SIMULTANEOUS METHOD DEVELOPMENT AND VALIDATION OF EPROSARTAN AND HYDROCHLORTHIAZIDE IN HUMAN PLASMA BY RP-HPLC METHOD

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Abstract

A new simple and precise stability indicating bioanalytical reverse-phase High Performance Liquid Chromatography (RP-HPLC) method was developedand validated for the simultaneous estimation of Eprosartan and Hydrochlorothiazidein their formulation and in human plasma.The developed method was successfully used for assaying drug contents in plasma. Isocratic elution mode was carried at Symmetry C 18 (150mm x 4.6 mm, 5μ) column using 0.1% Orthophosphoric acidpH (2.2): Acetonitrile (65:35v/v) as mobile phase at flow rate 1.0mlmin at detection wavelength 240 nm. Valsartan was taken as an internal standandard. The method was validated as per ICH guidelines. It is concluded that the present validated method can be successfully applied for the estimation of Eprosartan and Hydrochlorothiazidein human plasma over the concentration range of 80 to 3200 ng /ml of Eprosartan, 8.5 to 340 ng /ml of Hydrochlorothiazide. The method for determination of Eprosartan and Hydrochlorothiazidein human plasma using HPLC detection met the acceptance criteria with respect to selectivity, precision, accuracy, linearity, recovery.

Key words: *ICH guidelines, Eprosartan, Hydrochlorothiazide, K2EDTA Plasma,Valsartan, Internal standard, Validation*

Introduction

Eprosartan mesylate is a non-biphenyl non-tetrazole angiotensin II receptor (AT_1) antagonist[1-2]. A selective non-peptide molecule is chemically described as 4-({2-butyl-5-[(1E)-2-carboxy-2-(thiophen-2-ylmethyl)eth-1-en-1-yl]-1H-imidazol-1-yl}methyl)benzoic acid (Fig 1). Its Chemical Formula is $C_{23}H_{24}N_2O_4S•ECH_4O_3S$ and Eprosartan mesylate is a white to off-white free-flowing crystalline powder that is insoluble in water, freely soluble in ethanol. It performs 2 actions on the renin angiotensin system. By preventing the binding of angiotensin II to AT1, vascular smooth muscle relaxes and vasodilation occurs[3,4]. By inhibiting norepinephrine production, blood pressure is further reduced.

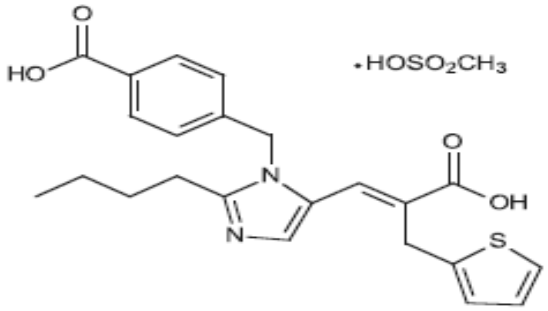

Fig 1: Chemical structure of Eprosartan

Hydrochlothiazide(HCT) is a thiazide diuretic is 6-chloro-3,4-dihydro-2 H 1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. Its empirical formula is $C_7H_8ClN_3O_4S_2$. It is a

white, or practically white, crystalline powder, slightly soluble in water. Hydrochlorothiazide inhibits sodium reabsorption in the distal tubules causing increased excretion of sodium and water as well as potassium and hydrogen ions[5-7.]

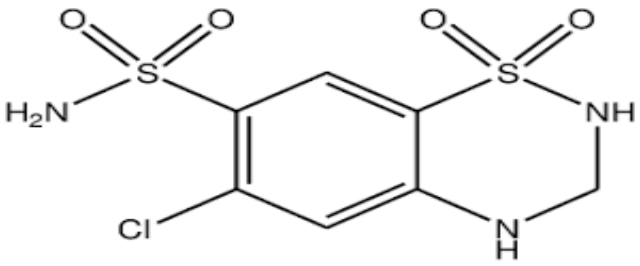

Fig 2: Chemical structure of Hydrochlorothiazide

Literature reveals that few methods have been reported for the simultaneous estimation of prazosin and polythiazide by using spectroscopic and chromatographic methods. But there is no reported for the bioanalytical methods. The main aim of the present study is to develop a precise, sensitive, accurate, selective, reproducible and rapid analytical technique for estimation of Eprosartan and Hydrochlorthiazide in human plasma.

Materials and Methods

Materials: API Eprosartan, Hydrochlorothiazide API was gifted by BMR chemicals, Hyderabad.

Human plasma: K_2 EDTA control plasma procured by Deccan Pathological labs, Hyderabad.

Table 1: List of Chemicals and Solvents

S.no	Chemical name	Grade	Manufacturing company
1	Distilled water HPLC water	HPLC	Rankem, Avantor performance material India limited
2	HPLC water	Analytical Reagent	Rankem, Avantor performance material India limited
3	Acetonitrile	Analytical Reagent	Rankem, Avantor performance material India limited
4	Phosphate buffer	Analytical Reagent	Rankem, Avantor performance material India limited
5	Methanol	Analytical Reagent	Rankem, Avantor performance material India limited
6	Sodium dihydrogen phosphate	Analytical Reagent	Rankem, Avantor performance material India limited
7	Ortho-phosphoric acid	Analytical Reagent	Rankem, Avantor performance material India limited

Table 2: List of Instruments

S.no	Instrument	Company name	Brand name
1	Electronic balance	Sartorious	Denver
2	pH meter	Metsar	BVK enterprises
3	Sonicator	Lab man	BVK enterprises
4	Centrifuge	Thermo Fisher	-
5	Vertex	Remi CM101	-
6	HPLC water	Alliance	Water HPLC 2695 SYSTEM

Method Development

Diluent: Based up on the solubility of the drugs, diluent was selected, 0.1% orthophosphoric acidand Acetonitrile taken in the ratio of 50:50.

Preparation of Eprosartan Stock solution (160 µg/ml):

Take 16 mg of Eprosartan in 100 ml volumetric flask and make the volume with diluent to produce 160µg/ml

Preparation of Eprosartan Spiking Solutions:

From the above Eprosartan stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 0.8µg/ml, 1.6µg/ml, 2.4 µg/ml, 9.6 µg/ml, 16 µg/ml, 19.2µg/ml, 25.6 µg/ml and 32.0µg/ml.Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 80ng/ml, 160ng/ml, 240ng/ml, 960ng/ml, 1600ng/ml, 1920ng/ml, 2560ng/ml and 3200ng/ml.

Preparation of Hydrochlorothiazide Stock solution (17µg/ml):

Take 1.7 mg of Hydrochlorothiazide in 100 ml volumetric flask and make the volume with diluent to produce 17 µg/ml.

Preparation of Hydrochlorothiazide Spiking Solutions:

From the above Hydrochlorothiazide stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 0.085 µg/ml, 0.17µg/ml, 0.255µg/ml, 1.02 µg/ml, 1.7 µg/ml, 2.04 µg/ml, 2.72 µg/ml and 3.4µg/ml.Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 8.5ng/ml, 17ng/ml, 25.5ng/ml, 102ng/ml, 170ng/ml, 204 ng/ml, 272ng/ml and 340ng/ml.

Preparation of internal standard Solution (Valsartan):

Stock-1: Take 50 mg of Valsartan in 100 ml volumetric flask and make up the volume with diluent to produce 500µg/ml.

Stock-2: From the above solution, take 1ml of solution into 10 ml volumetric flask and make up the volume with diluent to produce 50µg/ml solutions.

Final concentration: From the above solution, take 0.5ml of solution and spiking blank plasma with working stock dilutions of analyte to produce 10µg/ml ISD concentration

Extraction procedure:

Take 750µl of plasma and 500µl of internal standard, 250µl of Eprosartan and 250µl of Hydrochlorothiazide from the spiking solutions of both into a centrifuging tube and add 1 ml of Acetonitrile go for cyclomixer for 15 sec. Then vertex for 2 min and finally centrifuge for 5 min at 3200 rpm speed. After the centrifugation collect the sample and filter it directly inject 50 µL into HPLC.

Optimized Chromatographic Conditions:

Mobile phase : 0.1% Ortho phosphoric acid pH (2.2): Acetonitrile (65:35)
Flow rate : 1.0ml/min
Column : Symmetry c18 (150mm x 4.6 mm, 5µ)
Detector wavelength : 240nm
Column temperature : 30^0C
Injection volume : 20µL

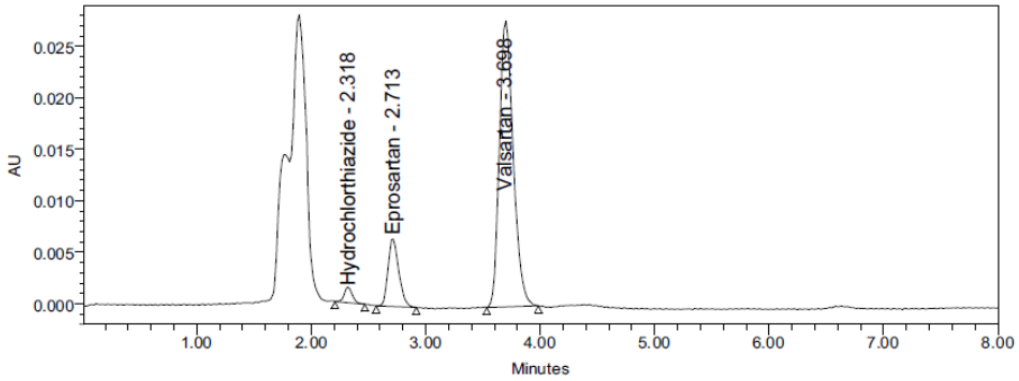

Fig 3: Optimized chromatogram for Eprosartan and Hydrochlorothiazide

Method Validation

System suitability:

All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The % CV for system suitability test was in the range of 0.94 for Retention time (RT) of Eprosartan, 0.26 for Retention time (RT) of Hydrochlorothiazide and 0.88% for the area ratio (analyte area/IS area) of Valsartan. The results were shown in table 3 & 4.

Auto sampler Carryover test:

Due to the auto-sampler was investigated by injecting a sequence of un-extracted and extracted samples. Results demonstrated that no significant carry over was observed during this experiment. The data was given in table 5 & 6.

Matrix factor evaluation:

Matrix effect is played a vital role in the assessment of pharmacokinetic studies. It was expressed as internal standard normalized matrix factor and it was varied from 0.90-0.99 which was close to 1 which indicates there is no ionization suppression or enhancement in plasma samples. The results were explained in table 7&8.

Quality control samples:

The chromatography observed during the course of Eprosartan and Hydrochlorothiazide was acceptable and representative chromatograms of standard Blank, standard zero (standard blank with internal standard) QC-LLOQ, QC-L, QC-M1, QC-M2 and QC-H samples. Sample chromatograms were shown in fig 4, 5,6,7,8 & 9.

Selectivity/Specificity:

To establish the selectivity of the method, possible interference at the retention time of Eprosartan, Hydrochlorothiazide and Internal standard due to endogenous plasma components were checked during the validation. Selectivity was performed by testing six batches of K_2EDTA blank plasma and the mass detection of extracted blank plasma gave good selectivity of both drug and internal standard. No interferences were found at the retention times of analytes and internal standard. Representative chromatograms of standard blank and blank with internal standard sample using pooled plasma.

Linearity:

Calibration was found to be linear over the concentration range of 80 to 3200ng /ml for Eprosartan, 8.5 to 340 ng /ml Hydrochlorothiazide. The coefficient correlation (r^2) value was found consistently greater than 0.999 in all the cases. This indicating linearity of results and an excellent correlation between peak area ratios for each concentration of analytes. The data was shown in table 9 & 10.

Precision and Accuracy:

The intraday and inter day accuracy and precision was assessed by analysing six replicates at five different QC levels like LLOQ, LQC, MQC and HQC. Accuracy and precision method performance was evaluated by determined by six replicate analyses for Eprosartan at four concentration levels, i.e., 80ng/ml(LLOQ), 640ng/ml (LQC), 1600ng/ml (MQC) and 2560µg/ml (HQC), Hydrochlorothiazide at 8.5ng/ml(LLOQ), 25.5 ng/ml (LQC), 170ng/ml (MQC) and 272ng/ml (HQC),The intra-day and inter day accuracy of plasma samples were assessed and

excellent mean % accuracy was obtained with range varied from 100.13 to 100.66%, and 99.16 to 100.87%for intraday and 99.72 to100.78%and 99.28 to 100.80% for inter day respectively. The precision (%CV) of the analytes and plasma samples were calculated and found to be 0.91 to 1.76% and 0.89 to 1.36%for intraday and 0.98 to 1.58% and 0.93 to 1.15% for inter day respectively. The results were given in table 11 & 12.

Recovery:

Recovery was determined by measuring the peak areas obtained from prepared plasma samples with those extracted blank plasma spiked with standards containing the same area with known amount of Eprosartan and Hydrochlorothiazide. The overall % mean recovery for Eprosartan and Hydrochlorothiazide was found to be 98.85% and 98.67%. The overall % mean recovery for Valsartan was found to be 98.11%. The results were demonstrated in table 13, 14 & 15.

Stabilities:

Long term stock solution stability for Eprosartan: In bench-top stability, six replicates of LQC & HQC samples (240 and 2560 ng/ml) were analyzed for 9 hours at room temperature on the laboratory bench. The % mean stability was calculated and found to 99.96% for LQC and 99.19% for HQC respectively.

Long term stock solution stability for Hydrochlorothiazide: In bench-top stability, six replicates of LQC & HQC samples (25.5 and 272 ng/ml) were analyzed for 9 hours at room temperature on the laboratory bench. The % mean stability was calculated and found to 99.69% for LQC and 99.89% for HQC respectively.

Matrix samples stability at -28±5 °C for 37 days & -80±5 ^{0}C:

Long term stock solution stability for the Eprosartan was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator. The % mean stability of the Eprosartan was found to be 99.90%, 100.10% at 28 ± 5°C and 100.16%, 100.84% at 80 ± 5°C respectively. Long term stock solution stability for the Hydrochlorothiazide was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator. The % mean stability of the Hydrochlorothiazide was found to be 100.94%, 100.42% at 28 ± 5°C and 101.11%, 100.24% at 80 ± 5°C respectively. The data was shown in table 17 & 18.

Results and Discussion

Table 3: System suitability of Eprosartan

Sample Name	File Name	Analyte Area	Analyte RT (min)	ISTD Area	ISTD RT (min)	Area Ratio
AQ MQC		53992	2.86	93279	2.31	0.5788
AQ MQC		53540	2.83	93088	2.33	0.5752
AQ MQC		54019	2.79	93705	2.35	0.5765
AQ MQC		53966	2.86	92324	2.34	0.5845
AQ MQC		54585	2.84	94280	2.32	0.5790
AQ MQC		53782	2.85	93794	2.33	0.5734
MEAN			2.839		2.329	0.57789
SD			0.0268		0.0152	0.003891
%CV			0.94		0.65	0.67

Table 4: System suitability of Hydrochlorothiazide

Sample Name	File Name	Analyte Area	Analyte RT (min)	ISTD Area	ISTD RT (min)	Area Ratio
AQ MQC		16814	3.39	93279	2.31	0.1803
AQ MQC		16781	3.38	93088	2.33	0.1803
AQ MQC		16884	3.36	93705	2.35	0.1802
AQ MQC		16754	3.38	92324	2.34	0.1815
AQ MQC		16698	3.37	94280	2.32	0.1771
AQ MQC		16723	3.38	93794	2.33	0.1783
MEAN			3.379		2.329	0.17960
SD			0.0087		0.0152	0.001588
%CV			0.26		0.65	0.88

Table 5: Auto sampler carryover of Eprosartan

Acquisition Batch ID			Date	
Sample ID	Peak Area		% Carryover	
	Drug	ISTD	Drug	ISTD
Unextracted samples				
RS	0	0	N/A	N/A
AQ ULOQ	108454	94128	0.00	0.00
RS	0	0		
AQ LLOQ	2785	94023	N/A	N/A
Extracted samples				

STD Blk	0	0	N/A	N/A
ULOQ	107879	93327	0.00	0.00
STD Blk	0	0		
LLOQ	2694	93388	N/A	N/A

Table 6: Auto sampler carryover of Hydrochlorothiazide

Acquisition Batch ID			**Date**	
Sample ID	Peak Area		% Carryover	
	Drug	ISTD	Drug	ISTD
Unextracted samples				
RS	0	0	N/A	N/A
AQ ULOQ	35158	94128	0.00	0.00
RS	0	0		
AQ LLOQ	868	94023	N/A	N/A
Extracted samples				
STD Blk	0	0	N/A	N/A
ULOQ	33686	93327	0.00	0.00
STD Blk	0	0		
LLOQ	843	93388	N/A	N/A

Table 7: Matrix factor evaluation of Eprosartan (absence of matrix factor)

Acquisition Batch ID		**Date**	
S. No.	**Plasma Lot No.**	**HQC**	**LQC**
		Nominal Concentration (ng/mL)	
		2560.000	**640.000**
		(2,176.000-2,944.000)	**(544.000-736.000)**
		Calculated Concentration (ng/mL)	
1	LOT1	2525.980	641.073
		2696.979	640.076
		2693.041	644.074
2	LOT2	2656.033	648.076
		2526.011	637.074
		2477.931	639.072
3	LOT3	2591.034	646.071
		2567.940	643.075
		2493.976	642.075
4	LOT4	2489.090	641.071
		2485.928	645.072

		2501.018	642.071
5	LOT5	2491.957	641.072
		2502.126	647.071
		2490.058	646.076
6	LOT6	2492.872	660.075
		2504.941	651.076
		2493.086	659.076
N		18	18
Mean		2537.7778	645.1848
SD		72.80512	6.26825
% CV		2.87	0.97
% Mean Accuracy		99.13	100.81
No. of QC Failed		0	0

Table 8: Matrix factor evaluation of Hydrochlorothiazide

Acquisition Batch ID		**Date**	
S. No.	**Plasma Lot No.**	**HQC**	**LQC**
		Nominal Concentration (ng/mL)	
		272.000	**25.500**
		Nominal Concentration Range (ng/mL)	
		(231.200-312.800)	**(21.675-29.325)**
		Calculated Concentration (ng/mL)	
1	LOT1	273.97	26.36
		271.59	24.54
		271.94	22.57
2	LOT2	273.91	24.60
		278.03	25.70
		276.88	27.36
3	LOT3	273.06	25.97
		279.83	25.97
		270.09	26.56
4	LOT4	278.81	25.48
		272.12	25.25
		279.77	26.32
5	LOT5	274.09	25.25
		271.74	25.36
		275.81	26.26
6	LOT6	271.78	25.78

		277.15	26.17
		274.01	25.48
N		18	18
Mean		274.6996	25.6109
SD		3.05969	1.02364
% CV		1.11	4.00
% Mean Accuracy		100.99	100.44
No. of QC Failed		0	0

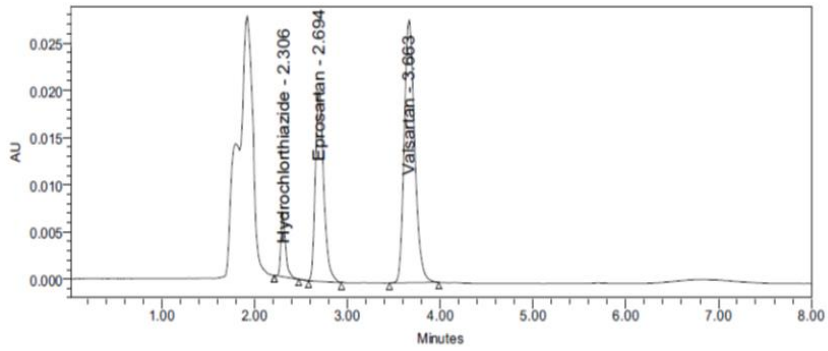

Fig 4: Chromatogram of standard Zero sample

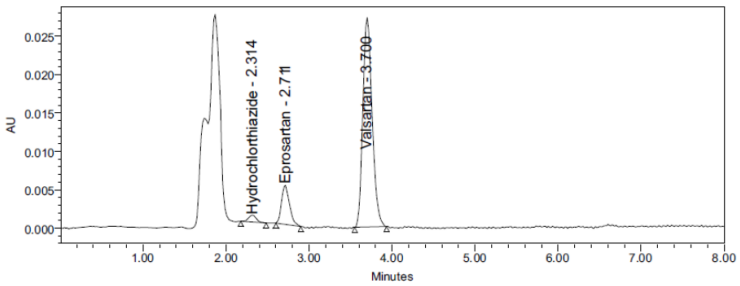

Fig 5: Chromatogram of QC-LLOQ sample Hydrochlorothiazide, Eprosartan

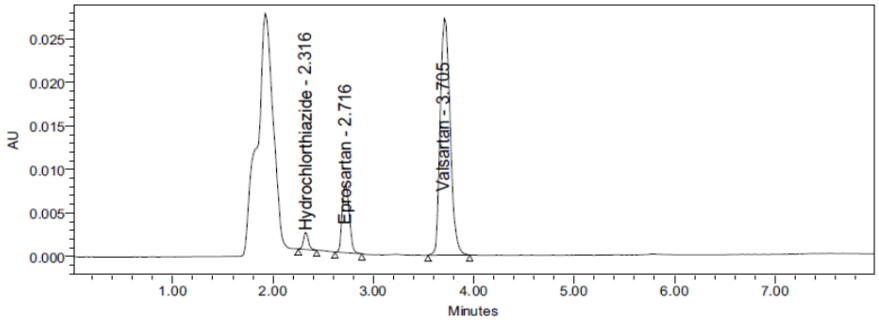

Fig 6: Chromatogram of QC-LQC sample Eprosartan, Hydrochlorothiazide and

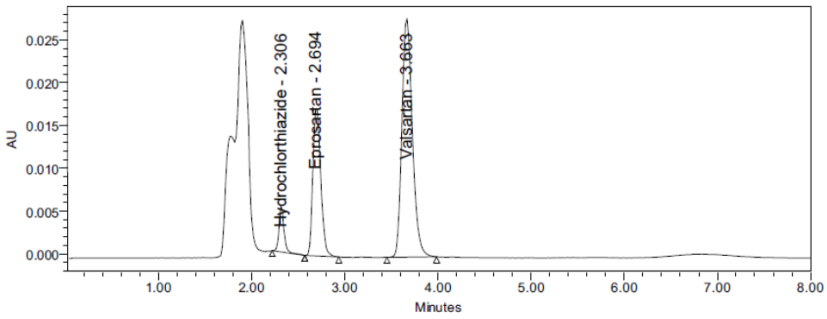

Fig 7: Chromatogram of QC-MQC sample Eprosartan,and Hydrochlorothiazide

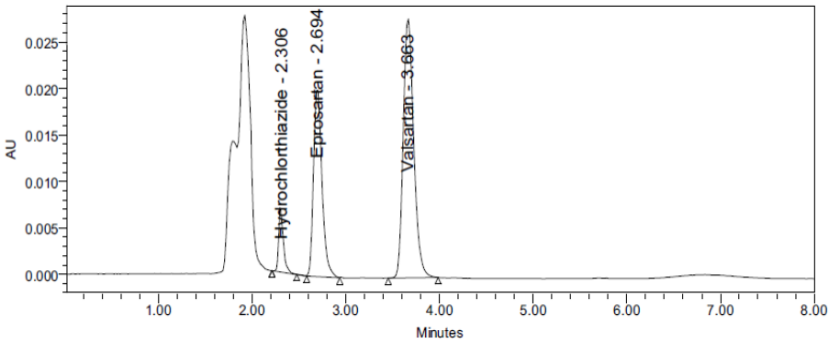

Fig 8: Chromatogram of QC-HQC sample Eprosartan,and Hydrochlorothiazide

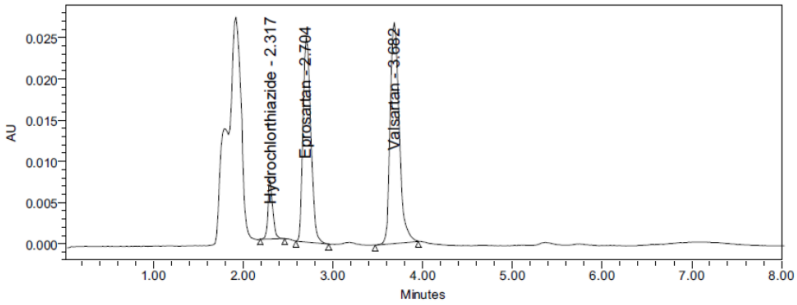

Fig 9: Chromatogram of ULOQ sample Eprosartan& Hydrochlorothiazide

Table 9: Linearity of Eprosartan

	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
Acquisition Batch ID	Nominal Concentration (ng/mL)							
	80.000	160.000	240.000	640.000	1600.000	1920.000	2560.000	3200.000
	Nominal Concentration Range (ng/mL)							
	(64.000-96.000)	(136.000-184.000)	(204.000-276.000)	(544.000-736.000)	(1,360.000-1,840.000)	(1,632.000-2,208.000)	(2,176.000-2,944.000)	(2,720.000-3,680.000)

	Back Calculated Concentration (ng/mL)							
P&A1	75.043	154.047	232.056	638.46	1573.45	1898.65	2543.66	3224.94
P&A2	79.041	161.032	238.074	642.63	1586.25	1916.35	2565.37	3231.68
P&A3	84.015	164.015	244.052	644.78	1595.84	1923.38	2578.35	3235.48
N	3	3	3	3	3	3	3	3
Mean	79.3663	159.6980	238.0607	641.9563	1585.1803	1912.7910	2562.4600	3230.6987
SD	4.49484	5.11610	5.99801	3.21651	11.22992	12.73843	17.52342	5.33394
%CV	5.66	3.20	2.52	0.50	0.71	0.67	0.68	0.17
% Mean Accuracy	99.21	99.81	99.19	100.31	99.07	99.62	100.10	100.96

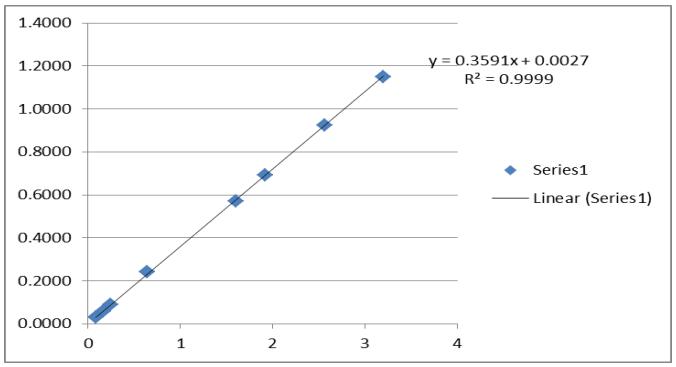

Fig 10: Calibration curve of Eprosartan

Table 10: Linearity of Hydrochlorothiazide

Acquisition Batch ID	**STD1**	**STD2**	**STD3**	**STD4**	**STD5**	**STD6**	**STD7**	**STD8**
	Nominal Concentration (ng/mL)							
	8.500	17.000	25.500	68.000	170.000	204.000	272.000	340.000
	Nominal Concentration Range (ng/mL)							
	(6.800-10.200)	(14.450-19.550)	(21.675-29.325)	(57.800-78.200)	(144.500-195.500)	(173.400-234.600)	(231.200-312.800)	(289.000-391.000)
	Back Calculated Concentration (ng/mL)							
P&A1	8.547	17.097	25.146	68.997	172.447	205.015	273.997	341.995

P&A2	8.452	17.132	25.349	67.532	170.532	204.986	267.698	339.992
P&A3	8.549	16.897	25.546	68.294	169.443	205.640	269.993	345.998
n	3	3	3	3	3	3	3	3
Mean	8.5160	17.0420	25.3470	68.2743	170.8073	205.2137	270.5627	342.6617
SD	0.05543	0.12679	0.20001	0.73270	1.52081	0.36950	3.18791	3.05800
%CV	0.65	0.74	0.79	1.07	0.89	0.18	1.18	0.89
% Mean Accuracy	100.19	100.25	99.40	100.40	100.47	100.59	99.47	100.78

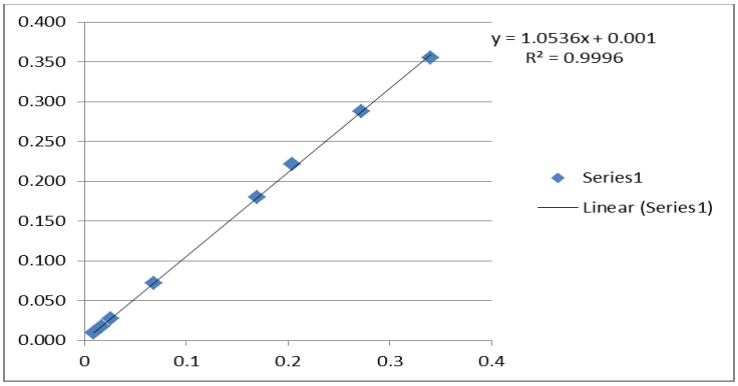

Fig 11: Calibration curve of HydrochlorothiazideTable 11: Precision&Accuracy of Eprosartan

Acquisition Batch ID	**Date**	**HQC**	**MQC1**	**LQC**	**LLOQ QC**
		Nominal Concentration (ng/mL)			
		2560.000	1600.000	640.000	80.000
		Nominal Concentration Range (ng/mL)			
		(2,176.000-2,944.000)	(1,360.000-1,840.000)	(544.000-736.000)	(64.000-96.000)
		Back Calculated Concentration (ng/mL)			
		2483.46	1556.44	632.032	79.013
		2523.26	1583.63	638.256	82.351
		2538.32	1563.98	643.035	79.451
		2587.46	1587.35	648.075	78.635
		2535.37	1617.46	653.234	81.483
		2579.13	1635.36	657.013	80.146
N		6	6	6	6
Mean		2541.1675	1590.7003	645.2742	80.1798

SD		38.14939	30.52685	9.36619	1.46237
%CV		1.50	1.92	1.45	1.82
% Mean Accuracy		99.26	99.42	100.82	100.22
		2532.12	1601.79	648.055	79.022
		2581.93	1604.89	650.059	82.362
		2543.57	1615.66	646.065	86.019
		2479.94	1595.79	645.066	84.020
		2575.78	1593.69	643.060	74.018
		2574.46	1591.43	640.058	72.460
N		6	6	6	6
Mean		2547.9667	1600.5403	645.3938	79.6502
SD		38.79897	8.96310	3.55869	5.49228
%CV		1.52	0.56	0.55	6.90
% Mean Accuracy		99.53	100.03	100.84	99.56

Table 12: Precision&Accuracy of Hydrochlorothiazide

Acquisition Batch ID	**Date**	**HQC**	**MQC1**	**LQC**	**LLOQ QC**
		Nominal Concentration (ng/mL)			
		272.000	170.000	25.500	8.500
		Nominal Concentration Range (ng/mL)			
		(231.200-312.800)	(144.500-195.500)	(21.675-29.325)	(6.800-10.200)
		Back Calculated Concentration (ng/mL)			
		273.997	171.997	25.348	8.463
		269.245	170.364	25.751	8.153
		273.993	174.994	24.949	8.244
		269.300	169.997	25.554	8.653
		267.990	172.336	25.445	8.541
		272.887	169.991	25.457	8.478
N		6	6	6	6
Mean		271.2353	171.6132	25.4173	8.4220
SD		2.69068	1.94441	0.26702	0.18782
%CV		0.99	1.13	1.05	2.23
% Mean Accuracy		99.72	100.95	99.68	99.08
		268.998	171.997	25.649	8.446
		271.995	169.585	24.952	8.649
		273.216	168.994	25.346	8.743
		274.998	171.347	25.455	8.452

		265.300	172.991	24.943	8.349
		267.000	170.888	25.158	8.555
N		6	6	6	6
Mean		2547.9667	270.2512	170.9670	25.2505
SD		38.79897	3.76877	1.49069	0.28353
%CV		1.52	1.39	0.87	1.12
% Mean Accuracy		99.53	99.36	100.57	99.02

Table 13: Recovery of Eprosartan

Acquisition Batch ID						
Replicate No.	**HQC**		**MQC1**		**LQC**	
	Un extracted Response	**Extracted Response**	**Un extracted Response**	**Extracted Response**	**Un extracted Response**	**Extracted Response**
1	87536	86420	54704	53658	2748	2702
2	87355	86574	54396	54157	2732	2706
3	87414	87248	54461	54030	2744	2693
4	87670	86981	54673	53914	2730	2708
5	87802	87055	54659	53440	2761	2716
6	87947	87301	54367	53646	2735	2713
N	6	6	6	6	6	6
Mean	87621	86930	54543	53808	2742	2706
SD	229.03	358.84	152.04	270.74	11.78	8.21
% CV	0.26	0.41	0.28	0.50	0.43	0.30
% Mean Recovery	99.21		98.65		98.71	
Overall % Mean Recovery	98.858					
Overall SD	0.3078					
Overall % CV	0.31					

Table 14: Recovery of Hydrochlorothiazide

Acquisition Batch ID						
Replicate No.	**HQC**		**MQC1**		**LQC**	
	Un extracted Response	**Extracted Response**	**Un extracted Response**	**Extracted Response**	**Un extracted Response**	**Extracted Response**

1	27136	26844	16846	16802	2601	2625
2	27399	26623	16961	16833	2620	2571
3	27539	27132	16807	16790	2627	2583
4	27683	26882	17082	16806	2602	2614
5	27566	27021	16997	16796	2619	2582
6	27413	26756	17164	16781	2636	2597
N	6	6	6	6	6	6
Mean	27456	26876	16976	16801	2618	2595
SD	188.69	182.22	136.17	17.86	13.81	20.75
% CV	0.69	0.68	0.80	0.11	0.53	0.80
% Mean Recovery	97.89		98.97		99.15	
Overall % Mean Recovery	98.671					
Overall SD	0.6833					
Overall % CV	0.69					

Table 15: Recovery of Valsartan (IS)

Acquisition Batch ID	**Date**	
S.No.	**Un extracted Area Ratio**	**Extracted Area Ratio**
1	94178	93309
2	94327	93985
3	94876	93464
4	94207	93885
5	94417	93449
6	93896	93529
N	6	6
Mean	94316.8	93603.5
SD	325.96	268.48
% CV	0.35	0.29
% Mean Recovery	99.24	

Table 16: Long term stock solution stability

Acquisition Batch ID	**Date**	
Replicate No.	**HQC**	**LQC**
	Nominal Concentration (ng/mL)	
	2560.000	**640.000**
	Nominal Concentration Range (ng/mL)	
	(2,176.000-2,944.000)	**(544.000-736.000)**
	Calculated Concentration (ng/mL)	
1	2512.997	647.073
2	2538.253	637.076
3	2549.994	639.070
4	2567.030	627.079
5	2519.991	645.067
6	2548.060	643.082
N	6	6
Mean	2539.3875	639.7412
SD	20.12853	7.22725
% CV	0.79	1.13
% Mean Accuracy	99.19	99.96

Table 17: Matrix samples stability at -28±5 °C for 37 days (Eprosartan)

Replicate No.	**HQC**		**LQC**	
	Nominal Concentration (ng/mL)			
	2560.000	2560.000	640.000	640.000
	Nominal Concentration Range (ng/mL)			
	(2,176.000-2,944.000)	(2,176.000-2,944.000)	(544.000-736.000)	(544.000-736.000)
	Calculated Concentration (ng/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	2562.996	2566.997	635.074	636.075
2	2553.999	2557.356	632.071	631.072
3	2560.993	2563.994	648.077	649.078
4	2553.002	2556.003	630.068	628.069
5	2562.993	2567.991	641.080	642.081
6	2564.005	2561.006	639.065	635.066
N	6	6	6	6

Mean	2559.6647	2562.2245	637.5725	636.9068
SD	4.88404	4.96094	6.59850	7.63076
% CV	0.19	0.19	1.03	1.20
%Mean Accuracy	99.99	100.09	99.62	99.52
% Mean Stability	100.10		99.90	

Table 18: Matrix samples stability at -28±5 °C for 37 days(Hydrochlorothiazide)

Acquisition Batch ID			**Date**	
Replicate No.	HQC		LQC	
	Nominal Concentration (ng/mL)			
	272.000	272.000	25.500	25.500
	Nominal Concentration Range (ng/mL)			
	(231.200-312.800)	(231.200-312.800)	(21.675-29.325)	(21.675-29.325)
	Calculated Concentration (ng/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	269.071	272.881	25.352	25.846
2	272.981	274.772	25.225	25.652
3	271.161	273.021	25.549	25.648
4	273.021	269.013	25.148	25.150
5	273.780	274.961	25.153	25.154
6	268.891	271.052	25.351	25.751
N	6	6	6	6
Mean	271.4842	272.6167	25.2963	25.5335
SD	2.12252	2.27197	0.15334	0.30437
% CV	0.78	0.83	0.61	1.19
%Mean Accuracy	99.81	100.23	99.20	100.13
% Mean Stability	100.42		100.94	

Conclusion

Based on the results obtained in this study, it is concluded that the present validated method can be successfully applied for the estimation of Eprosartan and Hydrochlorothiazide in human plasma over the concentration range of 80 to 3200 ng /m of Eprosartan, 8.5 to 340ng /ml of Hydrochlorothiazide. The method for determination of Eprosartan and Hydrochlorothiazide in

human plasma using HPLC detection met the acceptance criteria with respect to selectivity, precision, accuracy, linearity, recovery.Stability evaluations performed in k2EDTA human plasma, stock solutions and stock dilutions met the acceptance criteria, demonstrating insignificant degradation of Eprosartan and Hydrochlorothiazide over the specified storage durations and conditions.

Acknowledgement; The authors are thanful to Spectrum Research solution .Hyderabad,for providing gift samples of Eprosartan &Hydrochlorthiazide and thankful to Deccanpathological Labs.Hyderabad to provide K_2EDTA controlled plasma.The authors are Special thankful to Sree vidyanikethan college of pharmacy to provide the facilities to complete this research work.
Conflict of Interest; The authors declared no conflict of interest.

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