Research Article

Simultaneous Determination of Valsartan and Hydrochlorothiazide in Human Plasma by LC-MS/MS Method.

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ABSTRACT

A rapid, selective and specific liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was developed and validated for simultaneous quantification of valsartan and hydrochlorothiazide in human plasma. After a solid phase extraction, the analytes were separated on an Ascentis express C18 column using Acetonitrile: Methanol: 10 mM ammonium Formate (5:85:10) as mobile phase at a flow rate of 0.400 mL/min with 10 µL of sample. Valsartan and hydrochlorothiazide were eluted at 1.0 ± 0.30 min in the duration of 2.50 min and the selected reaction monitoring was specific for mass detection employing negative electro spray ionization. The precursor to product ion transitions of m/z 433.8 → 178.80 and m/z 295.80 → 204.90 were used to quantify valsartan and hydrochlorothiazide respectively. The method was linear in the concentration range of 20.00–10019.33 ng/mL for valsartan and 3.76–303.53 ng/mL for hydrochlorothiazide. Intra-day and inter day precision (%CV) and accuracy (% Nominal) for quality control samples (55.95, 4662.30 and 7875.51 ng /mL for valsartan and 11.22, 136.80 and 232.89 ng /mL for hydrochlorothiazide) ranged between 2.05 to 3.60% for valsartan 2.75-2.95% for hydrochlorothiazide. and % nominal ranged between 100.30 to 108.54% for valsartan and 95.05 to 101.14% for hydrochlorothiazide respectively. Extraction recovery of valsartan from plasma was in the range 69.84 – 71.05%, Mean recovery is 70.35% and 69.74 – 69.92%, mean recovery is 69.80% for hydrochlorothiazide respectively.

Keywords: Hydrochlorothiazide, Human Plasma, Tandem Mass Spectrometry, Valsartan.

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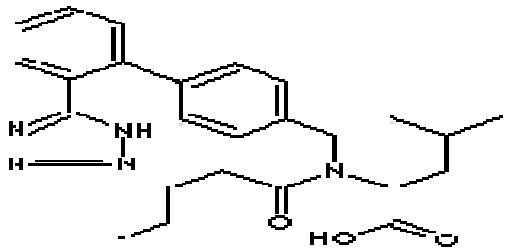
INTRODUCTION

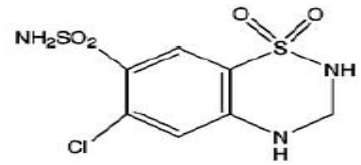
Valsartan, 7(S)-3-methyl-2-(N-{[2'-(2H-1, 2, 3, 4-tetrazol-5-yl) biphenyl-4- l] methyl} pentanamido) butanoic acid (**Figure 1**) belonging to the chemical class specific angiotensin II (Ang II) receptor antagonist. It acts selectively on the AT1 receptor subtype, which is responsible for the known actions of angiotensin II[1]. Valsartan does not exhibit any partial agonist activity at the AT1 receptor and has much (about 20,000 fold) greater affinity for the AT1 receptor than for the AT2 receptor. Valsartan is not known to bind to or block other hormone receptors or ion channels known to be important in cardiovascular regulation.

Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide (**Figure 1**) belonging to the chemical class thiazide diuretic. Thiazides affect the renal tubular mechanisms of electrolyte reabsorption, directly increasing excretion of sodium and chloride in approximately equivalent amounts. Indirectly, the diuretic action of hydrochlorothiazide reduces plasma volume, with consequent increases in plasma renin activity, increases in aldosterone secretion, increases in urinary potassium loss, and decreases in serum potassium. The renin-aldosterone link is

mediated by angiotensin II, so coadministration of an angiotensin II receptor antagonist tends to reverse the potassium loss associated with these diuretics[2]. The mechanism of the antihypertensive effect of thiazides is unknown.



(S)-3-methyl-2-(N-{[2'-(2H-1, 2, 3, 4-tetrazol-5-yl) biphenyl-4- l] methyl} pentanamido) butanoic acid



6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide

Figure 1: Chemical Structure valsartan and Hydrochlorothiazide

Only few methods appear in the literature for determination of valsartan individually based on HPLC [3-8] and GC-MS [9], spectrophotometry, LC [10-11]. HPLC & LC-MS, when in combination with hydrochlorothiazide [12-17].There have been several reports on the determination of hydrochlorothiazide individually or in its combination with other drugs, including the use of liquid chromatography [18-24] and spectrophotometry [25-29] and valsartan with other combination [30].

The above mentioned methods have low linearity range, more elution time, flow rate and injection volume. In this paper a rapid, specific and inexpensive high- performance liquid-chromatographic–electrospray mass spectrometric (LC–ESI–MS) method is developed for analysis of valsartan and hydrochlorothiazide in human plasma. It is less sample volume and simple solid phase extraction (SPE) technique with 20.00–10019.33 ng/mL for valsartan and 3.76–303.53 ng/mL for hydrochlorothiazide as LOQ and ULOQ for extraction and a short Elution time 1.00± 0.30 min with 0.400 ml/min flow rate for Quantification of valsartan and hydrochlorothiazide in human plasma.

MATERIALS AND METHODS

Chemicals and Reagents:

Valsartan Reference standard (Potency w/w 99.0%), was gifted sample from Hetero drugs, India. Hydrochlorothiazide (**Figure 1**) reference standard (IS) (Potency w/w is 99.6%) was was gifted sample from Dr.Reddy's Losartan reference standard (IS) (Potency w/w is 99.4%) was gifted sample from Hetero Drugs. Zidovudine reference standard (IS) (Potency w/w is 99.0%) was gifted sample from Dr.Reddy's. HPLC grade Methanol (HPLC grade) Acetonitrile and Ammonia Solution (GR grade) Ammonium formate (AR Grade), manufactured by Merck Ltd were purchased from Merck Specialties Ltd., (Mumbai, India). High purity water was prepared with Milli-Q water purification system obtained from Millipore PVT Limited (Bangalore, India). HLB 3cc/60mg Cartridges (Waters-Oasis) were procured from Waters Corporation, Ireland. Blank (drug free) human plasma was obtained from Vuppala Venkaiah Memorial Blood Bank, Hyderabad, India and stored at -20 °C prior to use.

HPLC operating conditions:

An HPLC system -Shimadzu interfaced with Sciex MS-MS was used as chromatographic separation module. The mobile phase

Acetonitrile: Methanol: 10 mM ammonium Formate (5:85:10] and drugs were eliminated within 2.50 min at a flow rate of 0.400 mL/min (gradient flow) into the mass spectrometer electro spray ionization chamber. The retention times of Valsartan and Hydrochlorothiazide is 1.00 ± 0.30 min and for losartan is 1.10 ± 0.35 min, for Zidovudine 1.20 ± 0.40 min respectively.

Mass spectrometry operating conditions:

Quantitation was achieved by using Sciex make LC-MS/MS detection in Negative ion mode for analyte and IS using a mass spectrometer, equipped with an ESI interface at 450°C desolvation temperature. The ion source parameters viz nebulizer gas 10.00 (kV), Curtain gas (CUR) 12.00 Collision gas (CAD) 6.00, Ion spray Voltage (IS) -4500, Temperature 450°C and compound related parameters of valsartan and Hydrochlorothiazide are Declustering Potential (DP) -30 & -45, Focusing Potential (FP) -260 & -240, Entrance Potential (EP) -10, Collision Energy (CE) -20 & -32, Collision cell exit potential (CXP) -15 &-5 analytes and IS. Detection of the ions was carried out in the multiple reaction monitoring modes (MRM), by monitoring the transition pairs of 433.80m/z →178.80m/z (V), 295.80m/z→204.90m/z (H) for analytes and 421.90 m/z →126.90 m/z, 265.80m/z→121.90m/z for the IS. The analysis data obtained were processed using Analyst Software (version 1.4.2).

Preparation of stock solutions of Valsartan and Hydrochlorothiazide and IS:

Primary stock solutions of valsartan and Hydrochlorothiazide for preparation of standard and quality control (QC) samples were prepared from separate weighing. Primary and stock solutions of valsartan and Hydrochlorothiazide (1 mg/mL) were dissolve in about 5 mL of methanol and make up to 10 mL with the same to get 1 mg/mL. Correct the final concentration of analytes accounting for its potency and the actual amount weighed. The primary stock solution of Losartan (IS) is (1 mg/mL) was prepared in water and Zidovudine (IS) is (1 mg/mL) was prepared in methanol. The stock solutions were stored at 2-8 °C, which were found to be stable for 5.81 days. One set of working stock solutions of valsartan and Hydrochlorothiazide made in 5mL of methanol (from primary CC stock) was successively diluted with methanol to prepare appropriate working solutions to prepare calibration curve (CC) standards. Another set of working stock solutions were made in methanol (from primary QC stock) was successfully diluted with 5mL of methanol to prepare appropriate dilutions for preparation of QC samples. A working IS solution was prepared in water for losartan and Methanol for zidovudine (w/v).

Preparation of calibration curve standards and quality control samples:

Calibration samples and quality control samples were prepared by spiking 20 µL of appropriate working solution into 980 µL of control human plasma. Calibration curve (CC) consists of a set of eight non-zero standard concentrations ranging from 20.00–10019.33 ng/mL for valsartan and 3.76–303.53 ng/mL for hydrochlorothiazide.The quality control samples were prepared at concentrations of 20.59 (LLOQ), 55.95 (LQC), 4662.30 (MQC) and 7875.51 ng/mL (HQC) for valsartan and 3.90 (LLOQ), 11.22 (LQC), 136.80 (MQC) and 232.89 ng/mL (HQC) for Hydrochlorothiazide Samples for the determination of precision and accuracy were prepared by spiking control human plasma in bulk with at appropriate concentrations and 550µL plasma aliquots were distributed into different tubes. All the spiked samples were stored at -70 °C.

Sample preparation:

A simple Solid Phase extraction method was followed for extraction of Valsartan and Hydrochlrothiazide from human plasma. Aliquot 0.500 mL plasma, add 50 µL of IS solution (25.000 µg/ml Losartan +300.000 µg/mL Zidovudine) Vortex, then add 0.250 mL of Diluted Ammonia solution, Vortex for 30 seconds. Condition the cartridge (HLB 3cc (60 mg) SPE Cartridge) with 2 mL of methanol and 2mL of water and apply total volume. Elute the samples completely and slowly under vacuum. Wash the cartridge with 2 mL of water for two times and dry for 2 minutes. Elute with 1 mL of Methanol for two times into Ria vial, Evaporate the eluted samples under dry nitrogen gas at

about 500C & 15psi for 35 minutes or till the samples are dried. Reconstitute the evaporated samples with 1 mL of mobile phase and vortex .Transfer into auto sampler vials and inject 10µL on to LC-MS/MS system.

Validation parameters:

Validation of analytical method for the assay in human plasma was carried out according to the USFDA guidelines.

Specificity and selectivity:

The specificity of the method was evaluated by analyzing human plasma samples from six different lots to investigate the potential interferences at retention times of analytes and IS. The responses of the interfering substances or background noises at the retention time of the valsartan and hydrochlorothiazide are acceptable if they are less than 20% of the response of the lowest standard curve point or LLOQ. The responses of the interfering substances or background noise at the retention time of the internal standard are acceptable if they are less than 5% of the mean response of internal standard in LLOQ samples.

Calibration curve:

Linearity was assessed in the concentration range of 20.00 to 10019.33 ng/mL for valsartan and 3.76 to 303.53 ng/mL for hydrochlorothiazide by weighted linear regression (1/X2) of analyte-IS peak area ratios based on four independent calibration curves prepared on two different days using eight-point calibration curve. The calibration curve had to have a correlation coefficient (r) of 0.99 or better. The acceptance limit of accuracy for each of the back-calculated concentration was ± 15 % except for LLOQ, where it was ± 20% (US DHHS, FDA, CDER). The calibrators used for analyte were 20.00, 50.00, 150.14, 500.47, 1000.93, 2502.33, 7514.50, 10019.33 ng/mL for valsartan and 3.76, 7.51, 15.02, 25.04, 50.08, 100.17, 200.33, 303.53 ng/mL for Hydrochlorothiazide. The samples were run in the order from low to high concentration. In addition, blank plasma samples were also analyzed to confirm the absence of direct interferences, but these data were not used to construct the calibration curve. For a calibration run to be accepted at least 75% of the standards, including the LLOQ and ULOQ, were required to meet the acceptance criterion, otherwise the calibration curve was rejected.

Precision and accuracy:

The intra-assay precision and accuracy were estimated by analyzing six replicates containing valsartan and Hydrochlorothiazide at four different QC levels viz., LLOQ, LQC, MQC, and HQC in human plasma. The inter-assay precision and accuracy was determined by analyzing six replicates at four different QC levels on four different runs. The acceptance criteria included accuracy within ± 15% deviation from the nominal values, except LLOQ QC, where it should be ± 20 % and a precision of ≤15% relative standard deviation (RSD), except for LLOQ QC, where it should be ≤20% (US DHHS, FDA, CDER, 2001).

Matrix Effect, Recovery and Process Efficiency

As per the new regulatory recommendations the relative Matrix effect, Recovery and Process efficiency were assessed for analyte(s) with aqueous (neat), Post-extracted and extracted samples. These parameters were evaluated in five different standard levels in six replicates. Preparation of neat (aqueous) standards in the mobile phase at five standard levels. Extracted 6 different blank matrix lots (as per the extraction procedure) and spiked the standards into the different lots (Post extraction spiking). Spiked the standards in to 6 different lots (same as used above) and extracted (Before extraction spiking).

The acceptance criteria for matrix effect was mean response obtained for post extracted samples should be within ±15% of the mean response of aqueous (neat) samples concentrations. %CV should not exceed 15%. Except STD1 where it is 20%.

For Recovery Percent recovery for analyte (s) or ISTD should not be more than 115%. %CV should not exceed 15%, Except STD1 where it is 20%. The %CV of the mean recovery of analyte(s) at standard levels should be within ± 15%.

For Process efficiency mean response obtained for extracted samples should be less than 115% of the mean response of aqueous (neat) samples Concentrations. %CV should not exceed 15%, Except STD1 where it is 20%.

Dilution Integrity:

The dilution integrity exercise was performed with the aim of validating the dilution test to be carried out on higher analyte concentrations above the ULOQ during real-time analysis of subject samples. Dilution integrity experiment was carried out at 2.0 times the ULOQ concentration for the analyte. Six replicates each of half and quarter concentrations were prepared by 2 times and 4 times dilution with blank plasma and their concentrations were calculated by applying the dilution factors 2 and 4.

Stability experiments:

The stability of valsartan, Hydrochlorothiazide, Losartan (IS) and Zidovudine (IS) in the injection solvent was determined periodically by injecting replicate preparations of processed plasma samples for up to 28.28 hrs (in the auto sampler at 15 °C) after the sample loading. Dry extract stability was successfully assessed by analyzing six replicates of wet extract stability samples stored at a temperature below 10°C for 26.58 hours at low and high concentrations. Stability of analyte (Valsartan and hydrochlorothiazide) in plasma during 7.95 hrs (bench-top) was determined at ambient temperature (± 25 °C) at two concentrations (LQC and HQC) in six replicates. The stability of Valsartan and hydrochlorothiazide in human plasma following four freeze-thaw cycles was assessed, where the samples were stored at - -70 ± 5°C between freeze/thaw cycles and the samples were thawed by allowing them to stand (unassisted) at room temperature for ~1.5 hrs. The samples were then returned to the freezer. Freezer stability (Long-term) of analyte in human plasma was assessed by analyzing the LQC and HQC samples stored at -20° ± 5°C and -70 ± 5°C.The samples were processed using the same procedure as described in the sample preparation section. Samples were considered stable if assay values were within the acceptable limits of accuracy (± 15%) and precision (≤15% RSD or CV %).

RESULTS

Mass spectrometry:

To obtain optimum sensitivity and selectivity, ESI technique operated in the Negative ion mode was used for the LC-MS/MS multiple reaction monitoring (MRM) analysis. Protonated form of analyte and IS [M + H] - ions were the parent ion in the Q1 spectrum and were used as the precursor ion to obtain Q3 product ion spectra. The product ion mass spectrum of Negative charged ion of analytes was monitored at 433.80m/z →178.80m/z (V), 295.80m/z→204.90m/z (H) and 421.90 m/z →126.90 m/z, 265.80m/z→121.90m/z for the IS.

Selectivity and Chromatography:

The degree of interference by endogeneous plasma constituents with analyte and IS was assessed by inspection of chromatograms derived from processed blank plasma sample. As shown in (**Fig. 2, 2A & 3, 3A**), no significant interferences in the blank human plasma traces were found from endogeneous components in drug-free human plasma at the retention times of the analyte and IS.

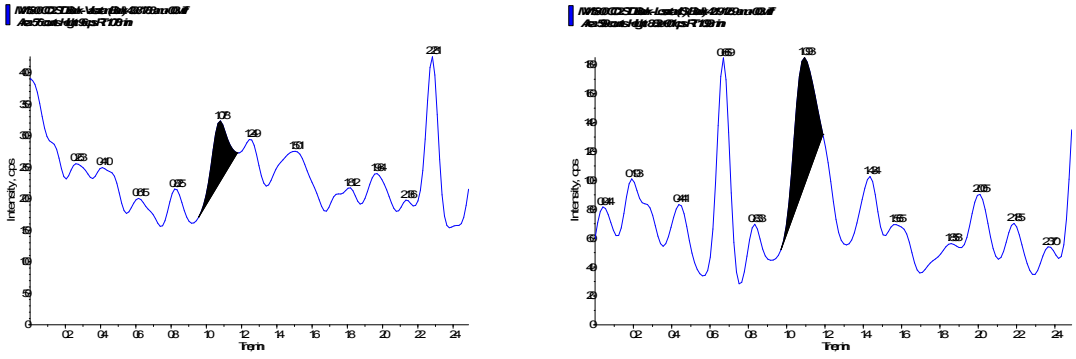


Figure 2: Extracted Blank Plasma (Valsartan & Losartan).

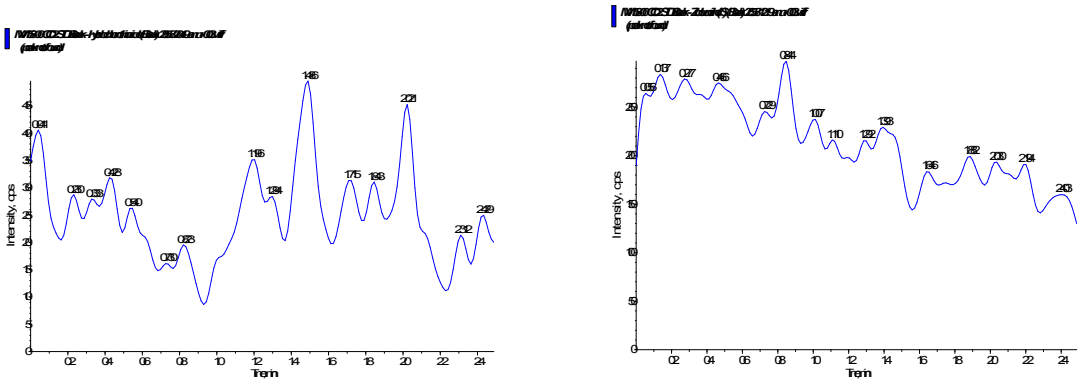


Figure 2 A: Extracted Blank Plasma (Hydrochlorothiazide & Zidovudine).

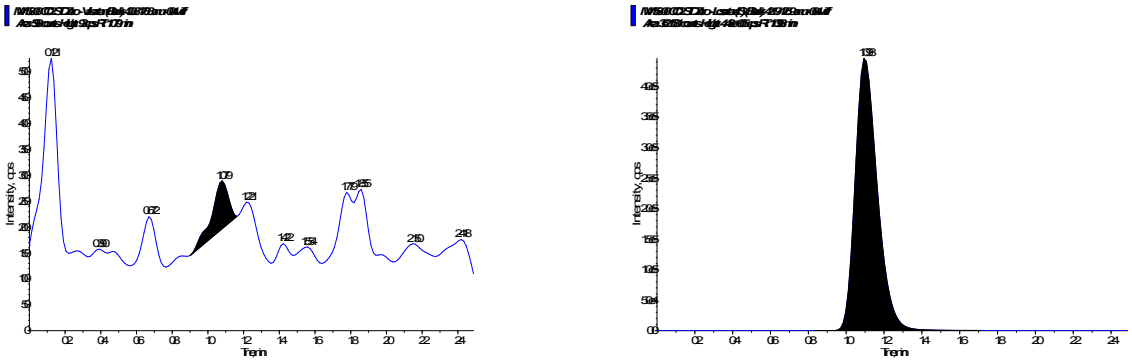


Figure 3: Extracted Blank Plasma with Internal Standard (Valsartan & Losartan).

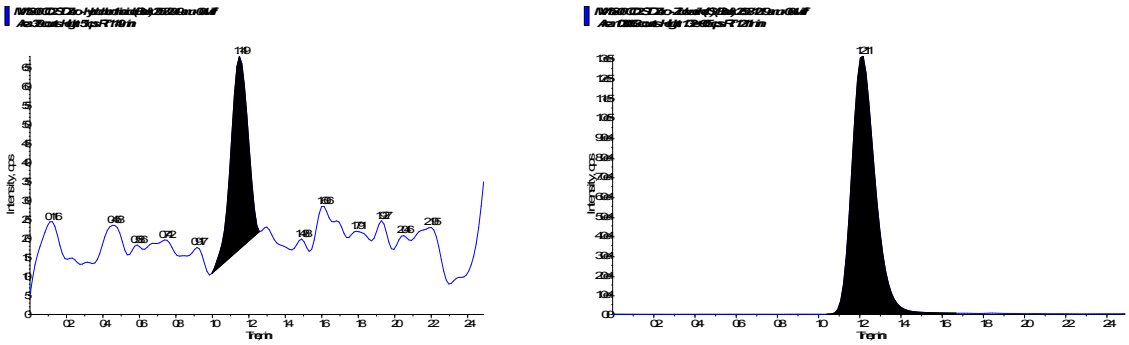


Figure 3 A. 3Extracted Blank plasma with Internal Standard (Hydrochlorothiazide & Zidovudine).

Sensitivity:

The lowest limit of reliable quantification for analyte was set as the concentration of the LLOQ. The precision and accuracy at LLOQ concentration were found to be 9.08% and 98.33%;

Extraction efficiency:

A simple solid phase extraction with 85 parts of Methanol, 5 parts of Acetonitrile and 10 parts of 10mM Ammonium formate as mobile phase proved to be robust and provided cleanest samples. The recoveries of analytes and IS were good and reproducible. The mean overall recoveries (with the precision range) of Valsartan were 79.83% ± 3.733 (1.27 – 5.44 %) and Hydrochlorothiazide 81.16% ± 3.156 (1.67 – 6.67 %). The recoveries of internal standards of losartan were 81.85 and Zidovudine 81.97%.

Matrix effect:
No significant matrix effect was observed in all the six batches of human plasma for the analytes at different standards level concentrations. The precision and %response for valsartan were found to be 0.61 to 8.85% and 97.43 to 104.30% and the precision and %response for Hydrochlorothiazide were found to be 1.36 to 6.66% and 99.07 to 102.69%.
Find the (**Table 1**) for the details.

Table 1: Comparison data of absolute matrix effect, relative recovery and process efficiency for Valsartan and Hydrovhlorothiazide.

Analyte / ISTD	A[a] (%CV)	B[b] (%CV)	C[c] (%CV)	Absolute matrix effect (%ME) [d]	Relative recovery (%RE) [e]	Process efficiency (%PE) [f]
STD 1						
Valsartan	7841(3.22)	7653 (8.85)	6444 (5.44)	97.60	84.20	82.19
Losartan	1692058(2.65)	1691663(1.53)	1395273(1.09)	99.98	82.48	82.46
Hydrovhlorothiazide	6849(9.82)	7033(6.66)	5623(6.67)	102.69	99.57	82.10
Zidovudine	587750(1.65)	585205(1.05)	492067(1.40)	84.08	79.95	83.72
STD 3						
Valsartan	55268(4.10)	57642 (1.42)	44624 (1.27)	104.30	77.42	80.74
Losartan	1683727(2.37)	1693456(3.34)	1354083(1.70)	100.58	79.96	80.42
Hydrovhlorothiazide	24206(4.38)	24757(6.52)	20081(1.67)	102.28	102.02	82.96
Zidovudine	584248(1.78)	596044(0.77)	480856(1.00)	80.67	81.11	82.30
STD 5						
Valsartan	38390(12.01)	385168 (2.68)	288902 (1.64)	100.33	75.01	75.25
Losartan	1720150(1.29)	1722505(3.26)	1338925(1.36)	100.14	77.73	77.84
Hydrovhlorothiazide	85455(3.56)	87500(1.89)	67005(2.96)	102.39	100.38	78.41
Zidovudine	616415(2.73)	618756(1.24)	485801(1.55)	78.51	76.58	78.81
STD 6						
Valsartan	939713 (1.80)	915591(0.61)	731984 (2.08)	97.43	79.95	77.89
Losartan	1653119(2.93)	1626115(2.78)	1364831(1.93)	98.37	83.93	82.56
Hydrovhlorothiazide	162625(1.67)	161511(1.69)	135100(3.08)	99.32	97.14	83.07
Zidovudine	634182(1.07)	616018(1.67)	512023(2.30)	83.12	83.65	80.74
STD 8						
Valsartan	3338867 (2.46)	3286578 (1.34)	2713700 (2.78)	98.43	82.57	81.28
Losartan	1331217(3.28)	1324490(1.45)	1127488(3.11)	99.49	85.13	84.70
Hydrovhlorothiazide	440241(2.78)	436152(1.36)	368569(3.14)	99.07	98.56	83.72
Zidovudine	625485(1.63)	616465(1.65)	514462(2.26)	83.45	84.50	82.25

A - Aqueous; B - Post Extracted; C – Extracted
a Mean area response of six replicate samples prepared in Mobile phase neat samples)
b Mean area response of six replicate samples prepared by spiking in post extracted blank
c Mean area response of six replicate samples prepared by spiking in plasma before extraction
d %Matrix effect: Post extracted mean response/Aqueous (Neat) mean response x 100
e %Recovery: Extracted mean response / Post extracted mean response x 100
f %Process efficiency: Extracted mean response / Aqueous Mean response x

Linearity:
After comparing the two weighting models i.e. 1/X and 1/X2 a regression equation with a weighting factor of 1/X2 of analyte to IS concentration was found to produce the best fit for the concentration-detector

response relationship for the analyte in human plasma. By using the recommended 1/X2 model, values for correlation coefficient (r2) were found ≥0.99 which indicate linearity over the whole calibration range for analyte. And also the mean value of r is greater than 0.99 in the concentration range of 20.00–10019.33 ng/mL for valsartan and 3.76–303.53 ng/mL for hydrochlorothiazide.

Precision and Accuracy:

Accuracy and precision data for intra- and inter-day plasma samples for valsartan and hydrochlorothiazide are presented in (**Table 2**). The assay values on both the occasions (intra- and inter-day) were found to be within the accepted variable limits.

Table 2: Intra and inter day precision of determination of Valsartan and Hydrochl-orothiazide.

Intra-batch and inter-batch precision and accuracy									
		Intra-batch				Inter-batch			
Level	Quality control samples (ng/mL)	*N*	Mean concentration observed (ng/mL)	Percentage bias	% CV	*N*	Mean concentration observed (ng/mL)	Percentage bias	% CV
Valsartan									
LLOQQC	20.59	6	24.40	18.50	9.35	24	22.94	11.41	10.58
LQC	55.95		60.52	8.17	5.73		60.73	8.54	3.60
MQC	4662.30		4972.49	6.65	1.51		4862.18	4.29	2.26
HQC	7875.51		7999.44	1.57	0.98		7899.2	0.30	2.05
Hydrochlorothiazide									
LLOQQC	3.90	6	4.07	4.36	5.45	24	3.79	-2.82	6.77
LQC	11.22		11.55	2.94	1.41		11.35	1.16	2.77
MQC	136.80		132.60	-3.07	2.28		130.02	-4.96	2.75
HQC	232.89		218.49	-6.18	1.94		222.07	-4.65	2.95

Dilution integrity:

The upper concentration limits can be extended to 19990.92 ng/mL Valsartan & 602.35 ng/mL Hydrochlorothiazide by a 1/2 or 1/4 dilution with screened human blank plasma. The mean back calculated concentrations for 1/2 and 1/4 dilution samples were within 85-115% of their nominal. The coefficient of variation (%CV) for 1/2 and 1/4 dilution samples were 0.57% and 0.34% for valsartan respectively and 1.01% and 0.80% for Hydrochlorothiazide. The %nominal for 1/2 and 1/4 dilution samples were 93.76% and 92.72 for valsartan and 93.80% and 96.98% for Hydrochlorothiazide respectively.

Stability studies:

The stability studies of analytes in human plasma over four freeze thaw cycles indicate that the analytes are stable in human plasma, when stored at below -70 ± 5 °C and thawed at room temperature. Results of bench top (7.95hr), auto-sampler (28.28 hr), Dry extract stability (26.58 hr), freeze-thaw stability (4 Cycles) were presented in (**Table 3**). The long term stability of the analyte in human plasma stored for a period of 48.80 days at -70°C ± 5 °C and 48.79 days at -20°C ± 5 °C compared with Zero day stability showed reliable stability behavior. The results of the tested samples were within the acceptance criteria.

DISCUSSION

Validated methods are essential for the quantitative estimation of valsartan and Hydrochlorothiazide concentrations in human plasma for clinical pharmacokinetic studies. The validated method is simple, rapid and specific range due to utilization of short elution time 1.00±0.30 min with duration of 2.50 min for each sample analysis. Here the developed and validated method for the determination of valsartan and Hydrochlorothiazide in human plasma with good / reasonable wider range (ULOQ 10019.33 ng/mL) for quantification of Valsartan in

combination of valsartan and Hydrochlorothiazide and individual estimation of valsartan in plasma samples. The method used very simple sample preparation procedure using Modern Solid phase extraction (SPE) method.

Table 3: Stability data of Valsartan and Hydrochlorothiazide under various conditions (n=6)

Storage condition	Valsartan			Hydrochlorothiazide		
	Nominal concentration (ng/mL)	Mean calculated conc. (ng/mL) ± SD	% Mean accuracy	Nominal concentration (ng/mL)	Mean calculated conc. (ng/mL) ± SD	% Mean accuracy
Bench top stability (7.95 hours)						
LQC	55.95	58.76± 3.473	105.02	11.22	10.98± 0.478	97.88
HQC	7875.51	8213.35± 67.104	104.29	232.89	218.25± 1.355	93.72
Dry extract stability (26.58 Hours)						
LQC	55.95	62.46± 6.820	111.63	11.22	11.07± 0.474	98.62
HQC	7875.51	7602.76± 88.962	96.54	232.89	201.84± 3.231	86.67
Auto sampler stability (24.00 Hours)						
LQC	55.95	61.59±2.283	110.09	11.22	10.66± 0.381	95.01
HQC	7875.51	7997.68± 202.544	101.55	232.89	208.66± 6.275	89.60
Freeze & thaw stability (Four cycles)						
LQC	55.95	57.92± 1.855	103.51	11.22	10.90± 0.703	97.12
HQC	7875.51	7646.45± 60.840	97.09	232.89	222.46± 3.139	95.52
Long term stability in plasma at -70°C (48.80 days)						
LQC	55.95	60.56± 3.494	106.86	11.22	11.20± 0.653	103.51
HQC	7875.51	7918.87± 117.200	98.40	232.89	225.49± 3.056	99.06
Long term stability in plasma at -20°C (48.79 days)						
LQC	55.95	59.39± 3.082	104.80	11.22	11.06± 0.794	102.22
HQC	7875.51	7783.16± 68.857	96.71	232.89	225.10± 2.455	98.89

CONCLUSION

In summary, the developed and validated a selective, rapid and specific range, high-throughput LC-MS/MS Tandem Mass Spectrometric Detection (Sciex) method to quantify valsartan and hydrochlorothiazide using Losartan and zidovudine as IS. To the best of knowledge, the cost-effectiveness, simplicity of the assay using Solid phase extraction and sample turnover rate of 2.50 min per sample with 0.400ml/min flow rate under wider Linearity range of analyte make it an attractive procedure in high-throughput bioanalysis of combination of valsartan and Hydrochlorothiazide and individual estimation of valsartan in plasma samples. From the results of the validation parameters, we can conclude that the developed method can be useful for BA/BE studies and routine therapeutic drug monitoring (TDM) with desired precision and accuracy.

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