Research Article

Development and Validation of a High-throughput LC–MS/MS Method for the Quantitation of Total Ezetimibe in Human Plasma.

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ABSTRACT

A sensitive and reliable analytical method for total ezetimibe in human plasma using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system was developed and validated for the pharmacokinetic study. Ezetimibe and internal standard, Ezetimibe-D4, were extracted by liquid-liquid extraction with Diethyl ether and Dichloromethane (70:30 v/v). The high performance liquid chromatographic separation was performed on a Discovery C18, 150x 4.6mm, 5µ column using a mobile phase composed of Acetonitrile 40:60 (v/v) - 10mM Ammonium formate buffer (pH 4.0 ± 0.3) at a flow rate of 1 mL/min. Tandem mass spectrometry was performed in the Electrospray ionization (ESI) negative ion mode, using multiple reaction monitoring (MRM) mode for the quantification. The mass transition pairs of m/z 408.40→271.0 for ezetimibe and m/z 412.10→275.10 for internal standard were used. The calibration curve was linear in the concentration range of 4.00ng/mL-400.00ng/mL for total ezetimibe with the lower limit of quantification of 4.00 ng/mL for total ezetimibe. The mean extraction recovery for total ezetimibe from plasma was 80.6%. Method validation investigated parameters, such as selectivity, recovery, accuracy, precision, linearity and sample stability, giving results within the acceptable range. This method was reproducible and reliable, and was successfully used to analyze human plasma samples for application in a bioequivalence study evaluating the pharmacokinetics of ezetimibe following single dose oral administration in 12 healthy male human volunteers in an open label, two-period, two sequence, randomized, crossover protocol under fasting conditions. Based on the 90% confidence interval of the individual ratios for Cmax and AUC0-inf, it was concluded that the method is efficient with a very short running time of 4 min, sufficiently sensitive and suitable for pharmacokinetic studies.

Keywords: Column liquid chromatography, mass spectrometry, MS-Liquid-liquid extraction, Ezetimibe, Method validation.

INTRODUCTION

The main risk factor for coronary heart disease is having elevated low-density lipoproteins. High cholesterol or Hyperlipidemia refers to a high degree of cholesterol in the blood, which raises the risk of heart disease, stroke, and atherosclerosis, plus other serious conditions.

The discovery of the novel cholesterol-lowering agent ezetimibe represents a different position from other developments.

The first new therapy for the treatment of hypercholesterolemia since the discovery of the statins, ezetimibe represents an important discovery.[1] Ezetimibe,[1-(4-fluorophenyl)-3(***R***)-[3-(4-fluorophenyl)-3(***S***)-hydroxypropyl]-4(***S***)-(4-hydroxyphenyl)-2-azetidinone], which selectively obstructs the absorption of bile and dietary cholesterol, as well as related phytosterols, from the intestine without affecting the concentration of fat-soluble vitamins, triglycerides, or bile acids, is the first member of a new class of lipid-lowering agents.[2,3]

After oral administration ezetimibe (EZM) is rapidly absorbed and extensively conjugated to Ezetimibe Phenoxy glucuronide (EZMG), which is pharmacologically active *in vivo*. Thus, EZM and EZMG are the major drug derived compounds detected in plasma, constituting approximately 10-20% and 80-90% of the total drug in plasma, respectively.[4]

To date there are few analytical methods reported for the estimation of EZM and EZMG in the form of total ezetimibe.

The reported bioanalytical methods were based either on HPLC coupled to UV detector[5,6]/radio detector[7] or by the use of tandem mass spectrometers.[8-11]

Thus the aim of the present study was to develop a more accurate and precise High Performance Liquid Chromatographic LC-MS/MS method for the determination of total EZM in human plasma for therapeutic drug monitoring and pharmacokinetic studies. As a part of it, developed and validated an isocratic LC-MS/MS method with simple and reproducible liquid-liquid extraction procedure.

The developed method was successfully used to study the pharmacokinetics of ezetimibe tablets (10mg), in healthy Indian human volunteers under fasting conditions.

MATERIALS AND METHODS

Chemicals & Reagents

Reference standard of SS was procured from USP and the working standards of SSA and ezetimibe were purchased

from Vivan Life Sciences Pvt. Ltd. (Mumbai, India). The working standards EZD and EZG were procured from Clearsynth Labs Pvt. Ltd. (Mumbai, India).

All the used standards have purity more than 99%. LC-MS grade methanol, acetonitrile and n-hexane were purchased from Thermo Fisher Scientific India Pvt. Ltd. (Mumbai, India). GR grade ammonium formate and sodium acetate were procured from Merck specialities Pvt. Ltd. (Mumbai, India).

HPLC type I water was obtained from Milli-Q A10 gradient water purification system (Millipore, Bedford, MA, USA). Drug free human plasma containing K_2EDTA anticoagulant was obtained from M/S Laxmi Sai Clinicals Labs (Hyderabad, India). B-Glucuronide type HP aq. Solution purchased from Helix Pomatia (USA).

Instrumentation

Modular HPLC system (Shimadzu, Kyoto, Japan) consisting of binary LC-20AD prominence pump, DGU-20A3 solvent degasser, CTO-ASvp column oven and high throughput SIL HTC auto sampler was used for the analysis. Mass spectrometric detection was performed on API-3000 triple quadrapole mass spectrometer (MDS SCIEX, Toronto, Canada) equipped with turbo ion spray interface. Quantification was performed in multiple reaction monitoring (MRM) mode with polarity switching. Analyst software version 1.4.2 was used for controlling the hardware and data handling. Samples were centrifuged with the refrigerated Centrifuge (Furlabo) with up to 10000 RPM capacity.

Chromatographic Conditions

Chromatographic separation was performed on Discovery® C18, 150 X 4.6 mm, 5µ analytical column, Isocratic mobile phase consisting of 10mM ammonium formate buffer (pH 4.0 ± 0.3): acetonitrile (40: 60, v/v) was delivered at a flow rate of 1mL/min.

The auto sampler was set at 10°C and the injection volume was 10µL. The column oven temperature was maintained at 35°C. The total chromatographic rum time was 4 min.

Mass Spectrometric Conditions

The turbo ion spray interface (TIS) was operating in negative ionisation mode to study the parent -> product ion (m/z) transition EZM and its internal standard (ISTD).

The MRM transitions monitored were m/z 408.40 → 271.10 (Ezetimibe), m/z 412.10 → 275.10 (EZM-D4) with a dwell time of 200 ms per transition and the quadrupoles 1 and 3 were set at unit resolution.

De-clustering potential (DP), entrance potential (EP), collision energy (CE) collision exit potential (CXP) were all optimized to allow the result with highest possible signal transduction with low background noise.

The pressure of drying gas was set at 7 Psi and temperature was kept 475°C. The Ion spray voltage was set at -4500V the pressure of collision gas (Nitrogen) was maintained at 7 Psi.

Preparation of Calibration Standards and Quality Control Samples

Individual standard stock solution of Ezetimibe, EZG and EZM-D4 (Figure 1) were prepared by dissolving their accurately weighed amounts in methanol to give final concentration of 1mg/mL. The solutions were stored in refrigerator at below 10°C and were brought to room temperature before use. Working solutions of analyte and ISTD were prepared by appropriate dilution of their stock solutions in 50% methanol. The working solutions were stored at room temperature and were prepared on day to day basis.

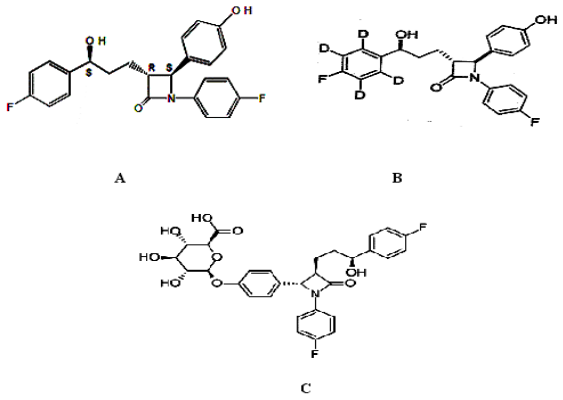

Figure 1: Structures of ezetimibe (A), ezetimibe D4 (B), Ezetimibe phenoxy Glucuronide (C).

K_2EDTA anticoagulant blank plasma collected from non-smoking healthy volunteers screened individually and pooled before use. Calibration standards and quality control (QC's) samples were prepared by spiking (0.1%) working solutions in screened blank plasma. Calibration standards were prepared at concentration of 4.00, 8.00, 20.00, 60.00, 100.00, 170.00, 300.00 and 400.00ng/mL for ezetimibe.

Quality control samples were prepared with both ezetimibe and EZMG with a ration of 5:95, both concentration were together denoted as total ezetimibe. Quality control samples were prepared at 4.00 ng/mL (LLOQQC), 12.00ng/mL (LQC), 170.00 ng/mL (MQC), 300.00ng/mL (HQC) for total ezetimibe. The bulk spiked samples were divided in to single aliquots and stored at -70°C in poly propylene tubes. Prior to analysis all frozen subject samples, calibration and quality control samples were thawed to room temperature and subjected to the sample preparation procedure as specified in section 2.6.

Sample Preparation

A 300 µL aliquot plasma sample was transferred to a 5 mL polypropylene tube and 50 µL of ISTD solution (1000 ng/mL of EZM-D4) was added followed by 0.3mL of 100mM sodium acetate buffer pH 3.5 ± 0.3 and 50 µL of β-Glucuronidase enzyme dilution (50000units/mL). The resulting samples was briefly mixed on a vortex and

incubated for 1 Hr at 35 °C in water bath, after samples came to room temperature added 2.5mL of Diethyl ether: Dichloromethane (70:30 %v/v) mixture and was again briefly mixed on a vortex. Later centrifuged the tubes at 4000rpm and flash freezed the samples in acetone and dry ice bath and transferred the supernant organic layer into another tubes. Using the nitrogen evaporator evaporate the supernant at 15psi and 50°C under stream of nitrogen gas for about 20min. Later reconstituted the residue with 0.3mL of reconstitution solution (10mM ammonium formate buffer: acetonitrile 40:60v/v) and transferred the solution into pre-labelled auto sampler vials.

Method Validation

A complete method validation of EZM in human plasma was done by following the USFDA and EMEA guidelines[12-15]. Validation runs were performed to evaluate selectivity, sensitivity, linearity, precision, accuracy, recovery, matrix effect, dilution integrity and sample stability. Each validation run was organized with a set of spiked standards, blank (with ISTD and without ISTD) and QC samples as per the validation parameters. Standard samples were analyzed at the beginning of the run and QC samples were distributed consistently throughout the validation runs. Selectivity of the method toward endogenous and exogenous components of plasma was evaluated in 14 different human plasma lots. The blank plasma lots were extracted (without addition of ISTD), and injected for LC-MS/MS detection. Later selectivity in each lot was evaluated by comparing the blank peak responses against the mean peak response observed in plasma spiked LLOQ sample (n = 6). The potential for interference from concomitant drugs was also investigated by spiking low quality control samples with aceclofenac, ibuprofen, paracetamol, ranitidine and ondansetron.

Linearity of the method was assessed using three calibration curves analyzed on two different days. Each plot was associated with an eight point non-zero concentrations spread over the dynamic range. A linear least squares regression analysis with weighing factor 1/X2 was performed on peak area ratios versus analyte concentrations. Peak area ratios for plasma spiked calibration standards were proportional to the concentration of analytes over the established range.

Intra batch (within day) and inter batch (between day) precision and accuracy were evaluated at four distinct concentrations (LLOQ, LQC, MQC and HQC) for each analyte. Precision and accuracy at each concentration level was evaluated in terms of %CV and relative error. The extraction recovery of total EZM was determined at LQC, MQC and HQC levels. The relative recoveries were evaluated by comparing the peak areas of extracted samples (spiked before extraction) with that of un-extracted samples (blank extracts spiked after extraction). The matrix effect was checked at low and high QC level using 8 different blank plasma lots (including two hemolytic and two Lipemic lots). Matrix factor for analyte and internal standard was calculated in each lot by comparing the peak responses of post extraction samples (blank extracts spiked after extraction) against the peak responses of equivalent aqueous samples prepared in mobile phase. Internal standard normalized matrix factor in each lot was later evaluated by comparing the matrix factor of analyte and internal standard.

Stability of analytes in both aqueous solutions and in biological matrix was evaluated after subjecting to different conditions and temperatures that could encounter during regular analysis. Stability in plasma was evaluated in terms of freeze–thaw stability, bench top stability, long-term stability, and extracted sample stability. Freeze–thaw stability was evaluated after six freeze (at −70 °C)–thaw (at room temperature) cycles. Dry extract stability was assessed at 10°C. Bench top stability was assessed at 10ºC and the long-term stability was evaluated at both −70 °C and −20 °C. Stability in whole blood was evaluated at 10ºC temperature. All the matrix related stability assessments were made at LQC and HQC level by comparing the stability samples concentrations against nominal concentrations. Stability of analytes in stock solutions and in working solutions was assessed at 1–10 °C. All comparisons were made against freshly prepared stock solutions or working solutions.

During routine analysis each analytical run was organized with a set of standard samples, a set of QC samples in duplicate and plasma samples to be determined.

Before each analytical run, system suitability was evaluated by injecting six replicates of ULOQ sample and two replicates of LLOQ sample, to check the system precision and chromatography. System suitability was considered acceptable when the coefficient of variation for response ratios was less than 4.0%.

Clinical Protocol and Pharmacokinetic Analysis

This validated method was successfully applied to An open label, randomized, two treatment, two sequence, two period, crossover, single dose, oral comparative bioavailability study of EZM 10 mg tablet for oral administration conducted in 12 healthy male human volunteers under fasting conditions. The age of the volunteers ranged from 23 to 44 years and the body weights of the subjects ranged from 54.86 to 71.80 kg. All subjects provided written informed consent. The study was conducted in accordance with the provisions of the Declaration of Helsinki. After an overnight fast for 10 h, all volunteers received a single dose of EZM 10 mg tablet orally with 240 mL of water. Blood samples (3 mL) from a suitable antecubital vein were collected into K2-EDTA Vacuntainers (BD; Franklin, NJ) at Pre-dose (0.00) and at 0.25, 0.50, 0.75, 1.00, 1.5, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 10.00, 12.00, 14.00, 18.00,24.00, 36.00, 48.00, 72.00, 96.00 hours post dose.

The blood samples were centrifuged at 2500 rpm for 10 min at 40°C temperature and the plasma was removed

and stored at −70°C until assayed for total EZM content. Plasma samples were spiked with the ISTD and processed as per the extraction procedure described earlier. Along with the clinical samples, the QC samples at low, middle 1, middle 2 and high concentration levels were also assayed in triplicate. All samples from a single volunteer were analyzed in the same run in order to avoid inter-assay variations.

Pharmacokinetic parameters from the human plasma samples were calculated by a noncompartmental model using the WinNonLin 5.3 software for. Blood samples were taken for a period of 96 hrs and it was considered as the area under the concentration–time curve (AUC) ratios was higher than 80% as per FDA guidelines. The first-order terminal elimination rate constant (K_{el}) was estimated by linear regression from the points describing the elimination phase on a log-linear plot. The maximum observed plasma concentration (C_{max}) and the time taken to achieve this maximum level (T_{max}) were directly obtained from the curves.

The areas under the time-concentration curve for EZM plasma concentration versus time for 96 h ($AUC_{0–96}$) were calculated using the trapezoidal method. Extrapolation of this area to infinity ($AUC_{0-\infty}$) was performed by adding the value $C96K_{el}$ to the calculated $AUC_{0–96}$. Where C96 is the EZM plasma concentration at 96 h. K_{el} is the first-order terminal elimination rate constant.

RESULTS AND DISCUSSION

Method Development

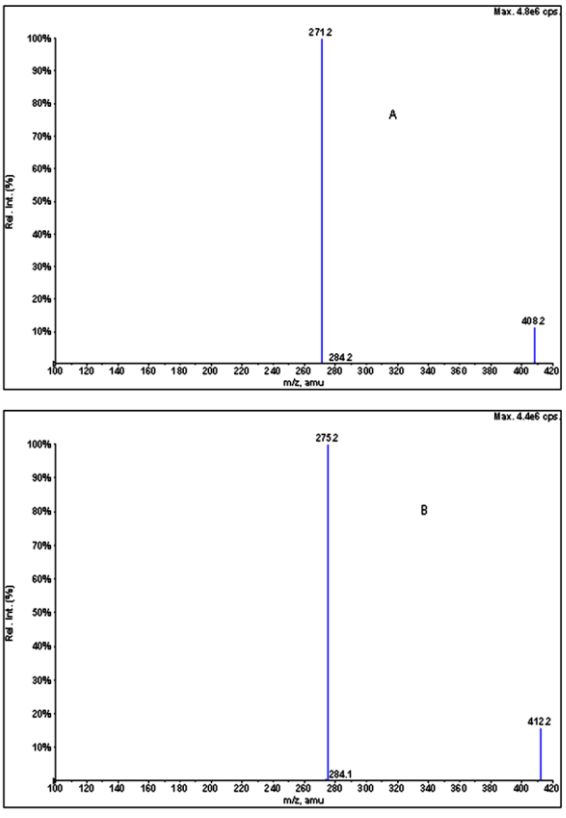

Figure 2: Product ion spectra of EZM (A) and EZM-D4 (B, ISTD)

For consistent and reliable estimation analytes it was necessary to give equal importance to optimize the extraction procedure as well as chromatographic and mass spectrometric conditions. All analytes were tuned in both positive and negative ionization modes using electro spray ionization technique, however based on sensitivity negative ionization mode was selected for EZM and EZM-D4. The Q1 and MSMS scans were performed using infusion technique and further compound and gas parameters were optimized using flow injection analysis. More emphasis was given in the optimization of chromatographic conditions as it is necessary to have sufficient chromatographic resolution. The $[M-H]^-$ peaks were observed at m/z 408.40→271.0 for ezetimibe and m/z 408.40→271.0 for internal standard.

Abundant and consistent product ions were found at m/z of 271.0 for EZM and at m/z of 275.0 for EZM-D4. (Figure 2) Collision gas at set point of 7 was resulted in maximum response for all analytes. A 10% change in source temperature, nebulizer gas, and curtain gas and ion spray voltage did not affect the signal intensities. A dwell time of 200 msec was used for each MRM channel and the both Q1 and Q3 quadrupoles were operated in low resolution mode.

Different make of analytical columns were tested in C18 and C8 phases and Discovery® C18, 150 × 4.6mm, 5µ was selected as it produced more symmetrical peaks with good chromatographic resolution and with a reasonable run time. Use of 10mM ammonium formate buffer in mobile phase has resulted consistently high peak responses for the analytes and lesser concentration buffer has shown diminished chromatographic resolution.

In the extraction procedure, Liquid –liquid extraction technique was selected to avoid unintended matrix effects. Direct elution technique was used to minimize the processing time and it does not affected the column life. During sample processing and analysis the samples were maintained at below 10 °C. Phenoxy glucuronide of ezetimibe was found relatively stable and no inter conversion was identified to form ezetimibe under the employed chromatographic and processing conditions.

Selectivity

Selectivity of the method in human K_2EDTA plasma was evaluated in 14 individual matrix lots including with two haemolysed and two lipemic lots.

Peak responses in blank lots were compared against the response of spiked LLOQ and negligible interference was observed at the retention time of analytes and ISTDs.

Linearity and Sensitivity

The linearity of each calibration curve was determined by plotting the peak area ratio (y) of analyte to ISTD verses the nominal concentration (X) of analyte. Calibration curves were linear from 4.00ng/mL to 400.00 ng/mL EZM with 'r' values more than 0.9987. The 'r' values, slopes and intercepts were calculated from three intra and inter

day calibration curves using weighed ($1/x^2$) linear regression for EZM. The observed mean back calculated concentration with accuracy (% RE) and precision (% CV) are presented (Table 1).

The lower limit of quantification (LLOQ) for determination was found to be 4.00 ng/mL for EZM. At LLOQ (n=6) accuracy (% RE) was in the range of -2.3 to 2.7 with a %CV of ≤ 2.3. At LLOQ the mean signal to noise ratio was found to be 232:1 for EZM.

Precision and Accuracy

Precision and accuracy was performed by performing intra and inter day precision and accuracy runs of 3 batches, each consisting of six replicates of quality control samples at four concentration levels (LLOQQC, LQC, MQC and HQC). The intra and inter batch precision was ≤7.4 for EZM with accuracy (%RE) between -8.2 to -3.2. The Precision and accuracy results were shown (Table 2).

Matrix Effect

Co-elution matrix components can suppress or enhance the ionization but might not result in a detectable response in matrix blanks due to selectivity of the MS detection; however they can affect the precision and accuracy of the assay. Therefore the potential for variable matrix related ion suppression was evaluated in eight independent sources (including two haemolytic and two lipemic lots) of human plasma, by calculating the IS normalized matrix factor. The mean IS normalized matrix factor for all analytes was ranged between 0.96 to 1.05 with a % CV of ≤2.9 as shown (Table 3).

Extraction Recovery and Dilution Integrity

The extraction recovery of analyte from K_2EDTA plasma was determined by comparing the peak response of plasma samples (n=6) spiked before extraction with that of plasma samples spiked after extraction. The mean recovery of EZM was found to be 80.83%, with % CV across the three levels was ≤ 0.2 as shown (Table 4).

Dilution integrity experiment was carried out at 2 times the ULOQ concentration. After the ¼ dilution the mean back calculated concentration for dilution QC samples was within 85-115% of nominal value with a % CV of ≤5.3. Similarly low QC samples spiked with concomitant drug were quantified within 15% of nominal value a % CV of ≤2.0.

Stability

Stability evaluations were performed in both aqueous and matrix based samples. All analytes and ISTDs in the stock solution were stable for 13.88 days in methanol at 1-10ºC and stock dilutions in methanol: water mixture (50:50v/v) were stable up to 23.08 hrs at 1-10°C. Stability in matrix was established up to 12.58 days at -70 °C and 12.61 at -20 °C. The results summary of stability evaluation was shown (Table 5).

Table 1: Summary of Calibration Standards

Analyte	Nominal	Mean*	% CV	% RE
Total Ezetimibe	4.00	3.99	0.9	-0.2
	8.00	7.96	1.8	-0.5
	20.00	20.54	0.7	2.7
	61.40	61.21	2.3	-0.3
	102.33	102.94	1.5	0.6
	170.55	171.09	1.5	0.3
	307.75	300.64	1.8	-2.3
	403.87	402.92	1.7	-0.2

*Mean of 3 replicates at each concentration (ng/mL); % CV= Percentage coefficient of variation.

% RE= Percent relative error.

Table 2: Inter Batch and Intra Batch precision and Accuracy

Analyte	QC level	Nominal*	Inter Batch			Intra Batch		
			Mean*	% CV	% RE	Mean*	% CV	% RE
Total Ezetimibe (TEZM)	LLOQQC	4.01	3.88	7.4	-3.2	3.81	4.4	-5.0
	LQC	12.00	11.34	2.7	-5.5	11.23	1.2	-6.4
	MQC	170.74	158.48	1.6	-7.2	156.77	1.2	-8.2
	HQC	307.14	290.27	1.8	-5.5	284.738	1.4	-7.3

* Concentration in ng/mL; % CV= Percentage coefficient of variation; % RE= Percent relative error.

Table 3: Matrix Effect Results

	LQC Level			HQC Level		
	MF for Analyte	MF for ISTD	IS normalized MF	MF for Analyte	MF for ISTD	IS Normalized MF
Lot 1	0.91	0.92	0.99	0.99	0.95	1.05
Lot 2	0.84	0.85	1.00	0.97	0.97	1.00
Lot 3	0.91	0.88	1.03	0.96	0.95	1.01
Lot 4	0.80	0.78	1.01	0.90	0.89	1.01
Lot 5*	0.87	0.87	1.00	1.00	1.02	0.98
Lot 6*	0.78	0.77	1.02	1.02	1.06	0.96
Lot 7#	0.90	0.90	1.00	1.09	1.05	1.03
Lot 8#	0.93	0.93	1.00	1.15	1.11	1.03
Mean			1.01			1.01
SD			1.01			0.03
% CV			1.4			2.9

* - Hemolyzed lot, # - Lipemic lot, % CV - Percent coefficient of variation, MF - Matrix factor

Table 4: Extraction Recoveries of Analyte and ISTD

Total Ezetimibe	LQC	37844.8	30628.7	80.9	80.83	0.2
	MQC	519201.0	42033.2	81.0		
	HQC	934838.8	753594.8	80.6		
Ezetimibe D4	MQC	500597.3	399637.7	79.8		

A = Mean areas of unextracted samples, B = mean areas of extracted samples.

Table 5: Stability Results

Stability Parameter	QC Level	A	B	% CV	% Stability
Bench top (24.65 hrs at below 25 °C)	LQC	12.00	12.067	2.1	100.6
	HQC	307.14	295.235	1.2	96.0
Freezer thaw stability (after10 cycles)	LQC	12.00	11.998	2.4	100.0
	HQC	307.14	293.950	1.1	95.7
In injector stability (46.88 hrs at 10 °C)	LQC	12.00	12.027	2.2	100.2
	HQC	307.14	292.120	1.7	95.1
Dry extract stability (47.50 hrs at 1-10 °C)	LQC	12.00	11.997	1.3	100.0
	HQC	307.14	292.090	0.7	95.1
Zero day stability in matrix	LQC	12.00	11.265	1.2	93.9
	HQC	307.14	288278	0.9	93.9
Long term stability (at -20 °C) for 12.58 days	LQC	12.00	11.997	0.8	99.8
	HQC	307.14	294.003	1.2	95.7
Long term stability (at -70 °C) for 12.61 days	LQC	12.00	12.035	0.8	100.03
	HQC	307.14	295.673	0.8	96.3

A = nominal concentrations (ng/mL) B = Mean concentrations (ng/mL) of analytes.

Stability evaluation in matrix was performed against freshly spiked calibration standards. EZM was stable up to 24.65 hrs on bench top at 25°C temperature and over 10 freeze-thaw cycles. The processed samples were stable up to 46.88 hrs in auto sampler at 10°C.

The evaporated dry extract sample were stable up to 47.50 hrs in refrigerator at 1-10 °C. No significant degradation of analyte was observed over the stability duration and conditions. The stability results presented were within 85-115%.

Stability in whole human blood was evaluated at both low and high QC level by comparing the mean response ratio of stability samples against the comparison samples. The stability of analytes in whole human blood at room temperature was within 85-115% up to 2.63 hrs.

Application

The validated method was successfully applied to 'An open-label, balanced, randomized, two treatment, two-period, two sequence, single-dose, crossover oral bioequivalence study of Ezetimibe Tablets 10 mg Tablets in normal healthy, adult, human subjects under fasting conditions' conducted over 12 healthy male human volunteers.

All 445 samples including the calibration, QC and volunteer samples were analyzed in 11 days using a two instruments and precision and accuracy of calibration and QC samples were within acceptance limit.

The chromatograms of EZM in real subject samples are presented. The mean plasma concentrations versus time profiles under fasting condition were represented (Figure 3). Incurred sample reanalysis results were within 20% of initial analysis values.

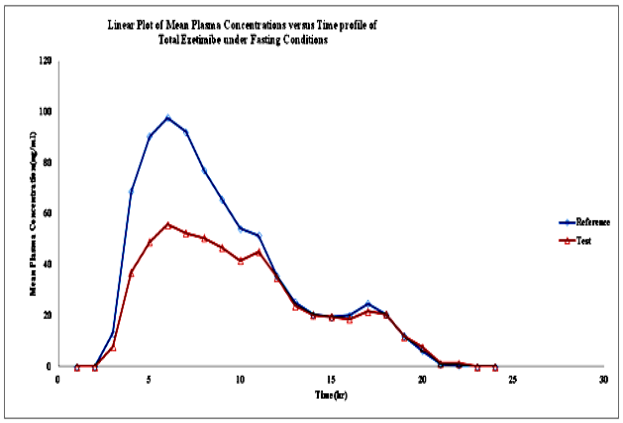

Figure 3: Plasma concentration versus time profile of Total Ezetimibe under Fasting Conditions.

CONCLUSION

A rapid, sensitive and accurate liquid chromatography with electro spray ionization tandem mass spectrometry method was developed for determination of TEZM in human plasma with short chromatographic runtime of 4.0 min and with high sensitivity. The extraction method utilizes low sample volume of only 300µL and shows consistent and reproducible recoveries for analyte and ISTD were with minimum plasma interference and matrix effect. The validated method can be used for the analysis of patient's samples receiving ezetimibe and to support the clinical pharmacokinetic studies.

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Abbreviations

CV: Coefficient Variation; HPLC: High Performance Liquid Chromatography; IS: Internal Standard; LC: Liquid Chromatography; LC-MS/MS: Liquid Chromatography – Mass Spectrometry / Mass Spectrometry LLOQ: Lower Limit of Quantitation; LQC: Low Quality Control; MQC: Medium Quality Control; HQC: High Quality Control; PK: Pharmacokinetics; QC: Quality Control; RP: Reverse Phase; CE: Collision energy; ESI: Electrospray Ionization; MRM: Multiple Reaction Monitoring; Cmax: The maximum plasma concentration of the drug; psi: Pounds per square inch.

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