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Simultaneous Quantitative Determination of Olmesartan and Hydrochlorothiazide in Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry

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ABSTRACT

A specific, sensitive and rapid LCMS/MS method was developed for simultaneous determination of olmesartan and hydrochlorothiazide in human plasma using olmesartan D4 and hydrochlorothiazide 13C6 as internal standards. Solid-phase extraction (SPE) method was used to extract the analytes from biological matrix. Analysis was carried out on phenomenex Luna C18 column with a flow rate of 0.600 mL/minute with 80% flow splitting. Detection was carried out on a triple quadrupole linear mass spectrometer, equipped with turbo ion spray source. The method was validated over the concentration range of 32.32 ng/mL to 2676.60 ng/mL for olmesartan and 5.12ng/mL to 423.83ng/mL for hydrochlorothiazide. Olmesartan and hydrochlorothiazide were found to be stable upto 75 days in K3EDTA based Human Plasma at -20ºC. Inter and intra-batch precision of olmesartan and hydrochlorothiazide were less than 15% and the accuracy was within 85–115% in plasma. The mean % recovery was 53.09 % for olmesartan and 59.12 % for hydrochlorothiazide in human plasma. The stability of olmesartan and hydrochlorothiazide in plasma were confirmed up to five freeze-thaw cycles at -20°C and on bench up to 24 hours and 15 minutes at ambient temperature. The method was validated satisfactorily and was suitable for the quantitation of olmesartan and hydrochlorothiazide from plasma samples in a pharmacokinetic study.

Keywords: Olmesartan, Hydrochlorothiazide, Edema, Hypertension, Liquid Chromatography, Mass Spectroscopy.

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INTRODUCTION

Olmesartan 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-2-(1H-1,2,3,4-tetrazol-5-yl)phenylphenyl}methyl)-1H-imidazole-5-carboxylic acid is an antihypertensive agent, which belongs to the class of medications called angiotensin II receptor blockers. It is indicated for the treatment of blood pressure alone or in combination with other thiazide diuretics. The FDA label includes a black-box warning of injury and death to the fetus, so women of child bearing age need to be warned and take the necessary precautions. Olmesartan is also contraindicated in diabetes mellitus patients taking aliskiren [1].

Hydrochlorothiazide 6-chloro-1,1-dioxo-3,4-dihydro-2H-1λ^6,2,4-benzothiadiazine-7-sulfonamide is a thiazide diuretic often considered the prototypical member of this class. It reduces the reabsorption of electrolytes from the renal tubules resulting in increased electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension, and diabetes hypoparathyroidism [2].

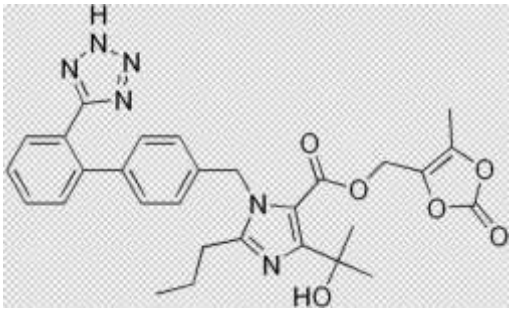
High blood pressure (BP) is a major risk factor for cardiovascular disease, contributing to the premature death of millions of patients worldwide every year. In the recent 2013 Guidelines of the European Society of Hypertension (ESH)/European Society of Cardiology (ESC) for the management of arterial hypertension, high blood pressure is defined, as a systolic blood pressure (SBP) $\geq$ 140 mmHg and/or a diastolic blood pressure (DBP) $\geq$ 90 mmHg. According to recent epidemiological surveys, the overall prevalence of hypertension in the general population ranges from 30 to 45%, and increases markedly with age.

Diuretics promote renal excretion of sodium and induce reflex activation of the renin-angiotensin system (RAS) via intrarenal mechanisms; such reflex activation makes BP more dependent on the RAS, thereby enhancing the antihypertensive efficacy of ARBs; on the other hand, ARBs can offset thiazide-induced potassium loss [3].

The aim of antihypertensive therapy is to minimize the risks associated with blood pressure (BP) elevation without adversely affecting quality of life. Epidemiologic studies and clinical trials have been used to define individual risk. Drug selection for antihypertensive therapy is based on efficacy in lowering BP and in reducing cardiovascular (CV) end points, including stroke, myocardial infarction, and heart failure [4].

Various methods for the analysis of olmesartan and hydrochlorothiazide have been published. These include UV spectrophotometric method [5-6], HPLC methods [7-10], HPTLC methods using human plasma [11-13] and liquid chromatography/ mass spectrometry methods [14].

The aim of this study was to develop and validate a sensitive and rapid bioanalytical method for quantification of olmesartan and hydrochlorothiazide in human plasma. Various available methods are intended to analyze single analyte and they did not have fast and sensitive method for simultaneous estimation.



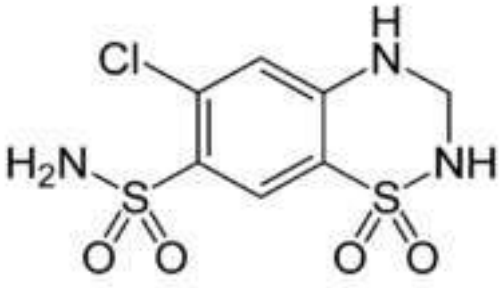


Figure 1A: Chemical structures of Olmesartan Figure 1B: Chemical structures of Hydrochlorothiazide

MATERIALS AND METHOD

Chemicals and materials

Working standards of olmesartan and hydrochlorothiazide were procured from Vivan Life Sciences Pvt. Limited. K3EDTA based Human Plasma was procured from Saibaba Health Care, Pune. The internal standard of Olmesartan D4 was purchased from clear synth labs limited. The internal standard of Hydrochlorothiazide 13C6 was purchased from Vivan Life Sciences Pvt. Limited. Acetonitrile and methanol (LC/MS grade) was purchased from J.T.Baker (Deventer, Netherlands). Formic acid, ammonia as well as all other chemicals and reagents were obtained from Sigma–Aldrich (Prague, Czech Republic).

Instrumentation for liquid chromatography–mass spectrometry

Analysis was conducted using a Shimadzu HPLC system with two pumps (Nexera X2, Shimadzu Corporation, Kyoto, Japan) coupled to a triple quadrupole linear mass spectrometer, equipped with Turbo Ion Spray source (API 4000; AB Sciex, Foster City, CA, USA) The MS/MS analysis was carried out using Multi-reaction monitoring (MRM) in negative ionisation mode. MRM conditions were established by infusing standards solutions into the MS/MS for optimization. Collision-induced dissociation (CID) of each M-H - was performed and the product ions giving the best signal to noise ratio were selected for the MRM analysis. Nitrogen was used as curtain gas and collision gas. The temperature of the vaporizer was set at 500°C. The mass spectrometer was operated at unit mass resolution with a dwell time of 100 ms per transition. All data acquisition and processing was performed using the analyst software.

Preparations of Standards and Intermediate solutions

Stock solutions of olmesartan and hydrochlorothiazide were prepared by dissolving approximately 2 mg of each compound in 2 mL of methanol. For the preparation of intermediate solutions, 0.850 mL olmesartan stock solution and 0.136 mL of Hydrochlorothiazide Stock Solution were dissolved in 5 mL of diluent. Final concentrations of olmesartan and hydrochlorothiazide intermediate solution were 172684.04ng/mL and 27343.95ng/mL respectively.

Preparation of Calibration Standards and Quality Control Samples

The spiking solutions of calibration curve standards and quality control samples were prepared by using the respective olmesartan and hydrochlorothiazide intermediate solutions and diluent. The calibration curve standards were in the concentration range of 32.32ng/mL to 2676.60ng/mL for Olmesartan and 5.12ng/mL to 423.83ng/mL for Hydrochlorothiazide. The calibration curve was generated using linear regression $y = ax + b$ with weighting $(1/x^2)$.

Sample preparation

The subject samples, calibration curve samples and quality control samples were vortex-mixed after thawing and aliquots of 200 µL were transferred into a respective pre-labelled sample tubes. Then 50 µL of Olmesartan D4 and Hydrochlorothiazide 13C6 Spiking Solution (5247.45 ng/mL and 603.57 ng/mL respectively) was spiked in all sample tubes (except in the Blank sample tube) and were vortex-mixed for about 30 seconds. Then 0.500 mL of 2.0% formic acid was added to all the samples and all the sample tubes were vortex-mixed for about 30 seconds. The samples were transferred to strata X-C, polymeric strong cation 30 mg, 1ml cartridges (SPE columns), which had been conditioned with 1.0 mL of 5% ammonia in methanol, followed by equilibration with 1.0 mL of 2 % formic acid solution. After application of the samples, the SPE columns were washed with 0.500 mL of 2 % formic acid, water followed by 25% methanol solution. Then SPE columns were dried for 3.0 min by applying positive pressure at maximum flow rate. The column was eluted with 250 µL of 2 % ammonia in 95% methanol solution (Twice). These samples were evaporated to dryness at about 50°C under a stream of nitrogen gas. The dried residue was reconstituted in 200 µL of reconstitution solution (0.2 % formic acid solution and methanol, 55:45 v/v) and vortex-mixed for about 30 seconds. The samples were transferred in individual pre-labelled auto-sampler vials for analysis purpose.

Analysis

The separation of the analytes were carried out, using Phenomenex Luna C18, (50×2 mm, 100Å, 5µ) column with binary flow of methanol and 0.2% formic acid solution (45:55 v/v) as Mobile Phase. An aliquot of 5.0µL of the sample was injected on the column. The separation was achieved

with isocratic elution at flow rate of 0.600 mL/minute with 80% flow splitting. The total analytical run time per sample was 1.60 min. The column oven temperature and auto-sampler temperature was maintained at 40ºC and 15ºC respectively. The LC/MS/MS analysis was performed using MRM transitions. Concentrations were determined by peak area ratios between analyte and internal standard.

Method validation

The method was validated for selectivity, sensitivity, matrix factor, matrix effect, precision and accuracy, linearity, recovery, and stability experiment according to the US Food and Drug Administration (FDA) guidelines[15].

For selectivity, six blank and six LLOQ level samples were processed and extracted as per the extraction procedure and interference was checked at the retention time of analytes or internal standards. For evaluation of matrix factor and matrix effect eight blank samples from eight different plasma lots, including one haemolyzed lot and one hyperlipidaemic plasma lot were spiked with spiking solution of LQC and HQC and were extracted as per extraction procedure. Matrix has a lot of inherent variability and can affect the response of analytes during the method validation and subsequently in subject analysis. The quantification of analytes from plasma can be grossly affected by a significant matrix effect. Quantitative measurement of matrix effect can be termed as matrix factor (MF) and defined as the ratio of an analytes peak or IS peak response in the presence of matrix ions to the analytes peak or IS peak response in the absence of matrix ions. Matrix effect was evaluated by calculating % RSD of IS-normalized matrix factor of analytes at LQC and HQC level. The % recovery of analytes and internal standard was determined by comparing the mean peak area of six extracted and six unextracted samples at three different concentration levels, (LQC, MQC and HQC). Calibration Curve Standards were prepared using pooled K3EDTA based Human Plasma. Calibration Curve Standard consisted of blank sample (matrix sample processed without Analyte and IS), Blank + IS (matrix sample processed with IS) and eight non-zero standards in the concentration range of 32.32 ng/mL to 2676.60 ng/mL for Olmesartan and 5.12 ng/mL to 423.83 ng/mL for Hydrochlorothiazide. For sensitivity experiment six samples of blank biological matrix from different lots were spiked with spiking solution standard A (LLOQ) and extracted along with precision and accuracy batch as per extraction procedure. Signal to Noise ratio (S/N), % RSD and % Nominal of back calculated concentration of analytes at LLOQ level was calculated.

Intra-batch and inter-batch accuracy and precision was evaluated from replicate analyses (n=6) of quality control samples containing olmesartan and hydrochlorothiazide at different concentrations

(LLOQ QC, LQC, LMQC, MQC and HQC). Data of precision and accuracy is summarized in table 02.

Table 01: Mass Parameters for Olmesartan, Hydrochlorothiazide and their Internal Standard

Compound Dependent Parameters	Olmesartan	Olmesartan D4	Hydrochlorothiazide	Hydrochlorothiazide 13C6
Q1	445.200	449.100	295.850	302.000
Q3	167.00	167.100	204.900	210.900
Declustering Potential (DP)	-79	-78	-91	-87.90
Entrance Potential (EP)	-9.84	-9.00	-13	-11.10
Collision Energy (CE)	-32.20	-32.00	-30	-31.93
Cell Exit Potential (CXP)	-13.71	-13.33	-15	-6.99
Source Dependent Parameters				
Curtain gas		25		
IS Voltage		-4500		
Temperature		500		
Collision Activation Dissociation (CAD)		10		
GS1		35		

Drug stability in a biological matrix is a function of the storage conditions, the chemical and/or physical properties of drug, the matrix and the container system. The stabilities of olmesartan and hydrochlorothiazide in biological matrix and working solutions at different storage conditions were evaluated. Stability of samples was evaluated on the basis of the comparison of various samples against freshly prepared samples of the same concentration. Percentage difference between the back calculated concentrations obtained for the samples under investigation and freshly prepared samples was evaluated. Six aliquots, each of LQC and HQC concentrations were used for stability study.

RESULTS AND DISCUSSION

Chromatographic conditions optimization

Initially plane solvents like methanol, formic acid, glacial acetic acid and ethyl acetate, were tried. The separation was tried with methanol and formic acid but no proper resolution observed between olmesartan and hydrochlorothiazide. Then separation was carried out on Kinetix C18 column using acetonitrile and 0.1 % formic acid (80:20 v/v) as mobile phase but again there is no proper resolution obtained. Then analysis was done on Phenomenex Luna C18 (50×2 mm, 100Å, 5µ) column using mixture of methanol and formic acid solution which showed good resolution. Finally

mobile phase consists of methanol and 0.2% formic acid solution (45:55 v/v) was selected, which gave good resolution of peaks for olmesartan and hydrochlorothiazide. Retention time for hydrochlorothiazide and olmesartan was 0.31 min and 0.71 min respectively.

Method validation

Method validation was performed for various parameters as per regulatory requirements and results obtained are defined as follows.

Selectivity and Specificity:

Selectivity was performed to assess that the quantitation of intended analytes was not affected by the presence of endogenous matrix components, metabolites, degradation products or co-administered drugs. Blank and LLOQ level sample from six different lots along with one Haemolysed and one hyperlipidemic lot were processed and extracted as per the extraction procedure. Interference was checked at the retention time of Analytes or internal standard. Quantitation of analytes was not affected by the presence of the endogenous compounds and No significant interference was observed at the RT of Olmesartan, Hydrochlorothiazide and their internal standards in blank samples, hence, the method is selective. Representative chromatograms of blank and LLOQ samples are presented in Figure 02 A, 02 B, 02 C, 02 D.

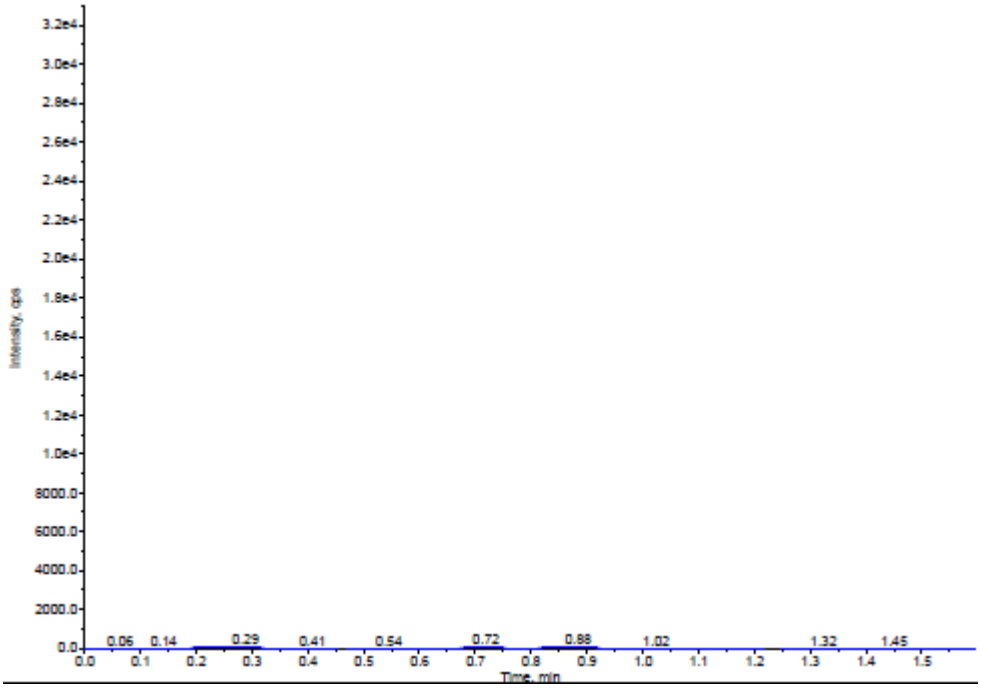


Figure 2A: Representative chromatogram of blank sample for Olmesartan

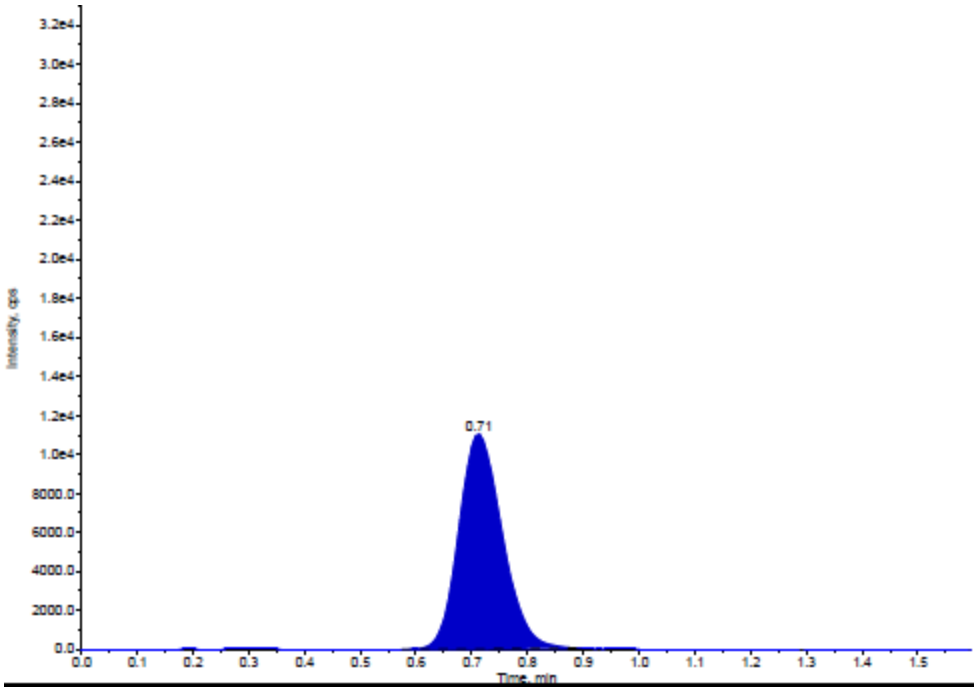


Figure 2B: Representative chromatogram of LLOQ sample for Olmesartan

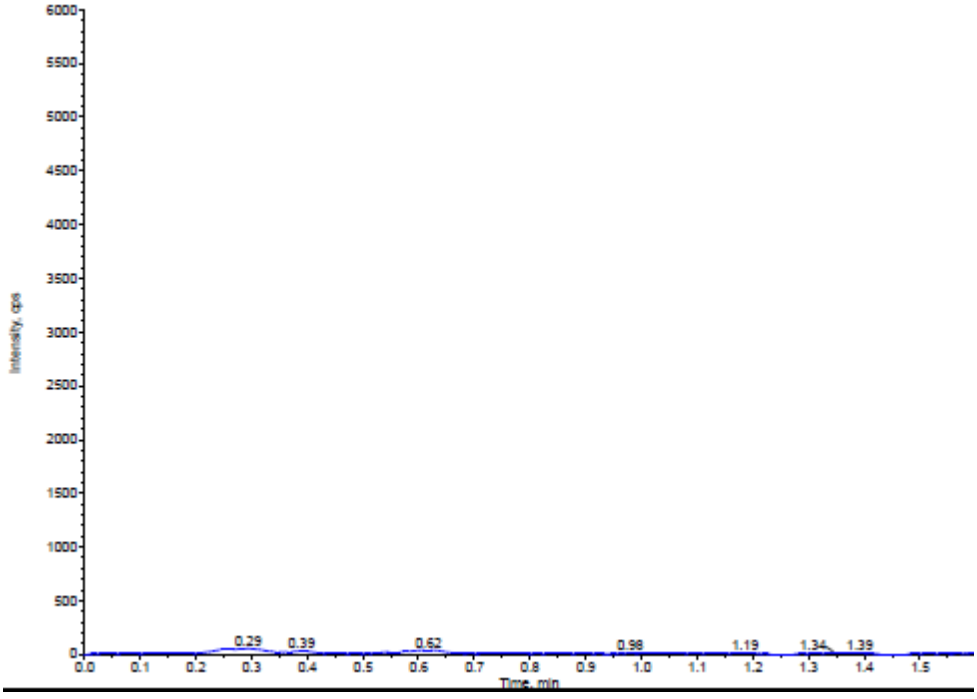


Figure 2C: Representative chromatogram of blank sample for Hydrochlorothiazide

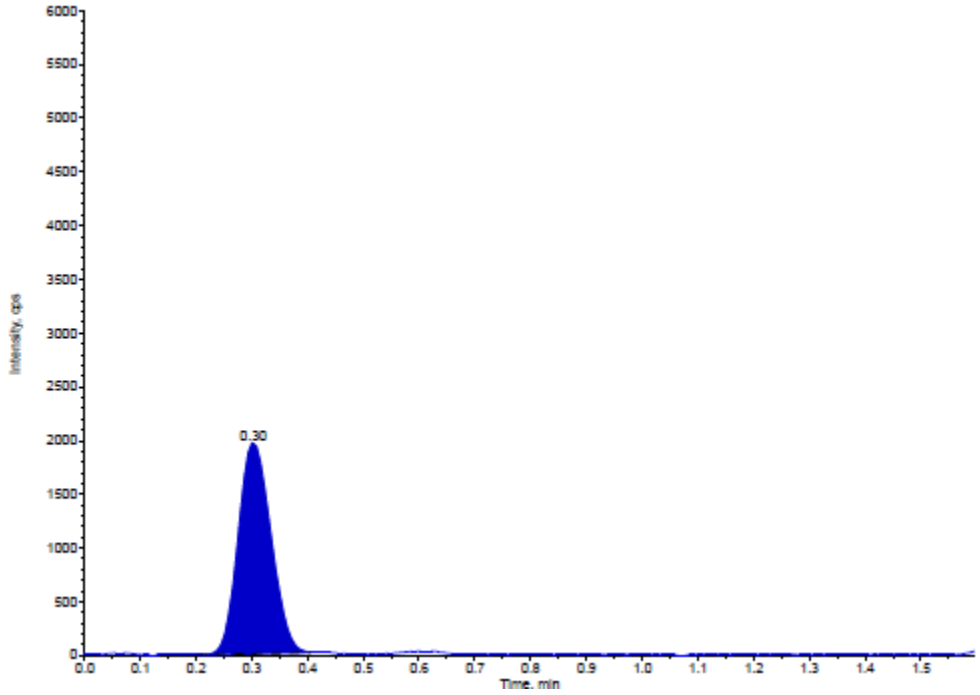


Figure 2D: Representative chromatogram of LLOQ sample for Hydrochlorothiazide

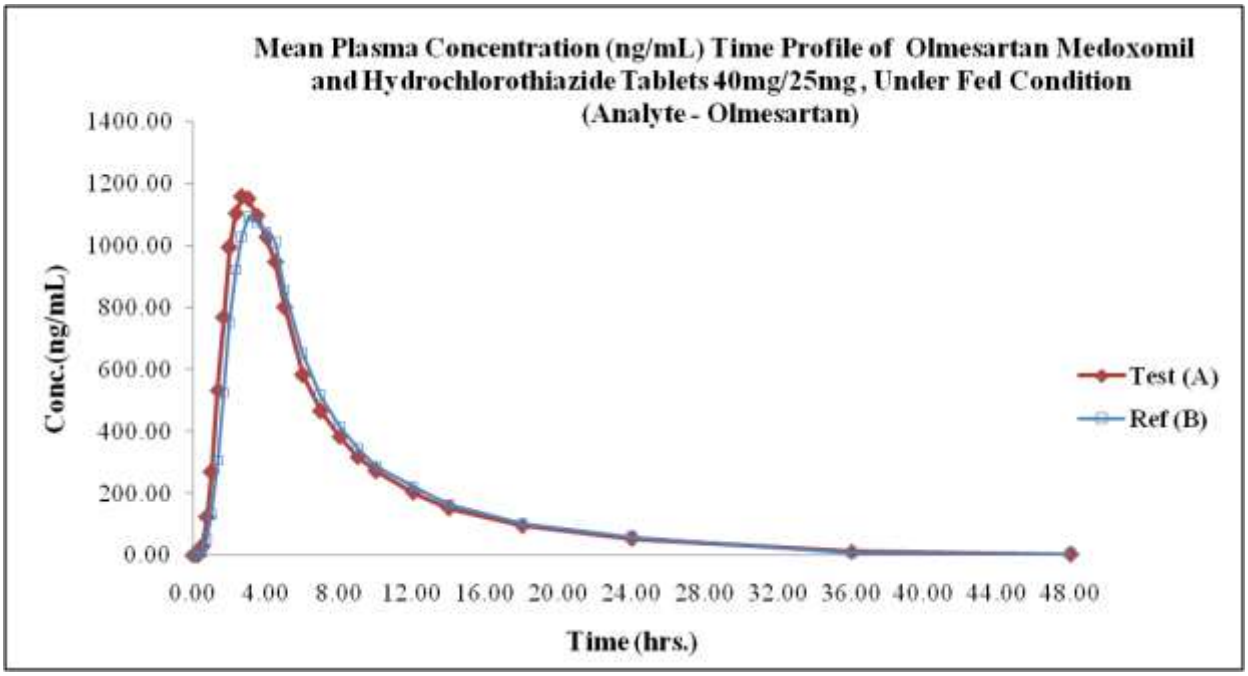


Figure 3A: Profile of mean plasma concentration vs time of Olmesartan

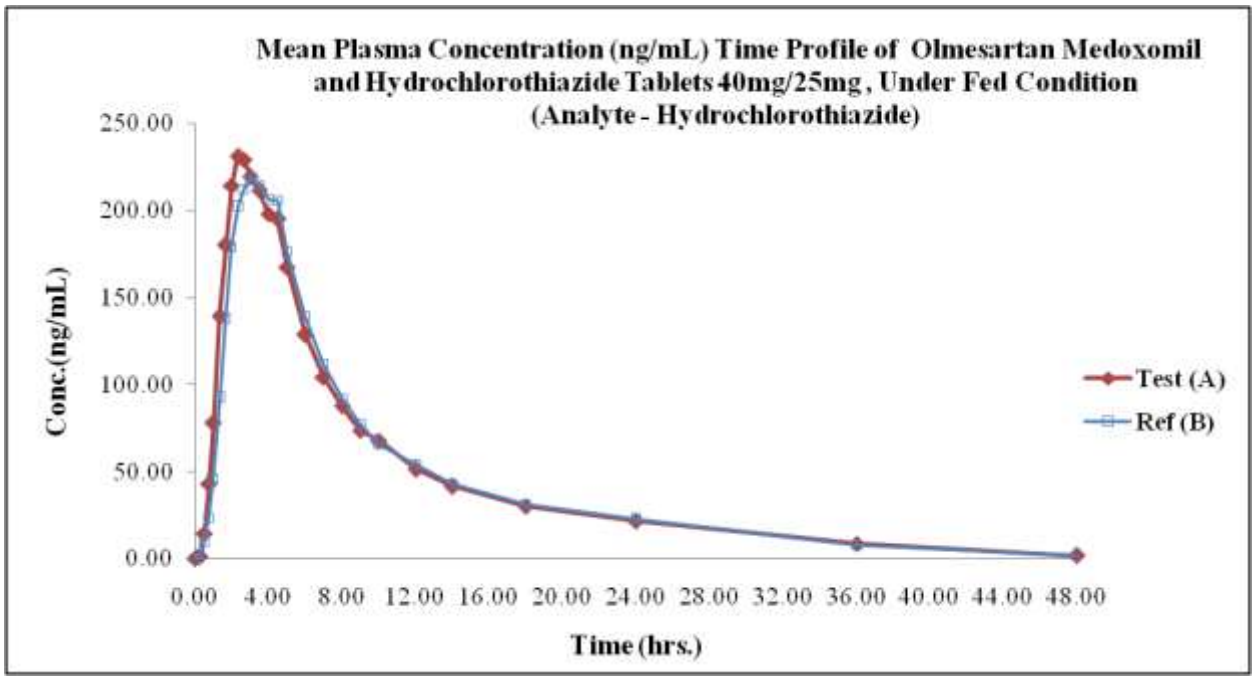


Figure 3B: Profile of mean plasma concentration vs time of Hydrochlorothiazide

Matrix Factor:

Matrix Effect was not observed for olmesartan and hydrochlorothiazide as % accuracy and % RSD data was within the acceptance criteria.% RSD of IS-normalized Matrix Factor of Analytes at LQC and HQC level were evaluated. Six normal plasma lots, one harmonized and one lipemic plasma lot were used for evaluation of matrix effect. The %RSD in matrix factor was 4.37% for Olmesartan and 3.27% for Hydrochlorothiazide at LQC level and 1.96% for Olmesartan and 3.63% for Hydrochlorothiazide at HQC level. The mean matrix factor for Olmesartan and Hydrochlorothiazide samples was 1.02 and 0.99 respectively at HQC level and 1.03 and 1.00 respectively at LQC level. The results of matrix factor were within the acceptance limit which shows that ionization and quantification of analyte is not effected by biological matrix.

Sensitivity:

Method was found to be sensitive as analyte peak (response) was identifiable, discrete and reproducible with precision of 20% and accuracy between 80% to 120%. Signal to Noise ratio (S/N) was calculated for each sensitivity sample. The % RSD and % Nominal of back calculated concentration of analytes at LLOQ level was calculated.

Linearity:

Calibration curves of olmesartan and hydrochlorothiazide in plasma were constructed using eight non-zero standard points covering the range of 32.32 ng/mL to 2676.60 ng/mL for olmesartan and 5.12 ng/mL to 423.83 ng/mL for hydrochlorothiazide. Coefficient of correlation of all calibration

curves were more than 0.99. The Calibration Curve was generated using linear regression y = ax + b with weighting (1/x2).

Table 02: Data of Inter-day and Intra-day Precision and Accuracy for Olmesartan and Hydrochlorothiazide

Olmesartan									
		Intra-batch				**Inter-Batch**			
QC ID	**Nominal Conc. (ng/mL)**	**N**	**Mean Calculated Conc. (ng/mL)**	**Accuracy (%)**	**CV (%)**	**n**	**Mean Calculated Conc. (ng/mL)**	**Accuracy (%)**	**CV (%)**
LLOQ QC	32.54	6	35.66	109.59	2.47	18	35.13	107.96	2.95
LQC	95.71	6	107.33	112.14	3.33	18	106.02	110.77	4.76
LMQC	534.68	6	572.96	107.16	2.39	18	586.46	109.68	2.85
MQC	1338.36	6	1406.25	105.07	1.51	18	1429.03	106.77	3.55
HQC	2141.38	6	2143.74	100.11	1.59	18	2197.70	102.63	3.86
Hydrochlorothiazide									
QC ID	**Nominal Conc. (ng/mL)**	**Intra-batch**				**Inter-Batch**			
		N	**Mean Calculated Conc. (ng/mL)**	**Accuracy (%)**	**CV (%)**	**n**	**Mean Calculated Conc. (ng/mL)**	**Accuracy (%)**	**CV (%)**
LLOQ QC	5.21	6	5.46	104.80	2.01	18	5.24	100.58	4.77
LQC	15.34	6	16.22	105.74	2.97	18	15.31	99.80	6.92
LMQC	85.67	6	86.29	100.72	1.36	18	86.17	100.58	2.91
MQC	214.45	6	217.16	101.26	2.92	18	215.64	100.55	3.08
HQC	343.11	6	343.35	100.07	2.15	18	338.80	98.74	2.64

n: Total number of observations

CV: Coefficient of Variation

Precision and Accuracy:

Inter batch and Intra-batch precision and accuracy experiments were done as a part of this validation exercise. A single precision and accuracy batch consisted of one set of calibration curve standards and six replicates of extracted samples for each concentration level were processed. Intra-day and inter-day accuracy and precision were evaluated from replicate analyses (n=6) of quality control samples containing Olmesartan and Hydrochlorothiazide at different concentrations (LLOQ QC, LQC, LMQC, MQC and HQC). Intra-batch and inter-batch accuracy and precision were also assessed from the analysis of the same QC samples on different days in replicate (n=6). QC samples were analyzed against calibration standards. Data of precision and accuracy is summarized in Table 02.

Recovery:

Average % recovery for olmesartan and olmesartan D4 at three different concentrations was 53.10% and 55.73% respectively. Average % recovery for hydrochlorothiazide and

hydrochlorothiazide 13C6 at three different concentrations was 59.12% and 60.61% respectively. The % recovery of analytes and IS from K3EDTA based human plasma are shown in table 03 04.

Table 03: Data of Recovery for Olmesartan and Hydrochlorothiazide

Olmeasartan		
QC ID	**% Mean Recovery**	**%CV**
LQC	50.23	1.43
MQC	54.36	2.68
HQC	54.70	2.05
Hydrochlorothiazide		
QC ID	% Mean Recovery	**%CV**
LQC	53.38	6.91
MQC	59.11	4.15
HQC	64.87	2.96

Table 04: Data of Recovery for Olmesartan D4 and Hydrochlorothiazide 13C6

Olmesartan D4		
QC ID	% Mean Recovery	%CV
LQC	56.29	0.85
MQC	56.40	1.00
HQC	54.51	2.37
Hydrochlorothiazide 13C6		
QC ID	% Mean Recovery	%CV
LQC	57.83	7.16
MQC	59.94	4.26
HQC	64.07	3.64

Haemolysis and Lipemic effect:

Haemolysis and lipemic effect of analytes was evaluated by calculating the % RSD and % nominal of back calculated concentration. From results it was observed that quantitation of Triamterene and Hydrochlorothiazide was not affected by haemolysis and lipid content of samples.

Ruggedness Test:

Ruggedness test was performed for different column, different equipment as well as different analyst. Precision and accuracy batches were processed for evaluation of ruggedness of the method at five different concentration levels (LLOQ QC, LQC, LMQC, MQC and HQC). All ruggedness tests were meeting the acceptance criteria of accuracy and precision.

Stability

Bench top stability was evaluated by thawing the samples at room temperature for more than 24 hours. No significant difference was observed between response of freshly prepared samples and stability samples of olmesartan and hydrochloroyhiazide after 24 h at −20°C temperature. Freeze-thaw stability was satisfactorily determined after five freeze- thaw cycles for six replicates of LQC

and HQC samples. The samples were stored at ambient temperature. Then samples were thawed at room temperature and processed with freshly prepared samples as per extraction procedure. No significant difference between freeze-thaw samples and freshly prepared samples was observed. For wet extract stability, samples were extracted and stored at 2-8ºC. After stipulated duration (22 hours and 51 minutes), Stability samples were processed along with fresh samples. % difference was calculated between stability samples and fresh samples. The auto-sampler stability was evaluated by keeping six replicates of extracted samples at LQC and HQC concentration levels in the auto-sampler up to 26 hours and 14 minutes at 15ºC. Freshly prepared plasma samples (six replicates) at LQC and HQC concentration levels were extracted along with one set of freshly prepared calibration curve standards as per the extraction procedure. These samples were then analyzed and compared with fresh samples for stability evaluation. Auto-sampler stability was evaluated with a single run of one set of freshly prepared calibration curve standards and six replicates of stability and fresh samples at LQC and HQC levels. The duration of auto-sampler stability of olmesartan and hydrochlorothiazide was calculated from the auto-sampler loading time of samples to the injection time of the First Stability sample. The % Difference, % RSD and % Nominal of back calculated concentrations of olmesartan and hydrochlorothiazide were calculated for the stability and fresh samples. All stability results met acceptance criteria. Results of stability experiments are shown in Table 05.

Table 05: Data of Stability for Olmesartan and Hydrochlorothiazide

Olmesartan					
Stability	**QC Level**	**Nominal Conc. (ng/mL)**	**Mean Calculated Conc. (ng/mL)**	**% Difference**	**% CV**
Bench Top	LQC	95.71	107.90	1.53	3.21
Stability	HQC	2141.38	2208.04	1.51	10.07
Freeze Thaw	LQC	95.71	105.34	1.91	3.31
Stability	HQC	2141.38	2151.72	2.09	1.55
Autosampler	LQC	95.71	104.03	-2.11	0.89
Stability	HQC	2141.38	2285.87	5.09	0.40
Wet Extract	LQC	95.71	99.60	-6.28	11.16
Stability	HQC	2141.38	2225.40	2.31	6.19
Dry Extract	LQC	97.17	98.44	1.11	1.23
	HQC	2149.48	2070.57	0.74	1.13
Long Term	LQC	95.71	101.17	-5.74	2.19
Stability	HQC	2141.38	2121.71	-1.03	0.92
Hydrochlorothiazide					
Stability	**QC Level**	**Nominal Conc. (ng/mL)**	**Mean Calculated Conc. (ng/mL)**	**% Difference**	**% CV**
Bench Top	LQC	15.34	15.82	-1.29	3.38
Stability	HQC	343.11	346.58	1.70	9.46

Freeze Thaw	LQC	15.34	16.10	1.40	2.24
Stability	HQC	343.11	347.83	0.56	1.21
Autosampler	LQC	15.34	14.24	-11.15	2.02
Stability	HQC	343.11	341.73	0.27	1.41
Wet Extract	LQC	15.34	13.99	-12.71	10.04
Stability	HQC	343.11	337.94	-0.84	7.80
Dry Extract	LQC	15.35	13.68	-3.54	1.85
	HQC	339.62	309.69	-0.07	3.28
Long Term	LQC	15.34	14.10	-13.07	3.93
Stability	HQC	343.11	317.67	-7.48	2.85

BIOEQUIVALENCE AND PHARMACOKINETIC STUDY:

The developed LC-MS/MS method was applied to the quantitation of olmesartan and hydrochlorothiazide in subject samples of the bioequivalence study. An open-labelled, balanced, randomized two-treatment, two-sequence, two period, single oral dose, two-way crossover design, bioequivalence study was carried out in 42 normal, healthy, adult, human subjects under fasting condition. Olmesartan medoxomil and hydrochlorothiazide 40/25 mg tablets were administered orally to all volunteers. The study was approved by Ethics Committee. The volunteers were selected on the basis of predetermined inclusion/exclusion criteria. Samples were analyzed and statistical evaluation was done to obtain Pharmacokinetic parameters. Table 06 and Table 07 shows evaluated pharmacokinetic parameters of Olmesartan and Hydrochlorothiazide in human subjects. Figure 3A and 3B shows profile of mean plasma concentration vs time respectively for Olmesartan and Hydrochlorothiazide.

Table 06: Pharmacokinetic parameters for Olmesartan

Parameter	**Geometric Least-Squares means[1]**		**Ratio[2]**	**CV%[3]**	**90% confidence interval limits[4]**		**Power (%)**
	Test	**Reference**			**Lower**	**Upper**	
Cmax (ng/mL)	1233.96	1216.27	101.45	11.50	96.92	106.2	100.00
AUC0-t (ng.hr/mL)	7832.49	7826.10	100.08	10.42	96.02	104.32	100.00
AUC0-inf npg.hr/mL)	8335.13	8319.48	100.19	10.53	96.08	104.48	100.00

Table 07: Pharmacokinetic parameters for Hydrochlorothiazide

Parameter	**Geometric Least-Squares means[1]**		**Ratio[2]**	**CV%[3]**	**90% confidence interval limits[4]**		**Power (%)**
	Test	**Reference**			**Lower**	**Upper**	
Cmax (ng/mL)	253.62	248.49	102.06	9.89	98.12	106.16	100.00
AUC0-t (ng.hr/mL)	1981.82	1952.21	101.52	6.75	98.82	104.29	100.00
AUC0-inf (ng.hr/mL)	2115.25	2079.53	101.72	6.85	98.98	104.53	100.00

CONCLUSION:

The proposed bioanalytical method for the quantitation of olmesartan and hydrochlorothiazide from K3EDTA based human plasma was satisfactorily validated using solid phase extraction procedure. The extraction procedure and LC-MS/MS conditions were optimized in order to improve the sensitivity and recovery of the method. No significant matrix effect was observed by analysing the plasma samples. Stability of olmesartan and hydrochlorothiazide was evaluated satisfactorily. Method is suitable for analysis of these analytes in their combined dosage forms, in a single isocratic run, in contrast with previous methods. This makes the method suitable for routine analysis in bioequivalence studies.

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