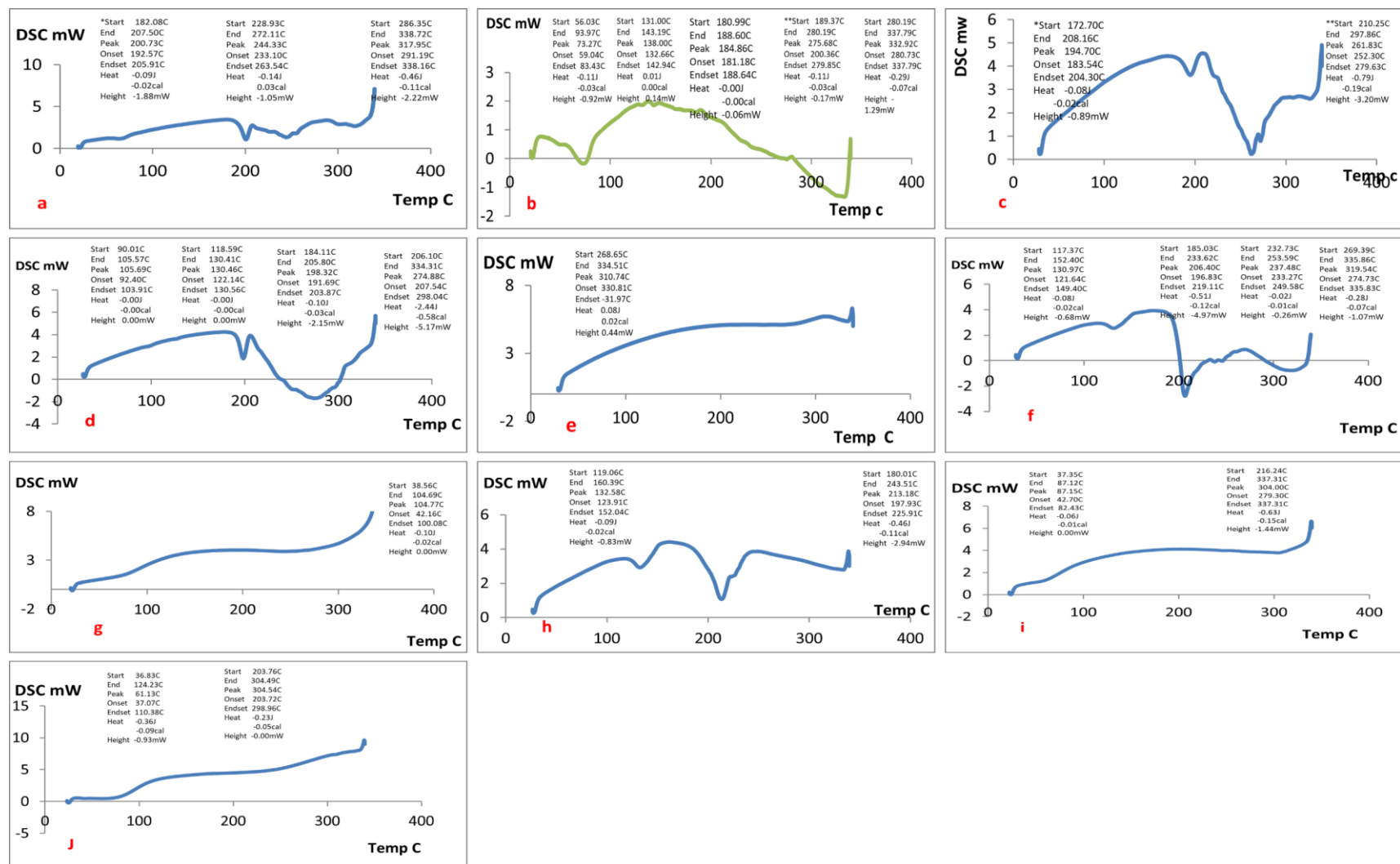
Figure 1: Differential Scanning Calorimetry of a) Fexofenadine Hydrochloride, b) Montelukast Sodium and c) 1:1 Physical Mixture of Fexofenadine & Montelukast, d) Montelukast Sodium and Sodium Starch glycolate, e) Montelukast Sodium and Aerosil, f) Montelukast Sodium and Magnesium Stearate, g) Montelukast Sodium and Avicel, h) Montelukast Sodium and Lactose monohydrate, i) Montelukast Sodium and Talc powder, j) Montelukast Sodium and Crospovidone.
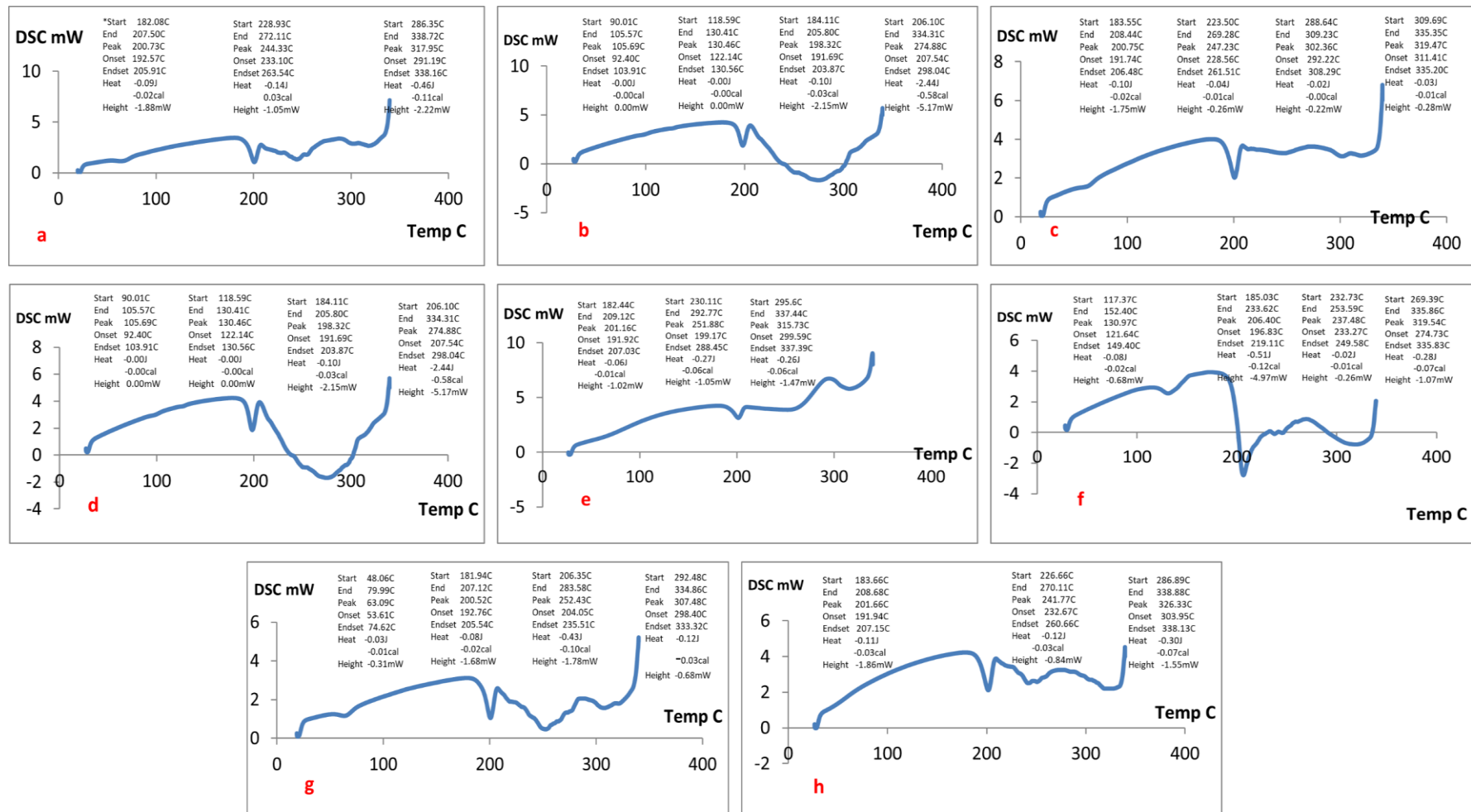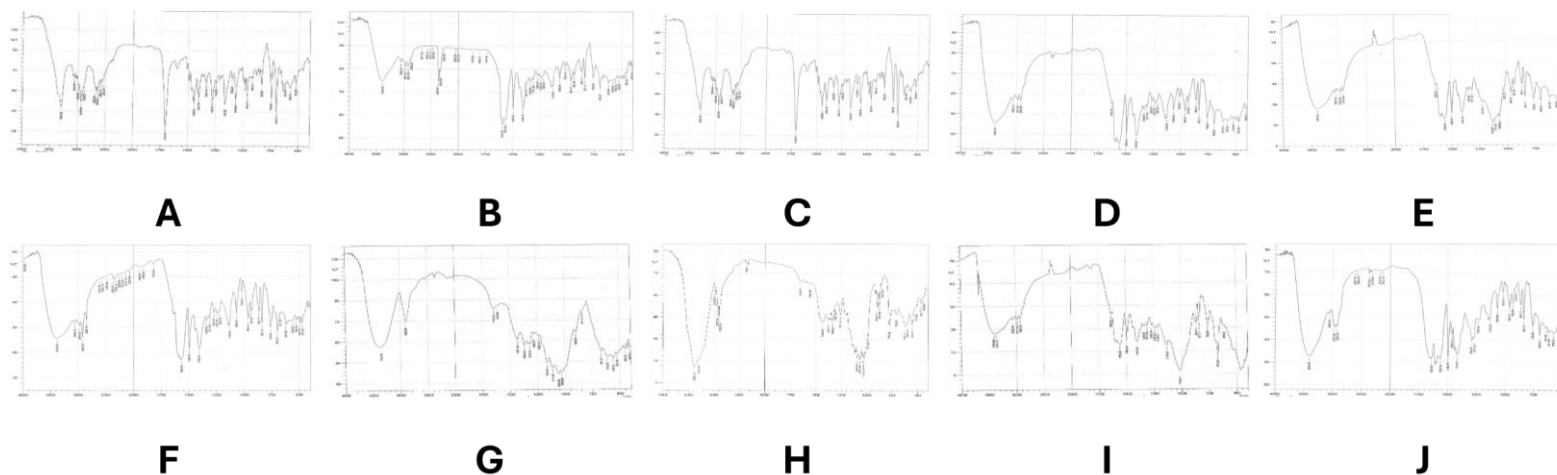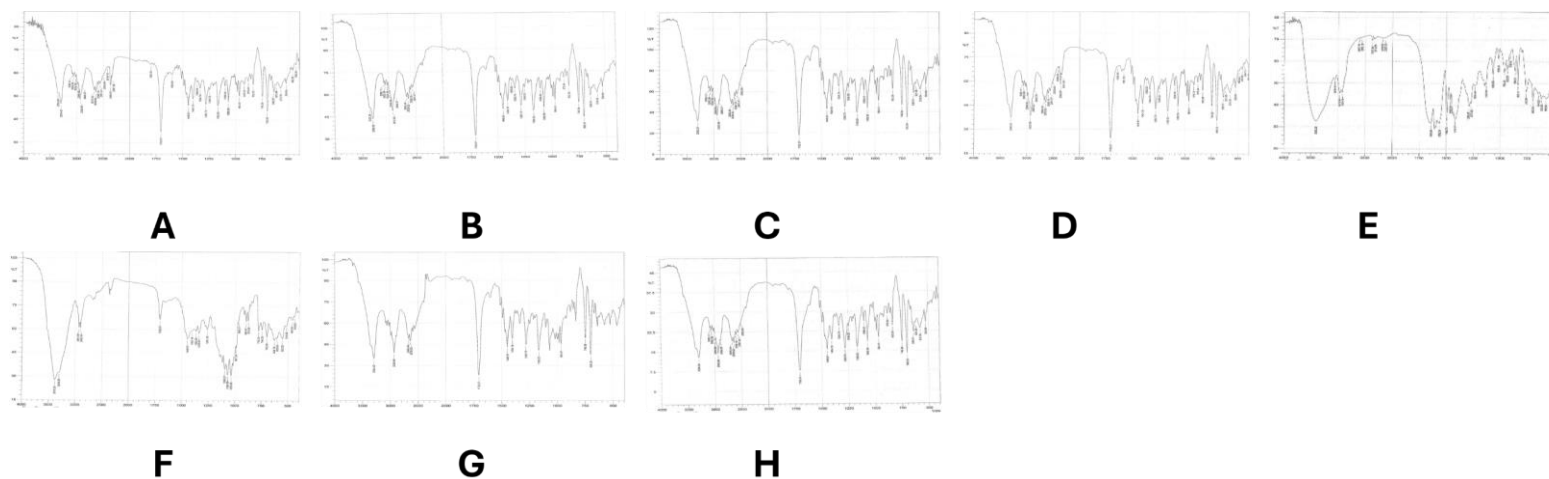
Figure 2: Differential Scanning Calorimetry of a) Fexofenadine Hydrochloride, b) Fexofenadine HCl and Sodium starch, c) Fexofenadine HCl and Aerosil, d) Fexofenadine and Magnesium Stearate, e) Fexofenadine HCl and Avicel, f) Fexofenadine and Lactose, g) Fexofenadine HCl and Talc, h) Fexofenadine HCl and Crospovidone.

Figure 3: FT-IR of a) Fexofenadine Hydrochloride, b) Montelukast Sodium and c) 1:1 Physical Mixture of Fexofenadine & Montelukast, d) Montelukast Sodium and Sodium Starch glycolate, e) Montelukast Sodium and Aerosil, f) Montelucast Sodium and Magnesium Stearate, g) Montelukast Sodium and Avicel, h) Montelukast Sodium and Lactose monohydrate, i) Montelukast Sodium and Talc powder, j) Montelukast Sodium and Crospovidone.

Figure 4: FT-IR of a) Fexofenadine Hydrochloride, b) Fexofenadine HCl and Sodium starch, c) Fexofenadine HCl and Aerosil, d) Fexofenadine and Magnesium Stearate, e) Fexofenadine HCl and Avicel, f) Fexofenadine and Lactose, g) Fexofenadine HCl and Talc, h) Fexofenadine HCl and Crospovidone.

Validation of LC-MS/MS analysis method

For the analysis of montelukast and fexofenadine in human plasma samples a LC-MS/MS method wasdeveloped. The analytical method met the studied validation criteria including linearity, precision and accuracy (Table 8). The recovery experiment for fexofenadine and levocetirizine as well as montelukast and levocetrizine showed a reproducible and consistent recovery at the three tested concentrations. The recovery results were in accordance with the consistency and
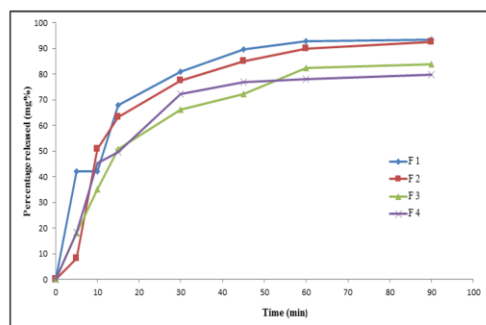
Fig. 5 Percentage of montelukast released from different tablet formulae in simulated gastric fluid (pH = 1.2).
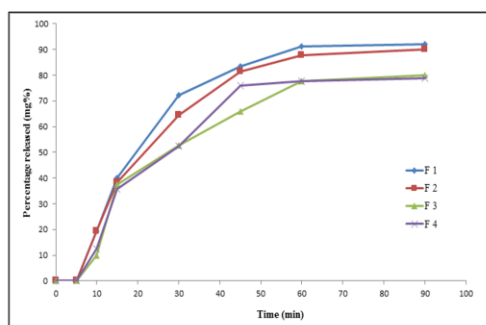
Fig. 6 Percentage of fexofenadine released from different tablet formulae in simulated gastric fluid (pH = 1.2).

reproducibility parameters and were considered suitable for the assay. The method demonstrated both selectivity and specificity, as no interfering peaks were observed at the retention times of either montelukast or fexofenadine. Similarly, no interfering peaks were detected at the retention time of the internal standard used in the study.

Bioequivalence study

The study participants exhibited a high tolerance for both drugs under investigation, and no adverse effects were reported. The pharmacokinetic parameters for montelukast and fexofenadine, for both the test and reference products, are presented in Table 9. Bioequivalence was established within the stipulated 90% confidence interval of 80% to 125% for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} with respect to the parametric method on log-transformed data. The test product examined in this study, montelukast-fexofenadine 10/120 mg tablets, was found to be bioequivalent to the reference products,

Table 7: Results of assay validation of the proposed HPLC methods for the analysis of montelukast sodium and fexofenadine hydrochloride

Parameters	Acceptance criteria	Montelukast sodium	Fexofenadine hydrochloride
Linearity and range	r ≥ 0.99	0.99985	0.99981
Precision and repeatability	RSD ≤ 1%	0.19%	0.11%
Accuracy and recovery*	97-103%	98.68-102.40	99.35-102.42
Specificity/ Selectivity	No interference, Resolution ≥ 2.0	There is no interference between fexofenadine hydrochloride and montelukast sodium and other excipients.	

* The intraday (n=3), average of three different concentrations repeated three times within the day.

Singular® 10 mg tablets and Telfast® 120 mg film-coated tablets. Plasma levels can serve as surrogate markers for clinical efficacy. As such, the data from this study, analyzed using appropriate statistical methods, demonstrate the essential similarity of plasma levels of montelukast-fexofenadine from the test product and the reference products, suggesting equal clinical efficacy of these two treatments (Fig. 9-12). Moreover, the mean plasma curves of both products are nearly identical, indicating that not only the Cmax and AUC, but also the time course of plasma levels throughout the entire sampling period, are the same. ANOVA of log-transformed data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and of the untransformed data for C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and t_{max} demonstrated that sequence effect, product effect, and period effect for all bioequivalence metrics did not significantly influence the outcome of the study.

Conclusion

From the present study it could be concluded that montelukast sodium and fexofenadine hydrochloride could be successfully formulated together in a single tablet dosage form. Compatibility testing showed neither interaction between the drugs nor between any of the drugs with any of the used additives. The selected tablet formula (F1), which revealed the highest release properties, was further studied for bioequivalence with the commercial products Singular® 10mg tablets and Telfast® 120 mg film coated tablets, in 8 healthy, adult,
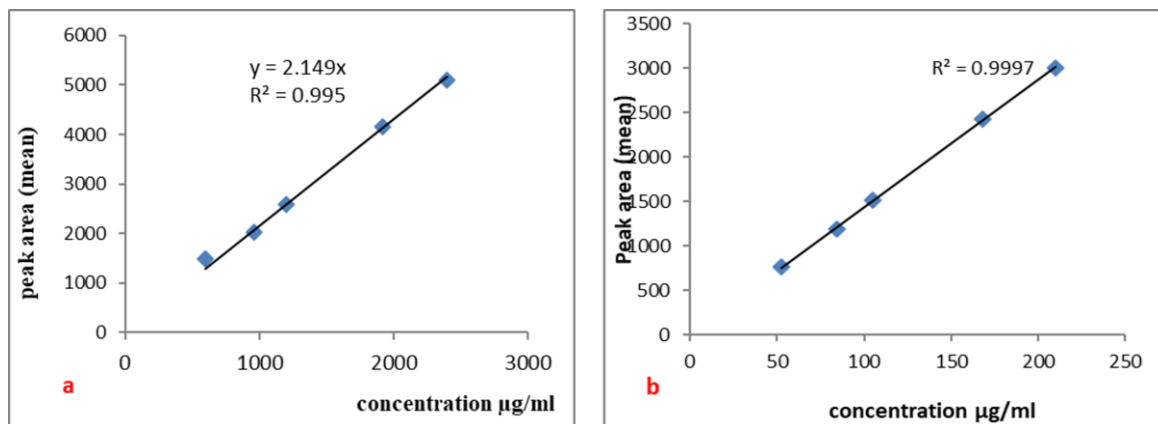
Figure 8: Linearity plot for a) fexofenadine HCl and b) montelukast sodium

male volunteers. The findings from this bioequivalence study demonstrated the similarity of the products under investigation in terms of absorption rate, as indicated by C_{max}, and absorption extent, as reflected by AUC_{0-t} and $AUC_{0-\infty}$. Given that plasma levels serve as a significant proxy for pharmacodynamic action and adverse effects, the results from this study suggest that the test tablet is expected to exhibit therapeutic activity and tolerance equivalent to those of the reference products.

Declaration of interest

The authors report no conflicts of interest.

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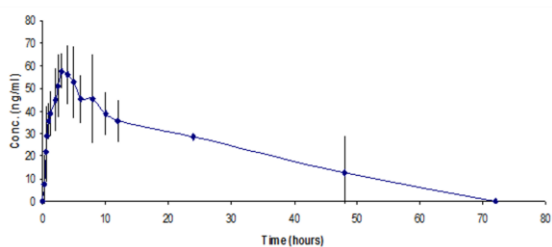8. Golightly LK, Greos LS. Second-generation

Fig. 9 Logarithmic plot for average montelukast plasma concentration (ng/ml) after single dose administration of reference product
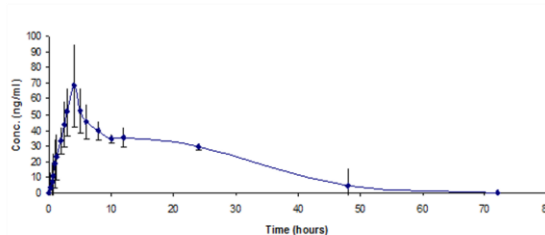

Fig. 10 Logarithmic plot for average montelukast plasma concentration (ng/ml) after single dose administration of test product
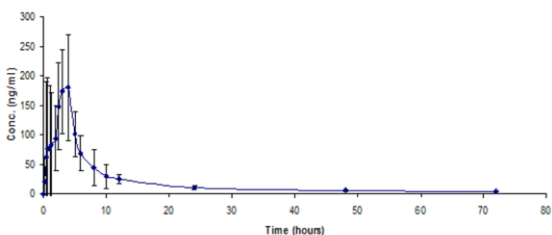

Fig. 11 Logarithmic plot for average fexofenadine plasma concentration (ng/ml) after single dose administration of reference product
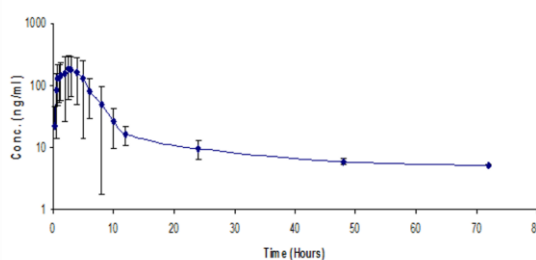

Fig. 12 Logarithmic plot for average fexofenadine plasma concentration (ng/ml) after single dose administration of test product

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