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Simultaneous Determination of Triamterene and Hydrochlorothiazide In Human Plasma by Liquid Chromatography-Mass Spectrometry

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ABSTRACT

Triamterene is a potassium-sparing diuretic which is commonly used in combination with Hydrochlorothiazide, a thiazide-type diuretic in clinical management of edema and moderate hypertension. In this study, a rapid and sensitive LC–MS/MS method was developed and validated for determination of Triamterene and Hydrochlorothiazide from K3EDTA based Human Plasma. Triamterene D5 and Hydrochlorothiazide 13C6 were used as an Internal Standards for analysis of Plasma Samples. The analytes and internal standards, were extracted by liquid–liquid extraction method using Se Quant®ZIC-HILIC, (5um,200A 150 X 4.6 mm) column with Acetonitrile and 2 mM Ammonium formate containing 0.1% formic acid (80:20 v/v) as the mobile phase. Linearity was assessed from 3.10ng/mL to 229.72 ng/mL for Triamterene and 5.47 ng/mL to 405.27 ng/mL for Hydrochlorothiazide in plasma. No significant matrix effects were observed by analysing the plasma samples on LC–MS/MS. The accuracy was in the range of 98.32 % to 102.86 % for both compounds. Triamterene and Hydrochlorothiazide were found to be stable up to 120 days in K3EDTA based Human Plasma at -20ºC. The stability of Triamterene and Hydrochlorothiazide in plasma was confirmed up to five freeze-thaw cycles (−20°C) and on bench up to 25 hours. The proposed bioanalytical method for the quantitation of Triamterene and Hydrochlorothiazide from K3EDTA based human plasma samples was satisfactorily validated. It can be used to include study data for quantitation of Triamterene and Hydrochlorothiazide from K3EDTA based human plasma in bioequivalence and bioavailability study.

Keywords: Triamterene, Hydrochlorothiazide, Edema, Hypertension, Liquid Chromatography, Mass Spectroscopy

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INTRODUCTION

Edema is an accumulation of fluid in the intercellular tissue that results from an abnormal expansion in interstitial fluid volume. The fluid between the interstitial and intravascular spaces is regulated by the capillary hydrostatic pressure gradient and the oncotic pressure gradient across the capillary. The accumulation of fluid occurs when local or systemic conditions disrupt this equilibrium, leading to increased capillary hydrostatic pressure, increased plasma volume, decreased plasma oncotic pressure (hypoalbuminemia), increased capillary permeability, or lymphatic obstruction [1].

Risk factors for cardio-vascular diseases (CVD) such as tobacco smoking, high level of blood cholesterol, diabetes, physical inactivity, and obesity, need to be taken into account when defining individual risk of coronary heart diseases (CHD) and when planning preventive and therapeutic intervention. There is a worrying increase in the distribution all of these risk factors among people in developing countries [2].

Triamterene (2, 4, 7-triamino-6-phenylpteridine) Figure 1A is a potassium-sparing diuretic commonly used in combination with Hydrochlorothiazide (6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide 1, 1-dioxide), a thiazide-type diuretic Fig. 1B in clinical management of edema and moderate hypertension

Hydrochlorothiazide is incompletely but fairly rapidly absorbed from the gastrointestinal tract. Peak plasma concentrations occur between 60 and 120 min. It is excreted unchanged into urine, 50% being recovered within the first 12 h. The serum half-life is estimated to be in the range of 3–4 h.

Triamterene is rapidly absorbed from the gastrointestinal tract but to a variable extent (30–70% of the oral dose). The peak plasma concentration of Triamterene is reached 2–4 h after an oral dose and the half-life of the Triamterene in plasma ranges from 1.5 to 2 h. Approximately 50% of the Triamterene in plasma is protein bound. Triamterene undergoes metabolism in the liver. Triamterene and its metabolites are then excreted really utilizing the processes of filtration and tubular secretion. About 20% of an oral dose appears unchanged in the urine, 70% as the sulphate ester of hydroxytriamterene, and 10% as free hydroxytriamterene and Triamterene glucuronide [3].

Various methods for the analysis of Triamterene and Hydrochlorothiazide have been published. These include HPLC-UV method [3, 4], HPTLC method [5], derivative Spectrophotometry method [6], liquid chromatography/tandem mass spectrometry method [7-8]. Various available methods are intended to analyze single analyte and they did not have fast and sensitive method for simultaneous

estimation. As these two analytes are having different ionization polarity in mass spectrometer, it is a challenge to develop simultaneous method. The aim of this study was to develop and validate a sensitive LC/MS/MS method for simultaneous quantification of Triamterene and Hydrochlorothiazide from human plasma.

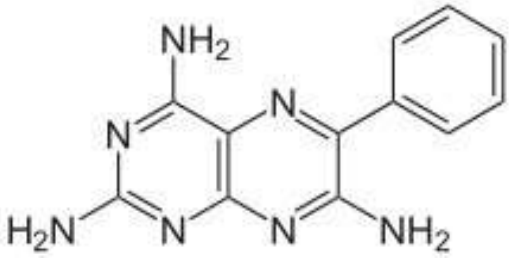

Figure 1A: Chemical structure of Triamterene

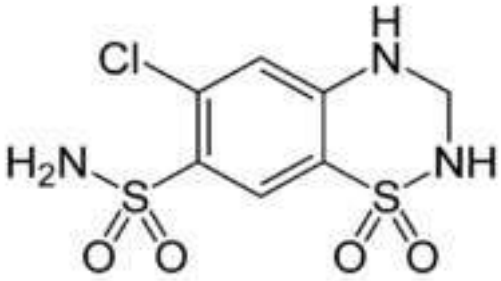

Figure 1B: Chemical structure of Hydrochlorothiazide

MATERIALS AND METHOD

Chemicals and materials

Working standard of Triamterene was procured from Clearsynth labs limited. Working standard of Hydrochlorothiazide was procured from Micro labs limited. K3EDTA based Human Plasma was procured from Saibaba Health Care, Pune. Acetonitrile and methanol (LC/MS grade) was purchased from J.T.Baker (Deventer, Netherlands). The internal standards Triamterene D5 and Hydrochlorothiazide 13C6 were purchased from Clearsynth labs limited. Formic acid, ammonium formate 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 triple quadrupole mass spectrometer, equipped with Turbo Ion Spray source (API 4000; AB Sciex, Foster City, CA, USA) coupled to a liquid chromatography system with two pumps (Nexera X2, Shimadzu Corporation, Kyoto, Japan). Pure nitrogen was used as curtain gas and collision gas. Air was used as nebuliser and auxiliary gas. The temperature of the auxiliary gas was set at 500°C and the ion spray voltage at 5500 V. The MS/MS analysis was

carried out using Multi-reaction monitoring (MRM) in positive ionization mode for Triamterene and negative ionization mode for Hydrochlorothiazide. Detailed mass parameters are provided in Table 01. To establish the appropriate MRM conditions, standard solutions were infused into the MS/MS for optimization. Collision-induced dissociation (CID) of each M+H and M-H was performed and the product ions giving the best signal to noise ratio were selected for the MRM analysis. All data acquisition and processing was performed using the analyst software. The solutions used for liquid-liquid extraction were centrifuged using Cooling Centrifuge (Remi instruments, CPR-23 Plus, C-23BL).

Preparations of Standards and Intermediate solutions

Stock solutions of Triamterene and Hydrochlorothiazide were prepared by dissolving approximately 2 mg of Triamterene and Hydrochlorothiazide in 2 mL of dimethyl sulfoxide (DMSO) and Methanol respectively. Intermediate Solution was prepared by dissolving respective stock solutions in 10 mL of diluents (Methanol: Water: 50:50).

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 Triamterene and Hydrochlorothiazide intermediate solutions and diluent. The calibration curve standards were in the range of 3.10 ng/mL to 229.72 ng/mL for Triamterene and 5.47 ng/mL to 405.27 ng/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 300 µL were transferred into a respective pre-labelled poly propylene tubes. Then 50 µL of IS solution, 100 µL of 0.1% Formic Acid Solution and 3.000 mL of Extraction Mixture (Tertiary butyl methyl ether and Ethyl Acetate 50:50 v/v) was added. The samples were vortex-mixed for about 30 seconds after addition of each solution. All the sample tubes were rotated on extractor for 10 minutes at 45 RPM and centrifuged at 4500 rpm for 7 minutes at 4ºC. Then 2.4 mL of the supernatant organic layer was transferred in other pre-labelled pp tubes. These samples were evaporated to dryness at about 45°C under a stream of nitrogen gas and dried residue was reconstituted in 200 µL of mobile phase. All the pp tubes were vortex-mixed for about 30 seconds and the samples were transferred in individual pre-labelled Autosampler vials for analysis purpose.

Analysis

The separation of the analytes was carried out, using Se Quant®ZIC-HILIC, (5um,200A 150 X 4.6 mm) column with Acetonitrile and 2 mM Ammonium formate containing 0.1% formic acid (80:20 v/v) as the 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 1.2 mL/minute. The total analytical run time per sample was 4.50 min. The column oven temperature was maintained at 45ºC and autosampler temperature was maintained at 15 ºC. The LC/MS/MS analysis was performed using MRM transitions. Concentrations were determined by peak area ratios between analyte and internal standard.

Method validation

This bioanalytical method was validated as per USFDA guidelines. Parameters like sensitivity, precision, accuracy, recovery, selectivity were evaluated during method validation. The method was also evaluated for matrix effect and Stability of analytes in refrigerated condition as well as ambient condition. For extracted samples, stability was evaluated at room temperature, in deep freezer, stability after freeze thaw cycles, dry extract stability, wet extract stability, whole blood stability, autosampler stability. Various other experiments like lipemic effect, haemolysed effect, dilution integrity, ruggedness and long batch performance were evaluated as per the USFDA guidelines [9].

RESULTS AND DISCUSSION

Method development

To develop a simple and popular LC-MS/MS method for quantitation of Triamterene and Hydrochlorothiazide, wide spectrum of organic solvents from different physicochemical categories with different volume fractions and combinations were tested. In terms of the analysis condition, various mobile phases, in different proportions, buffered and non-buffered at various pH were attempted to provide concomitantly the best peak resolution and retention times. After considering all the data, the optimum method conditions described earlier were selected for method validation.

Method validation

The LLOQ established in Method Validation was 3.10 ng/mL for Triamterene and 5.47 ng/mL for Hydrochlorothiazide. Figures of Blank and LLOQ sample are given in Figure 2A and 2B for Triamterene and 2C and 2D for Hydrochlorothiazide. The Linear range was determined from eight concentration levels covering the range from 3.10 ng/mL to 229.72 ng/mL for Triamterene and 5.47 ng/mL to 405.27 ng/mL for Hydrochlorothiazide. Intra-day and Inter-day accuracy and precision were evaluated from replicate analyses (n=6) of quality control samples containing

Triamterene and Hydrochlorothiazide at five different concentrations. QC samples were analyzed against calibration standards. The percent recovery of analytes and IS from K3EDTA based human plasma was determined by comparing the mean peak area of six extracted and six unextracted samples at three different concentrations (LQC, MQC and HQC). Percent recovery for Triamterene and Hydrochlorothiazide was ranged from 56.17% to 69.93% (Table 03). Percent recovery for Triamterene D5 and Hydrochlorothiazide 13C6 was ranged from 61.29% to 77.81% (Table 04). Haemolysis and lipemic effect was also evaluated by calculating the % RSD and % nominal of back calculated concentration. 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. Stability experiments were performed based on the comparison of stability samples against freshly prepared samples of the same concentration. Stability was evaluated by calculating percentage difference between the back calculated concentrations of stability samples and freshly prepared sample.

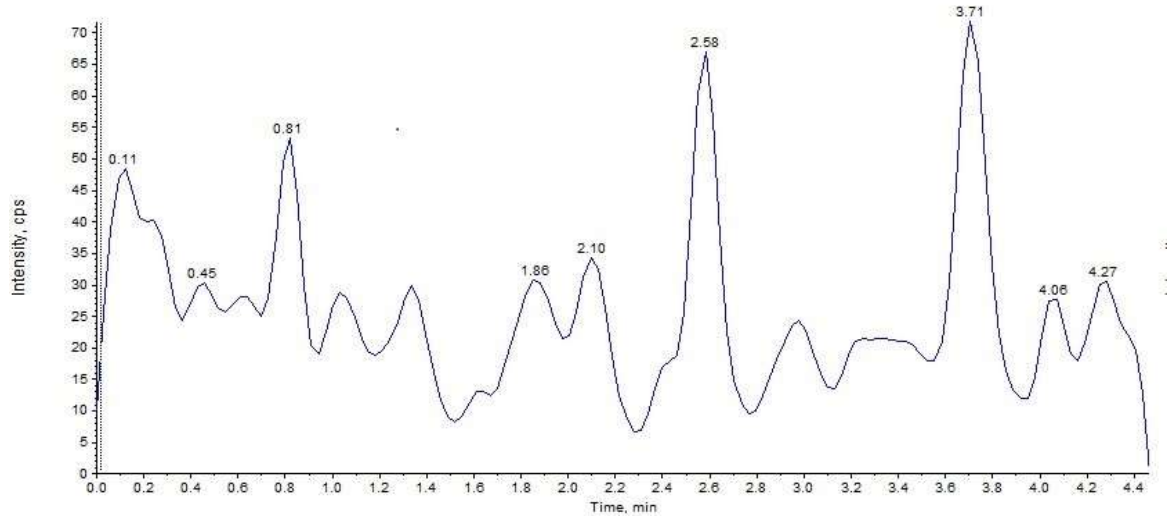

Figure 2A: Representative chromatogram of blank sample for Triamterene

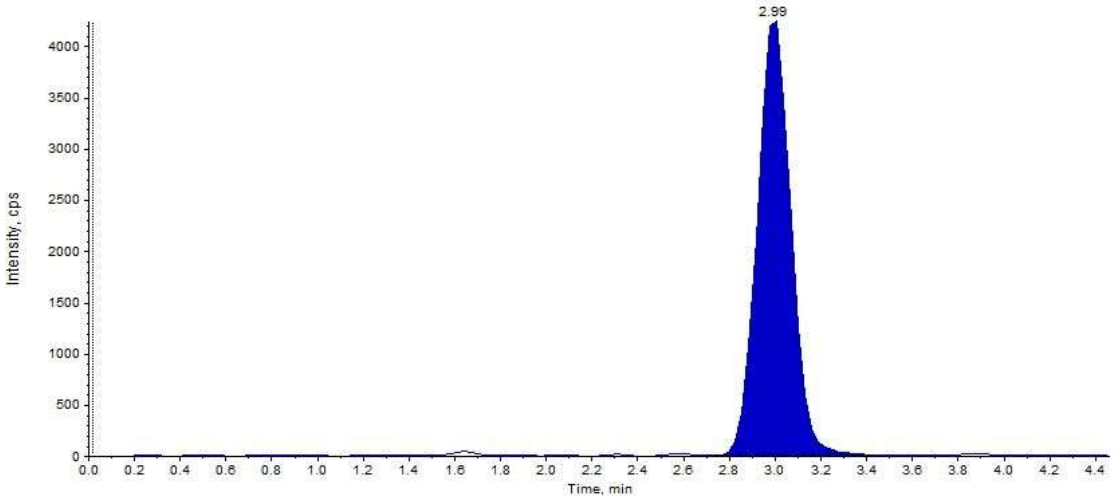

Figure 2B: Representative chromatogram of LLOQ sample for Triamterene

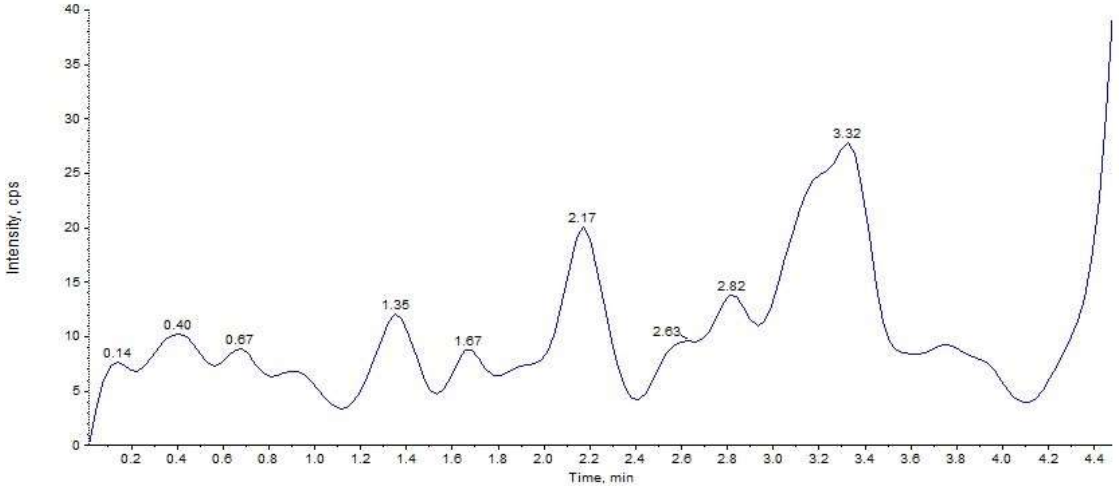

Figure 2C: Representative chromatogram of blank sample for Hydrochlorothiazide

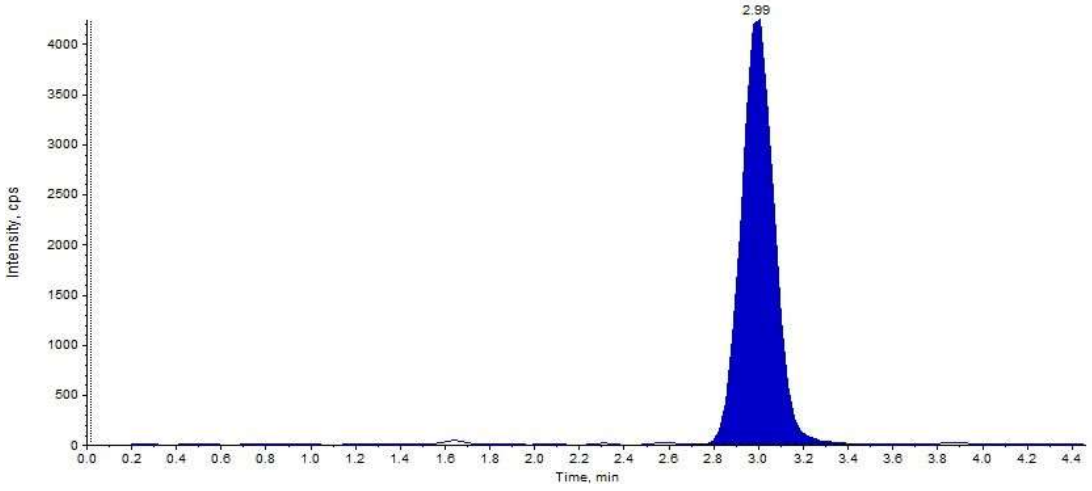

Figure 2D: Representative chromatogram of LLOQ sample for Hydrochlorothiazide

Selectivity and Specificity:

Selectivity was assessed to show 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 biological matrix and there was no interference of the biological matrix in the quantitation of Triamterene and Hydrochlorothiazide, hence, the method is selective.

Matrix Factor:

% RSD of IS-normalized Matrix Factor of Analytes at LQC and HQC level were evaluated. Six normal plasma lots, one haemolized and one lipemic plasma lot were used for evaluation of matrix effect. The %RSD in matrix factor was 2.69% for Triamterene and 4.57% for Hydrochlorothiazide at LQC level and 2.53% for Triamterene and 6.05% for Hydrochlorothiazide at HQC level. The mean matrix factor for Triamterene and Hydrochlorothiazide samples was 1.01 and 1.03 respectively at HQC level and 0.99 and 1.04 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.

Auto sampler Carryover Test:

The carryover response in the reconstitution solution (RS) injection as well as in Blank Sample at the retention time of analytes and IS was evaluated. No peak was found at the retention time of the analytes in reconstitution solution (RS) injection or blank sample injected after aqueous STD H and STD H respectively, its area response was less than 20.00% of analytes area response of aqueous STD A or extracted STD A respectively. No peak was found at the retention time of the IS in Reconstitution Solution (RS) injection or Blank sample, its area response was less than 5.00% of IS area response of an Aqueous STD A or extracted STD A respectively.

Sensitivity:

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) was calculated for each sensitivity sample. The % RSD and % Nominal of back calculated concentration of analytes at LLOQ level was calculated.

Linearity:

Linearity was evaluated using freshly prepared spiked plasma samples and calibration curves were constructed using eight non-zero standard points covering the range of 3.0 to 400 ng/ml. The Calibration Curve was generated using linear regression y = ax + b with weighting (1/x2).

Precision and Accuracy:

For all precision and accuracy experiments % RSD, % bias and % accuracy of back calculated concentration of analytes was calculated. Intra-day and inter-day accuracy and precision were evaluated from replicate analyses (n=6) of quality control samples containing Triamterene 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:

The % recovery of analytes and IS from K3EDTA based human plasma was determined by comparing the mean peak area of six extracted and six unextracted samples at three different concentrations (LQC, MQC and HQC). The data of recovery is summarized in Table 03 and 04.

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

Compound Dependent Parameters	Triamterene	Triamterene D5	Hydrochlorothiazide	Hydrochlorothiazide 13C6
Q1	254.100	259.200	295.800	301.700
Q3	104.200	242.100	204.900	210.700
Declustering Potential (DP)	35	96.00	-91	-100.00
Entrance Potential (EP)	6	10.00	-13	-10.00
Collision Energy (CE)	50	45.00	-30	-38.00
Cell Exit Potential (CXP)	10	11.00	-15	-15.00
Source Dependent Parameters				
Curtain gas		20		
IS Voltage		5500		
Temperature		550		
Collision Activation Dissociation (CAD)		10		
GS1		35		
GS2		60		

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

Triamterene									
		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	3.16	6	3.12	98.73	4.03	18	3.02	95.57	4.39
LQC	9.30	6	9.07	97.53	2.16	18	9.14	98.28	2.46
LMQC	46.50	6	45.20	97.20	2.25	18	45.87	98.65	2.26
MQC	116.25	6	109.57	94.25	1.74	18	112.32	96.62	2.58
HQC	184.53	6	173.38	93.96	2.57	18	177.57	96.23	2.66
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	5.62	6	4.82	85.77	10.67	18	5.03	89.50	8.22
LQC	16.53	6	15.05	91.05	3.68	18	15.64	94.62	7.28

LMQC	82.65	6	76.71	92.81	4.12	18	79.76	96.50	5.38
MQC	206.61	6	197.05	95.37	4.44	18	201.28	97.42	4.62
HQC	327.96	6	305.71	93.22	2.67	18	317.38	96.77	4.61

n: Total number of observations

CV: Coefficient of Variation

Table 03: Data of Recovery for Triamterene and Hydrochlorothiazide

Triamterene		
QC ID	% Mean Recovery	**%CV**
LQC	56.17	2.59
MQC	62.29	1.27
HQC	65.56	1.37
Hydrochlorothiazide		
QC ID	% Mean Recovery	**%CV**
LQC	65.93	5.47
MQC	69.93	1.61
HQC	67.80	0.81

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.

Dilution Integrity:

The effect of dilution on the analysis of Triamterene and Hydrochlorothiazide was evaluated by diluting the Dilution Integrity sample by 2 and 10 times. Dilution Integrity sample was having concentration 367.55 ng /mL for Triamterene and 648.44 ng /mL for Hydrochlorothiazide. Six replicates of each diluted DI samples were analysed for dilution integrity. % RSD and % Nominal of back calculated concentration of Analytes was calculated for the diluted samples. From the data it was concluded that analytes can be accurately and precisely quantified after dilution up to 10 times.

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.

Long Batch Performance:

The long batch performance experiment was performed to evaluate any trends over time within one run and to demonstrate accuracy and precision of QC samples with a size equivalent to a prospective analytical run. The experiment was performed with multiple sets of QC samples at five different concentrations (LLOQ QC, LQC, LMQC, MQC and HQC). The data generated in this Long Batch indicates acceptable and satisfactory instrument performance over the period of time for which the Long batch (consisting of 170 samples) was run. This Long batch was a simulation of the proposed subject sample analysis batch. Also no trend within a run was observed over the period in which 170

samples of the Long Batch were analyzed. Hence analysis of plasma samples of the subjects from the proposed Triamterene and Hydrochlorothiazide bio-study can be successfully done in analytical runs comprising of 170 samples.

Stability

Evaluation of the stability of samples was based on the comparison of stability samples against freshly prepared samples of the same concentration. Percentage difference between the back calculated concentrations obtained for the sample under investigation and freshly prepared sample was evaluated. Six aliquots, each of LQC and HQC concentrations were used for stability study. In bench top stability the low and high QC sample were thawed and left at room temperature for 25 hours. Comparison of the results for QC sample (low and high) with freshly prepared samples showed that there was no significant difference between response of freshly prepared samples and samples of Hydrochlorothiazide and Triamterene after 25 hours. Freeze-thaw stability was determined after five freeze- thaw cycles for six replicate of low and high QC sample. The samples were stored at −20°C 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. Dry extract stability was evaluated by extracting samples till step of drying. Dry extract samples were stored at 2-8ºC in refrigerator for 24 hours. After stability duration (24 hours) these samples are reconstituted and analysed along with fresh samples for stability evaluation. For wet extract stability, samples were extracted and stored at 2-8ºC. After 24 hours, stability samples were processed along with fresh samples. % difference was calculated between stability samples and fresh samples. All stability results met acceptance criteria. Results of stability experiments are shown in Table 05.

Table 04: Data of Recovery for Triamterene D5 and Hydrochlorothiazide 13C6

Triamterene D5		
QC ID	**% Mean Recovery**	**%CV**
LQC	61.29	1.64
MQC	65.50	1.78
HQC	69.68	1.12
Hydrochlorothiazide 13C6		
QC ID	**% Mean Recovery**	**%CV**
LQC	75.85	1.59
MQC	77.81	2.03
HQC	76.21	2.71

Table 05: Data of Stability for Triamterene and Hydrochlorothiazide

Triamterene					
Stability	**QC Level**	**Nominal Conc. (ng/mL)**	**Mean Calculated Conc. (ng/mL)**	**% Difference**	**% CV**
Bench Top Stability	LQC	9.30	9.16	1.54	2.53
	HQC	184.53	176.25	-0.08	2.59
Freeze Thaw	LQC	9.30	9.32	0.32	2.24
Stability	HQC	184.53	177.86	1.49	2.29
Autosampler	LQC	9.30	9.14	1.32	1.73
Stability	HQC	184.53	177.26	0.49	1.94
Wet Extract	LQC	9.30	9.23	1.09	1.70
Stability	HQC	184.53	175.22	-0.99	3.71
Dry Extract	LQC	9.30	9.03	-1.10	2.44
	HQC	184.53	179.66	1.51	0.90
Long Term Stability	LQC	9.30	9.31	0.21	2.34
	HQC	184.53	176.99	0.99	1.83
Hydrochlorothiazide					
Stability	**QC Level**	**Nominal Conc. (ng/mL)**	**Mean Calculated Conc. (ng/mL)**	**% Difference**	**% CV**
Bench Top Stability	LQC	16.53	17.85	12.29	5.80
	HQC	327.96	313.50	-3.85	4.24
Freeze Thaw	LQC	16.53	15.54	-5.82	4.94
Stability	HQC	327.96	312.63	-2.69	4.30
Autosampler	LQC	16.53	16.16	1.66	6.99
Stability	HQC	327.96	312.85	-4.05	2.87
Wet Extract	LQC	16.53	15.28	-7.00	6.18
Stability	HQC	327.96	304.33	-3.76	3.32
Dry Extract	LQC	16.53	16.05	-2.31	3.20
	HQC	327.96	325.59	2.96	2.22
Long Term Stability	LQC	16.53	16.08	-2.54	3.79
	HQC	327.96	316.76	-1.41	1.72

BIOEQUIVALENCE AND PHARMACOKINETIC STUDY:

The developed bioanalytical method was applied to the quantitation of Triamterene and Hydrochlorothiazide in plasma samples generated during the bioequivalence study. An open-labelled, balanced, randomized two-treatment, two-sequence, four period, single oral dose, fully replicate crossover design, bioequivalence study was carried out in 40 normal, healthy, adult, human subjects under fasting condition. During the pharmacokinetic studies Triamterene-Hydrochlorothiazide 75/50 mg tablets was administered orally. 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 Triamterene

and Hydrochlorothiazide in human subjects. Figure 3A and 3B shows profile of mean plasma concentration vs time respectively for Triamterene and Hydrochlorothiazide.

Table 06: Pharmacokinetic parameters for Triamterene

Parameter	Geometric Least-Squares means[1]		Ratio[2]	CV%[3]	95% Upper Confidence Bound[4]	Power (%)
	Test	Reference				
Cmax (ng/mL)	209.50	169.43	123.65	58.50	-0.1107	93.37
AUC0-t (ng.hr/mL)	645.57	604.50	106.79	34.39	-0.0555	99.67
AUC0-inf npg.hr/mL)	681.81	657.43	103.71	39.95	-0.0806	98.75

Table 07: Pharmacokinetic parameters for Hydrochlorothiazide

Parameter	Geometric Least-Squares means[1]		Test-to-Ref Ratio[5]	CV%[6]	90% confidence interval limits[7]		Power (%)
	Test	Reference			Lower	Upper	
Cmax (ng/mL)	481.08	448.36	107.30	15.24	102.61	112.20	100.00
AUC0-t (ng.hr/mL)	3200.51	3203.37	99.91	8.28	96.62	103.32	100.00
AUC0-inf (ng.hr/mL)	3321.29	3326.00	99.86	8.01	96.74	103.08	100.00

1. For log e-transformed results (Ln), value is the least-squares geometric mean.
2. Ratio calculated as Test-squares mean divided by the Reference least-squares mean.
3. Estimated intra-subject coefficient of variation.
4. 95% Upper Confidence Bound on linearized statistic.
5. Ratio% of least-squares means or least-squares geometric means for log e-transformed results.
6. Intra-subject CV% calculated from the mean square term of the ANOVA as 100% × Sqrt (eMSE-1).
7. Confidence interval on ratio.

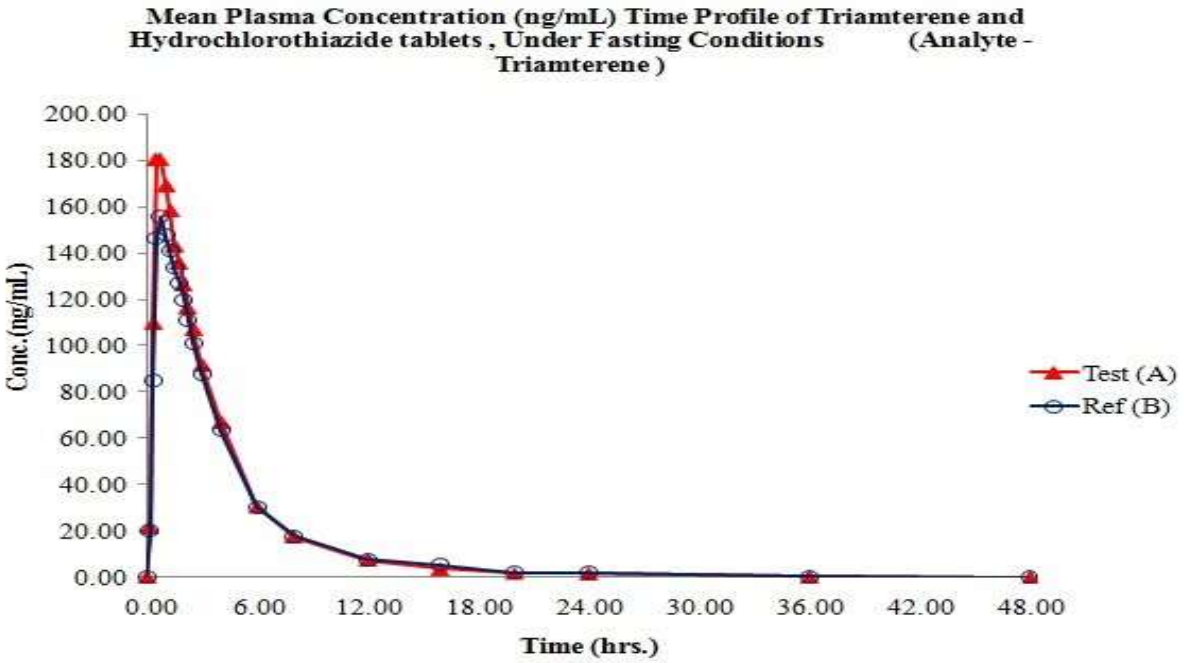

Figure 3A: Profile of mean plasma concentration vs time of Triamterene

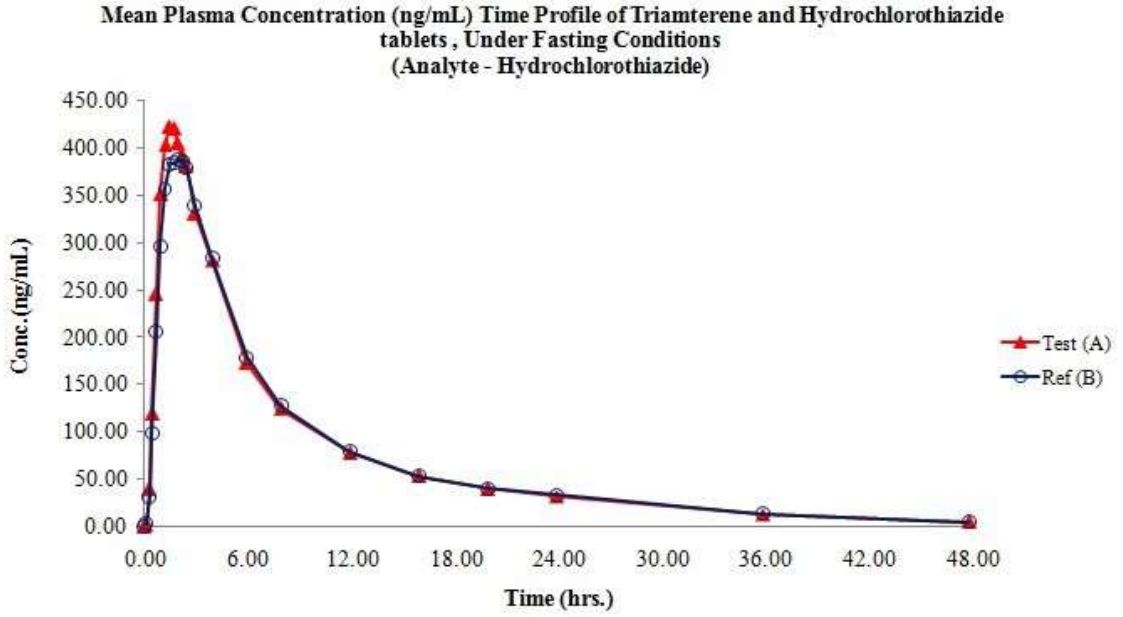

Figure 3B: Profile of mean plasma concentration vs time of Hydrochlorothiazide

CONCLUSION:

A rapid and sensitive liquid chromatography-mass spectroscopy method was developed with polarity switching without compromising on sensitivity. This method was validated for simultaneous quantification of Triamterene and Hydrochlorothiazide in K3EDTA based Human Plasma. The method has excellent precision and accuracy which enables detection of Triamterene and Hydrochlorothiazide. No significant matrix effects were observed by analysing the plasma

samples on LC–MS/MS. Stability data of Triamterene and Hydrochlorothiazide was found to be satisfactory. This liquid-liquid extraction method has several advantages, including rapid analysis, a simple mobile phase, simple sample preparation, and improved sensitivity. It 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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