Semi-sensitive LC-MS/MS-ESI method for simultaneous determination of montelukast and fexofenadine in human plasma: application to a bioequivalence study

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ABSTRACT: A rapid, simple, sensitive and selective LC-MS/MS method was developed and validated for simultaneous quantification of montelukast (MT) and fexofenadine (FF) in human plasma (200 μL) using montelukast-d6 (MT-d6) and fexofenadine-d10 (FF-d10), respectively as an internal standard (IS) as per the US Food and Drug Administration guidelines. The chromatographic resolution was achieved on a Chromolith RP18e column using an isocratic mobile phase consisting of 20 mM ammonium formate–acetonitrile (20:80, v/v) at flow rate of 1.2 mL/min. The LC-MS/MS was operated under the multiple-reaction monitoring mode using electrospray ionization. The total run time of analysis was 4 min and elution of MT, FF, MT-d6 and FF-d10 occurred at 2.5, 1.2, 2.4 and 1.2 min, respectively. The standard curve found to be linear in the range 2.00–1000 ng/mL with a coefficient of correlation of ≥0.99 for both the drugs. The intra- and inter-day accuracy and precision values for MT and FF met the acceptance as per FDA guidelines. MT and FF were found to be stable in a battery of stability studies viz., bench-top, auto-sampler and repeated freeze-thaw cycles. The validated assay was applied to an oral bioequivalence study in humans. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: montelukast; fexofenadine; human plasma; method validation; LC-MS/MS; pharmacokinetics; bioequivalence

Introduction

Allergic rhinitis is a medical condition, in which the nasal airways are blocked because of the presence of an allergen. The conditions manifests itself when an allergen (such as pollen, dust, animal dander) sets off the production of the antibody immunoglobulin E (IgE) that binds to mast cells and basophils containing histamine. The initiation and regulation of allergic inflammation provides rich cellular sources of various mediators such as histamine and cysteiny1 leukotrienes (CysLTs), which play an important role in the pathogenesis of allergic airway inflammation. Histamine and CysLTs have diverse roles in the pathogenesis of allergic rhinitis (Peters-Golden and Sampson, 2003).

Montelukast (MT, Fig. 1, CAS no. 158966-92-8) is a CysLT1 type-1 (CysLT1) receptor antagonist used for the maintenance treatment of asthma, to relieve symptoms of seasonal allergies and urticaria. It inhibits the physiological actions of leukotriene D4 at the CysLT1 receptors without any agonistic activity (SINGULAIR, 2008). This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation (Jones et al., 1995). Fexofenadine (FF, Fig. 1, CAS no. 153439-40-8) is a nonselecting antihistaminic drug used in the treatment of hay fever, allergic rhinitis and chronic urticaria. FF is a highly selective second-generation peripheral histamine-1 receptor antagonist, of gastrointestinal tract, large blood vessels and bronchial smooth muscle (Simpson and Jarvis, 2000). This blocks the endogenous action of histamine, which subsequently leads to temporary relief of nasal congestion associated with allergy.

Fixed-dose combination and combination of drugs with complementary mechanism of action have been observed and reported for improved efficacy with lesser side effects and better patient tolerability. In combination therapy, lower doses of individual drugs are administered to produce a similar pharmacological effect (because of additive or synergistic effect) to the higher doses used in monotherapy. This provides the pharmacological rationale of combining a CysLT1 antagonist (MT) and anti-histaminic drug (FF) for the treatment of allergic rhinitis. Cingi et al. (2010) have demonstrated that CysLT1 antagonist and anti-histamine combination therapy is more effective than anti-histamine alone in the control of allergic rhinitis treatment.

Abbreviations used: CysLTs, cysteiny1 leukotrienes; FF, fexofenadine; IgE, immunoglobulin; ISR, incurred samples reanalysis; LLE, liquid–liquid extraction; MT, montelukast

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An extensive literature survey revealed that several LC-MS/MS methods have been published for the quantitation of MT (Papp et al., 2007; Bharathi et al., 2009; Challa et al., 2010; Liu et al., 2012) and FF (Hofmann et al., 2002; Fu et al., 2004; Isleyen et al., 2007; Nirogi et al., 2007; Teng et al., 2007; Yamane et al., 2007; Guo et al., 2010; Stanton et al., 2010; Flynn et al., 2011) individually or with some other drugs (Nirogi et al., 2006; Bharathi et al., 2008) in various biological matrices. It is pertinent that a sensitive, rapid and reliable bioanalytical method is evolved for simultaneous quantitation of two or more drugs in combination therapy in the human plasma. To date, there is no LC-MS/MS method reported for the simultaneous determination of MT and FF in any biological matrix. It is suggested that a simultaneous estimation method would help researchers as both the drugs used in this method are available in the market (Telekast F, Montair FX, Montolife FX, Montymo FX, Montemac FX, Monti FX and Uniair FX contain 10 mg of MT and 120 mg of FF) as a fixed dose combination and has application in pharmaco kinetic and bioequivalence studies. The present work describes a simple, selective and sensitive method that employs a simple liquid–liquid extraction technique for sample preparation and liquid chromatography with electrospray ionization–tandem mass spectrometry for simultaneous quantitation of MT and FF in human plasma. The application of this assay method to a bioequivalence study in healthy male volunteers following oral co-administration of MT (10 mg) and FF (120 mg) is described.

**HPLC operating conditions**

A Shimadzu LC-20 AD Series HPLC system (Shimadzu Corporation, Kyoto, Japan) was used to inject 4 µL aliquots of the processed samples on a Chromolith RP18e (4.6 × 100 mm, 5 μm), which was kept at 5 ± 1°C. The isocratic mobile phase, a mixture of 20 mM ammonium formate–acetonitrile, 20:80 (v/v) was then degassed ultrasonically for 10 min and delivered at a flow rate of 1.2 mL/min with 80% splitting into the mass spectrometer electrospray ionization chamber.

**Mass spectrometry operating conditions**

Quantitation was achieved with MS/MS detection in positive ion mode for the analytes and IS using a MDS Sciex API-4000 mass spectrometer (Foster City, CA, USA) equipped with Turboionspray™ interface at 400°C. The ion spray voltage was set at 5500 V. The source parameters, viz. the nebulizer gas, curtain gas and CAD gas were set at 40, 30 and 5 psi, respectively. The compound parameters, viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP), for MT and MT-d<sub>6</sub> were similar and were 105, 32, 10 and 14 V. For FF and FF-d<sub>10</sub> the DP, CE, EP and CXP were 100, 36, 10 and 15 V. Detection of the ions was carried out in the multiple-reaction monitoring mode, by monitoring the transition pairs of m/z 586.2 precursor ion to the m/z 422.2 for MT and m/z 592.2/574.2 for MT-d<sub>6</sub>. FF was monitored with m/z 502.4 precursor ion to the m/z 466.3 product ion and m/z 512.7/476.3 for FF-d<sub>10</sub>. Quadrupoles Q1 and Q3 were set on unit resolution. The analysis data obtained were processed by Analyst software™ (version 1.4.2).

**Preparation of extraction buffer**

About 1.26 g of ammonium formate was weighed accurately and transferred into a 1000 mL beaker and dissolved in ~900 mL of Milli-Q water. The contents were transferred into a 1000 mL measuring cylinder. The final volume was made up with water and the pH was adjusted to 6.4 with formic acid.

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**Chemicals and reagents**

MT sodium (purity 99.5%), FF (purity 99.9%), MT-d<sub>6</sub> sodium (purity 100%, Fig. 1) and FF-d<sub>10</sub> (purity 98.4%, Fig. 1) were purchased from Vivan Life Sciences, Mumbai, India. HPLC-grade acetonitrile and methanol were purchased from Spectrochem (Phillipsburg, USA). Analytical-grade ammonium formate was purchased from Merck (Mumbai, India). Water used for the LC-MS/MS analysis was prepared from Milli-Q water purification system procured from Millipore (Bangalore, India). All other chemicals and reagents were of analytical grade and used without further purification. The control human K<sub>3</sub>-EDTA plasma sample was procured from Supratech Voluntary Blood Bank (Ahmedabad, India).

**Experimental**

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**Figure 1. Chemical structures of montelukast (MT), fexofenadine (FF), montelukast-d<sub>6</sub> (MT-d<sub>6</sub>) and fexofenadine-d<sub>10</sub> (FF-d<sub>10</sub>)**

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Preparation of stock solutions of analytes and IS

Primary stock solutions of MT and FF for preparation of standard and quality control (QC) samples were prepared from separate weighings. The primary stock solutions of MT, FF and ISs were stored at 2–8°C, and were found to be stable for a week (data not shown). They were consecutively diluted with methanol–water (50:50, v/v) to prepare working solutions for the calibration curve. Another set of working stock solutions of MT and FF was made in methanol–water (50:50, v/v) from primary stock for preparation of QC samples. A working mixed IS solution (500 ng/mL MT-d6 and 200 ng/mL FF-d10) was prepared in methanol–water (50:50, v/v). Working stock solutions were found to be stable when stored at 2–8°C for a week (data not shown). Working solutions of MT and FF were prepared in combination.

Preparation of calibration curve and quality control samples

Calibration curve samples were prepared by spiking 200 μL of control human plasma with the appropriate working solutions of the MT and FF prepared in combination (20 μL). Calibration curve standards consisting of a set of nine non-zero concentrations ranging from 2.00 to 1000 ng/mL for both were prepared. Samples for the determination of precision and accuracy were prepared by spiking control human plasma in bulk with MT and FF at appropriate concentrations and 200 μL plasma aliquots were distributed into different tubes. The QCs were prepared at 2.00 (lower limit of quantitation, LLOQ), 6.00 (low quality control, LQC), 50.0 (medium quality control, MQC1), 400.0 (MQC2) and 800 ng/mL (high quality control, HQC) for both the drugs. All the samples were stored at −78 ± 8°C.

Sample preparation

A simple liquid–liquid extraction (LLE) method was followed for extraction of MT and FF from human plasma. To an aliquot of 200 μL plasma, IS working solution (20 μL of mixed IS) was added and mixed for 30 s on a cyclomixer (Remi Instruments, Mumbai, India). After the addition of 100 μL of extraction buffer (20 μL of 20 mM ammonium formate in water [pH 6.4]) the samples were mixed for 30 s. Then 2.5 mL ethyl acetate was added and samples placed on a reciprocating shaker for 20 min at 40 rpm, followed by centrifugation for 5 min at 4000 rpm on an Eppendorf 5810 R (Eppendorf, India). The organic layer (2 mL) was separated and evaporated to dryness at 40°C using a gentle stream of nitrogen (Turbovap®, Zymark®, Kopkinton, MA, USA). The residue was reconstituted in 300 μL of the mobile phase and a 4 μL was injected into the column.

Method validation

A systematic validation of the method was carried out as per the US FDA guidelines (US DHHS et al., 2001) in human plasma.

Selectivity. Selectivity of the method was assessed by analyzing six different lots of blank human plasma samples. The responses of the interfering substances or background noises at the retention time of the MT, FF and IS were acceptable if they were <20% of the mean response of the lowest standard curve point or LLOQ. The responses of the interfering substances or background noise at the retention time of the IS were acceptable if they were <5% of the mean response of the working ISs.

Sensitivity. The sensitivity was established from the background noise or response from six spiked LLOQ samples. The six replicates should have a precision of ±20% and an accuracy of ±20%.

Matrix effect. The matrix effect is investigated to ensure that precision, selectivity and sensitivity are not compromised by the matrix. Matrix effect was checked with six different lots of K2EDTA plasma. Three replicate samples each of LQC and HQC were prepared from different lots of plasma (36 QC samples in total).

Calibration curve. Linearity was tested for both MT and FF in the concentration range of 2.00–1000 ng/mL. For the determination of linearity, standard calibration curves containing at least nine points (non-zero standards) were plotted and checked. In addition, blank plasma samples were also analyzed to confirm the absence of direct interferences, but these data were not used to create the calibration curve. The acceptance limit of accuracy for each of the back-calculated concentrations is ±15% except for the LLOQ, where it is ±20%. For a calibration run to be accepted at least 67% of the standards, including the LLOQ and upper limit of quantitation (ULQQ), are required to meet the acceptance criterion, otherwise the calibration curve is rejected. Five replicate analyses were performed on each calibration standard. The samples were run in the order from high to low concentration.

Precision and accuracy. Intra-assay precision and accuracy were determined by analyzing six replicates at five different QC levels. Inter-assay precision and accuracy were determined by analyzing six replicates at five different QC levels on four different runs. The acceptance criteria included accuracy within ±15% standard deviation (SD) from the nominal values, except for the LLOQ, where it should be ±20% and a precision of 15% relative standard deviation (RSD), except for the LLOQ, where it should be 20%.

Recovery. Recovery of the analytes from the extraction procedure was determined by comparing the peak areas of the analytes in spiked plasma samples (six each of LQC, MQC2 and HQC) with those of the analytes in samples prepared by spiking the extracted drug-free plasma samples with the same amount of the analytes at the step immediately prior to chromatography. Similarly, recovery of the IS was determined by comparing the mean peak areas of the extracted QC samples (n = 6) with those of the IS in samples prepared by spiking the extracted drug-free plasma samples with the same amounts of IS at the step immediately prior to chromatography.

Dilution integrity. The dilution integrity experiment was carried out at 2.0 times the ULOQ concentration for both the analytes. Six replicates each with 3- and 10-fold diluted concentrations were prepared and their concentrations were calculated by applying the dilution factor 3- and 10-fold, respectively.

Stability experiments. Stability tests were conducted to evaluate the analytes stability in stock solution and in plasma samples under different conditions. The stock solution stability at room temperature and refrigerated conditions (2–8°C) was performed by comparing the area response of the analytes (stability samples) with the response of the sample prepared from fresh stock solution. Bench-top stability (6 h), autosampler stability (53 h), freeze–thaw stability (five cycles) and long-term stability (at −78 ± 8°C for 93 days) were performed at LQC and HQC levels using six replicates at each level. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15% SD) and precision (15% RSD).

Incurred samples reanalysis. The recent EMA and FDA guidelines have emphasized on the necessity of ensuring incurred sample reproducibility (EMA, 2011; US DHHS et al., 2001). EMA 2011 guideline on bioanalytical method validation provided the rational and procedure for conductance of incurred sample reanalysis (ISR). As per the guidance, the difference in concentrations between the initial value and the ISR should be less than ±20% of their means for at least 67% of the repeats. Large differences between results may indicate analytical issues and should be investigated. In total 280 samples out of 2770 samples were re-assayed under a separate batch.

Bioequivalence study

A bioequivalence study was performed in healthy, male, adult human volunteers (n = 60) under fasting conditions. The ethics committee
approved the protocol and the volunteers were provided with informed written consent. This was a single-dose, single-blind, randomized, two-way crossover trial in which the reference formulation [Allegra™ tablets (FF 120 mg), and Singular® (MT 10 mg), Merck and Co., India] and test formulation (fixed dose combination of FF 120 mg and MT 10 mg tablets of Merck and Co., India) were alternately administered in randomized dosing sequences, under fasting conditions, with 7 days wash-out period between dosing periods. In each treatment period, volunteers were required to fast for approximately 10 h prior to the dosing. The tablet(s) was/were administered with 240 mL water, and then water was not allowed until 1 h post-dosing. Subjects received a standard meal at about 4, 8, 12, 24, 28, 32 and 36 h after dosing in each period.

For the measurement of pharmacokinetic parameters, venous blood samples (4 mL at each time point) were collected at pre-dose and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.33, 2.67, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 36 and 48 h after dosing in K3EDTA vacutainer collection tubes (BD Franklin, NJ, USA) during each treatment period. The tubes were centrifuged at 4000 rpm for 10 min at 4°C and the plasma was collected. The collected samples were stored at $-78 \pm 8°C$ until analysis. An aliquot of 200 $\mu$L of thawed plasma samples were spiked with pooled ISs and processed as mentioned in the Sample Preparation section. Along with clinical samples, QC samples at LQC, MQC1, MQC2 and HQC concentrations were assayed in triplicate and were distributed among unknown samples in the analytical run; 67% of the QC samples accuracy must be within 85–115% of the nominal concentration and not less than 50% at each QC concentration level must meet the acceptance criteria. The plasma concentration vs time profiles of MT and FF were analyzed by noncompartmental method using WinNonlin Version 5.2 (Pharsight Corporation, Mountain View, CA, USA). According to the guidance from the FDA, product bioequivalence was based on the 90% confidence interval for the ratios of least squares means of the log-transformed values for $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{\text{max}}$.

**Statistical analysis**

The statistical comparison of the ln-transformed $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ and untransformed $T_{\text{max}}$, $t_1/2$ and $K_{el}$ for MT and FF was carried out using SAS® Version 9.2 (SAS Institute Inc., USA). The sequence effect

![Figure 2](https://example.com/figure2.png)  
**Figure 2.** Typical multiple reaction monitoring (MRM) chromatograms of MT (left panel) and IS (right panel) in (a) human blank plasma, (b) human plasma spiked with IS, (c) a lower limit of quantitation (LLOQ) sample along with IS and (d) a 1 h plasma sample showing MT peak obtained following an oral dose of MT (10 mg) and FF (120 mg) tablets to a healthy volunteer along with IS.
will be tested at the 0.10 level of significance using the subjects nested within sequence mean square from the ANOVA as the error term. All other main effects were tested at the 0.05 level of significance against the residual error (mean square error, MSE) from the ANOVA as the error term. The above analyses were done using procedure PROC GLM in SAS, version 9.2.

Results

Method development

Mass parameters were optimized in both positive and negative ionization modes for the analytes and IS. Good response was found in positive ionization mode. Data of the multiple reaction monitoring was considered to get better selectivity.

Separation of MT, FF and ISs was attempted using various combinations of acetonitrile, methanol and buffers with varying contents of each component on different columns. Use of 20 mM ammonium formate with acetonitrile helped in achieving good response for MS detection in the positive ionization mode. A mobile phase consisting of 20 mM ammonium formate–acetonitrile, 20:80 (v/v) was found suitable, as the analytes were protonated and well separated in this mobile phase. A Chromolith RP18e (100 × 4.6 mm, 5 μm) column gave a good peak shape and response even at LLOQ level for both analytes and ISs. The mobile phase was operated at a flow rate of 1.2 mL/min. The retention times of MT, FF, MT-d6 and FF-d10 were 2.5, 1.2, 2.4 and 1.2 min, respectively, which allowed a small run time of 4.0 min.

Selectivity and chromatography

The degree of interference by endogenous plasma constituents with the MT, FF and ISs was assessed by inspection of chromatograms derived from a processed blank plasma sample. As shown in Figs. 2 and 3, no significant direct interference in the blank plasma traces was observed from endogenous substances in drug-free plasma at the retention times of the MT, FF and their corresponding ISs. The capacity factor ($K'$) for MT and FF was 1.74 and 0.32, respectively.

Figure 3. Typical MRM chromatograms of FF (left panel) and IS (right panel) in (a) human blank plasma, (b) human plasma spiked with IS, (c) an LLOQ sample along with IS and (d) a 1 h plasma sample showing FF peak obtained following an oral dose of MT (10 mg) and FF (120 mg) tablets to a healthy volunteer along with IS.
Sensitivity

The lowest limit of reliable quantification for the analytes was set at the concentration of the LLOQ. The precision and accuracy at LLOQ concentration were found to be <10.1 and 104%, and <13.9 and 103% for MT and FF, respectively. We have also found that, in presence of MT HQC concentration, no interference was observed at the retention time of FF at LLOQ. Similarly the sensitivity of FF was not affected by HQC concentration of MT (data not shown).

Extraction efficiency

LLE technique was employed for the sample preparation in this validation work. LLE is helpful in producing a spectroscopically clean sample and avoiding the introduction of nonvolatile materials onto the column and MS system and also minimizing the experimental cost. Clean samples are essential for minimizing ion suppression and matrix effect in LC-MS/MS. Among the different solvents checked alone and in combination for their suitability, ethyl acetate proved to be robust and provided the cleanest samples. The recoveries of the analytes and the ISs were good and reproducible. The mean recoveries for MT, FF, MT-d6, and FF-d10 were 85.8, 70.3, 87.7 and 68.3%, respectively.

Matrix factor

No significant matrix effect was observed in all the six batches of human plasma for the analytes at LQC and HQC concentrations. The precision and accuracy for MT and FF at LQC concentration were 1.07 and 99.3%, and 1.46 and 99.2%, respectively. Similarly, the precision and accuracy for MT and FF at HQC concentration were 1.12 and 99.1%, and 0.37 and 99.4%, respectively. The overall IS normalized matrix factors for MT and FF were 0.56 and 0.52, respectively.

### Table 1. Precision and accuracy of the method for determining montelukast (MT) and fexofenadine (FF)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration (ng/mL)</th>
<th>Concentration found (mean; ng/mL)</th>
<th>Precision (%)</th>
<th>Accuracy (%)</th>
<th>Concentration found (mean; ng/mL)</th>
<th>Precision (%)</th>
<th>Accuracy (%)</th>
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<td>2.00</td>
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### Table 2. Stability samples result for MT and FF (n = 6)

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<tr>
<th>Analyte</th>
<th>Stability test</th>
<th>QC (nominal concentration (ng/mL))</th>
<th>Mean ± SD (ng/mL)</th>
<th>Stability (%)</th>
<th>Precision (% CV)</th>
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<td>MT</td>
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<td>6.77 ± 0.21</td>
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<td>800</td>
<td>894 ± 7.86</td>
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<td>6.08 ± 0.18</td>
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<td>800</td>
<td>755 ± 9.35</td>
<td>98.8</td>
<td>1.24</td>
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</table>

aAfter 6 h at room temperature;  
bAfter five freeze and thaw cycles;  
cAfter 53 h of injection;  
dAt −78 °C for 93 days.
Linearity

The nine-point calibration curve (2.00, 4.00, 10.0, 25.0, 50.0, 125, 250, 500 and 1000 ng/mL for both analytes) was constructed by plotting the peak area ratio of analyte–corresponding deuterated IS against the nominal concentration of calibration standards in human plasma. Following the evaluation of different weighing factors, the results were fitted to linear regression analysis with the use of $1/X^2$ (where $X$ is the concentration) weighting factor, giving a mean linear regression equation for the calibration curve of $y = 0.0021(\pm 0.0001)x - 0.0003(\pm 0.0003)$ and $y = 0.0035(\pm 0.0001)x + 0.0002(\pm 0.0007)$ for MT and FF, respectively. The mean correlation coefficient of the weighted calibration curves generated during the validation was $\geq 0.99$.

Figure 4. Mean plasma concentration–time profile of (a) MT and (b) FF in human plasma following oral co-administration of MT (10 mg) and FF (120 mg) tablets to healthy human volunteers ($n = 6$).
Precision and accuracy
As shown in Table 1, the precision and accuracy of each analyte in the intra- and inter-day runs were within ±15% at LQC, MQC-1, MQC-2 and HQC concentrations and within ±20% at LLOQ QC.

Dilution integrity
The mean back-calculated concentrations for 3- and 10-fold dilution samples were within 85-115% of their nominal value. The coefficients of variation (CV) for 3- and 10-fold dilution samples were <10% for both analytes.

Stability studies
In the different stability experiments carried out, viz. bench-top stability (6 h), autosampler stability (53 h), repeated freeze–thaw cycles (five cycles at –78 ±8°C) and long-term stability at –78 ±8°C for 93 days, the mean percentage nominal values of the analytes were found to be within ±15% of the predicted concentrations for the analytes at their LQC and HQC levels (Table 2). Thus, the results were found to be within the acceptable limits during the entire validation.

Incurred samples reanalysis
All of the 280 samples selected for ISR met the acceptance criteria for FF; however, 275 samples met the acceptance criteria for MT. The back-calculated accuracy values ranged from –17.91 to 11.32 and from –19.49 to 17.94 for FF and MT, respectively.

Bioequivalence study
The present method was applied to the analysis of plasma samples obtained from 60 healthy human volunteers following oral administration of test and reference formulations as a part of the bioequivalence study. The sensitivity and specificity of the assay were found to be sufficient for accurately characterizing the plasma pharmacokinetics of MT and FF in humans. Fig. 4(a and b) depicts the mean ± SD plasma concentration vs time profile of MT and FF, respectively, in these volunteers (for greater clarity in these figures, a few time points were not included). Both MT and FF were detectable in human plasma up to 48 h. The key pharmacokinetic parameters for both the analytes in reference and test formulations are shown in Table 3. Following ANOVA analysis it was concluded that the test formulation (for both the MT and FF) when compared with reference formulation meets the bioequivalence criteria in terms of Cmax, AUC0–t and AUC0–∞. The 90% confidence intervals for Cmax, AUC0–t and AUC0–∞ were 101–116, 102–113 and 102–112%, respectively, for MT and 91.6–106, 92.4–105 and 92.3–105%, respectively, for FF. The results indicate that two formulations are bioequivalent.

Discussion
So far there are no published methods available for the simultaneous quantification of MT and FF in any of the biological matrices. To the best of our knowledge, this is the first report on the simultaneous analysis of MT and FF in human plasma with good sensitivity (LLOQ 2.00 ng/mL for both analytes). This validated method was used in a bioequivalence study to quantify the concentrations of MT and FF following oral administration. The derived pharmacokinetic parameters were in close agreement with earlier reported values for both the drugs, which proves the robustness of the method, although there was a little compromise on LLOQ for both the drugs (compared with the earlier reported methods).

Conclusion
A rapid, simple, specific and sensitive LC-MS/MS-ESI method was developed and validated for quantification of MT and FF in human plasma. The extraction method gave consistent and reproducible recoveries for MT, FF and corresponding deuterated ISs from plasma. The cost-effectiveness, simplicity of the assay, the use of liquid–liquid extraction and sample turnover rate of less than 4 min per sample are innovative features that make it an interesting procedure for high-throughput bioanalysis of MT and FF. Based upon the assessment of the validation parameters and derived results, it could be conclude that the developed method can be useful for BA/BE studies with the desired precision and accuracy.

References


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