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Bioanalytical method development and validation of simultaneous analysis of telmisartan and hydrochlorthiazide in human plasma using LC-MS/MS

Mathew George[1]*, Lincy Joseph[1], Arpit Kumar Jain[2], Anju V.[1]
[1]Pushpagiri College of Pharmacy, Thiruvalla, Kerala, India.
[2]Jaipur National University, Jagatpura, Jaipur, Rajasthan 302017, India.

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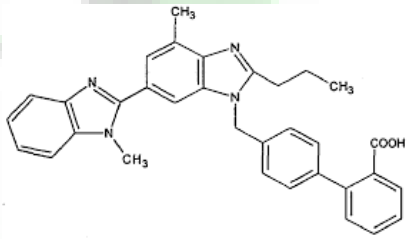
Abstract: A simple, sensitive, rapid and economic high performance thin layer chromatographic method and a mass spectroscopic assay method has been developed for the quantification of telmisartan and hydrochlorthiazide combination in human plasma. The internal standards and analytes were extracted from human plasma by solid-phase extraction with HLB Oasis1cc (30mg) catridges. The scanning and optimization for the samples are done using methanol: water (50:50). The samples were chromatographed using reverse phase chromatography with C-18 column of different manufacturers like Ascentis C18 (150×4. 6, 5µ) using the buffer system Acetonitrile: Buffer (80:20%v/v) which consist of 2±0. 1Mm ammonium format at a flow rate of 0. 7ml/min at a column oven temperature 35±1⁰c. The internal standard used was hydrochlorthiazide13c1, d2 and telmisartand3. The extraction techniques include conditioning, loading, washing and elution, drying followed by reconstitution of the dried samples. The volume injected was 10µl with the retention time of 3-4 min for telmisartan, 1-2 min for hydrochlorthiazide and for the internal standards the retention time was 3-4 min for telmisartand3 and 1-2 min for hydrochlorthiazide c13d2. The rinsing solution was Acetonitrile: HPLC grade water in the ratio (50:50). The above developed method was validated using various parameters like selectivity and sensitivity, accuracy and precision, matrix effects, % recovery and various stability studies. The method was proved to be sensitive, accurate, precise and reproducible. The preparation showed high recovery for the quantitative determination of telmisartan and hydrochlorthiazide in human plasma.

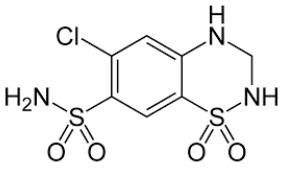
Key word: Telmisartan, Hydrochlorthiazide, Solid phase extraction, Liquid chromatography –Mass spectroscopy, Bioanalytical validation

Introduction

Telmisartan (4-[6-(1-TELhyl-1H-benzimidazole-2-yl)2-propyl-1H-benzi- midazole-1-yl] methyl] biphenyl-2-carboxyllic acid is a selective angiotensin II receptor blocker used for the treatment of hypertension and heart failure. They block the vasoconstrictor and aldosterone secreting effects of angiotensin II by blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscles and adrenal glands. It is a poorly water soluble drug and its bioavailability is 42-58% and are highly bound to plasma proteins (>99. 5%) and has a half-life of 24 hrs. [1, 2]



Telmisartan



Hydrochlorthiazide

Hydrochlorthiazide (6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide-1, 1-dioxide) is one of the oldest thiazide diuretic used to treat hypertension. They act by depleting the sodium stores in the body. The drug inhibits cells reabsorption of sodium chloride and they bind to the site of chlorine on the sodium chloride transporter molecule preventing it from taking up sodium. This depletes the Nacl stores in the body which reduces blood pressure and cardiac output. [3]

Thizide diuretics are prescribed in combination with other antihypertensive drugs like β- blockers, angiotensin converting enzyme inhibitors or angiotensin II receptor blockers. A combination of HCTZ with TEL is more effective than either drug alone for the management of hypertension. A method was developed using HPLC/MS system using these drug combinations and was validated using various parameters by several sets of analysis.

***Corresponding Author:**
Prof. Dr. Mathew George,
Pushpagiri College of Pharmacy
Medicity Tiruvalla – 7,
Kerala 689107, India.

Materials and Methods

Chemicals and reagents: The internal standards like telmisartand3, hydrochlorthiazide13C, d2were purchased from Ranbaxy research lab. The reference sample of telmisartan and hydrochlorthiazide were also purchased from Ranbaxy research labs. HPLC grade water for the LC-MS analysis were prepared from Milli Q; a water purification system from the Millipore (Bangalore). Methanol and Acetonitrile were of HPLC Grade and the former was purchased from Fine chemicals and the latter from Spectrochem Pvt. Ltd., the analytical grade buffer adjusters like formic acid were purchased from Qualigens fine chemicals and ammonium formate and ammonia solution from Fluka Pvt. Ltd. and the control. K3 EDTA plasma from Yash laboratories. The column was chosen as the HLB Oasis 1cc (30mg)

Instrumentation and chromatographic conditions: An HPLC system consisting of reverse phase chromatographic C18 column of Ascentis (150×4. 6 mm, 5µm) with the prominence pump, an auto sampler and a solvent degasser was used for the study. A set of calibration curve standards, quality control samples and plasma samples were used for the study. Aliquots of the samples (10µl). were injected into the column which was kept at (35±1^{0}C) and the mobile phase used was Acetonitrile: Buffer in the ratio (80:20). The samples were delivered at a flow rate of 0. 7ml/min into the electron spray ionization chamber of the mass spectrophotometer. The rinsing solution was Acetonitrile: HPLC grade water in the ratio (50:50). The quantitation was carried out using MS/MS detection using positive ionization mode for telmisartan and telmisartan d3 and negative ionization mode for hydrochlorthiazide and hydrochlorthiazide C13d2using a mass condition (API 4000). The source parameters for (hydrochlorthiazide, telmisartan) like curtain gas (15, 17), collision associated dissociation (6, 6), ion source gas1 (50, 41), ion source gas 2 (50, 60) and the temperature550^{0}C were set. The compound parameters like declustering potential (DP), electron potential (EP), collision energy (CE) and the Collision exit potential (CXP) of telmisartan and telmisartand3 was found to be 65v, 15v, 50v, 20v respectively and that for hydrochlorthiazide and hydrochlorthiazidec13d3 was found to be -40v, -10v, -30v, -20v. The ions were detected by using multiple reaction monitoring (MRM) having the m/z values of hydrochlorthiazide and their internal standard hydrochlorthiazide 13cd2 by Q1 and Q3 scans was from 296 to 269. 1 and 299 to 270.1 respectively and for telmisartan and their internal standard telmisartand3 were found to be 515. 3, 276. 2 and 518. 5, 279. 5 respectively. [4, 5, 6. , 8, 9]

Preparation of standard solution

Primary stock solution of telmisartan and hydrochlorthiazide for preparation of standard calibration curve and quality control (QC) samples were prepared from separate weighing

Preparation of telmisartand3and hydrochlorthiazide 13cd2 stock solution: Accurately weighedtelmisartand3 (5. 224mg) and hydrochlorthiazide 13c, d2 (5. 0881mg) and dissolved in methanol (5ml) to a concentration of 1mg/ml or 1000000ng/ml. The solution is protected from light and stored in a refrigerator at 1-10^{0}C.

Preparation of dilutions for the stock: From the stock solutions dilutions were made using methanol. 0. 02 ml of the solution was taken and made up to 50 ml to get a concentration of 400 ng/ml.

Preparation of calibration curve standards and quality control samples: The calibration dilutions were prepared by transferring 0. 1 ml from the standards and made upto 10 ml with plasma to get quality control dilutions (spiking dilutions). The calibration curve standard consist of a set of nine non-zero concentrations ranging from 1. 09-601. 86 ng/ml for telmisartan and 0. 809 -350. 03 ng/ml for hydrochlorthiazide. Samples for the determination of precision and accuracy were prepared by spiking control human plasma in bulk with telmisartan and amlodipine at appropriate concentrations. The quality control samples prepared for each analytes are; for telmisartan 1. 16 (LOQQC), 3. 08 (LQC), 228. 2 (MQC) and 456. 4 (HQC); for hydrochlorthiazide 0. 813 (LOQQC), 2. 36 (LQC), 134. 7 (MQC) and 269. 4 (HQC)[6, 7]

Sample processing method (solid phase extraction): One set of calibration curve standards, quality control sample were withdrawn and subjected to plasma samples from the freezer. Allowed to stand at room temperature by keeping them for some time. The samples were vortexed to ensure complete mixing of contents. Pipetted out 50µl of Internal standard stock dilution approximately 400ng/ml of telmisartan d3 and HCTZ C13 d2 into the poly propylene tubes. Added 500µl of the plasma samples and vortexed. 300µl of formic –water mixture was added to this. The sample mixture was loaded onto an HLB catridge which was preconditioned with 1 ml methanol followed by 1 ml HPLC grade water. It was centrifuged for 1 min at 1500rpm. The samples were loaded into the catridge by running the centrifuge for 2 min. Finally, the centrifuge was washed with methanol-water mixture and again washed and centrifuged at 1500rpm. The samples were eluted out with 1 ml methanol and dried under nitrogen at 50^{0}C±2^{0}Cat 15 psi. Reconstituted the dried residue with 500µl of

reconstitution solution and transferred into vials for analysis using LC-MS/MS system. [10, 11]

Bioanalytical method validation studies

The validation for a bioanalytical procedure is to demonstrate the performance and reliability of the method. The fundamental parameter to ensure the acceptability of a performance of abioanalytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility and stability

Selectivity: It is the ability to differentiate and quantify the analyte in the presence of other components in the sample. Analysis of blank samples of the human plasma was obtained from 6 sources and the lower limit of quantification was noted. There are no interfering peaks observed at the retention time of drugs and internal standards. The peaks showed a good shape and are completely resolved from the plasma components at the retention time of 3-4 min for hydrochlorthiazide and 2-3 min for telmisartan.

Accuracy and precision: Accuracy describes the closeness of mean test result to the true value and precision is the closeness of individual measurements of the same analyte done under similar conditions. The intraday and interday assay and precision were determined by analyzing 6 replicates at 4 QC levels using a minimum of five determinations per concentration. At least 3 concentrations should be in the range of expected concentration. The acceptance criteria included for accuracy is that the mean value should be within the range of ±15% standard deviation from the nominal values except LLOQ; which can deviate upto 20% and the acceptance criteria for precision should be such that concentration level should be <15% of the relative standard deviation except LLOQ; where it can exceed upto 20%.

Linearity: The linearity was determined using a least square regression analysis of standard plot associated with a nine-point standard curve. The linearity of telmisartan and HCTZ was tested in the concentration range of 0. 809-350. 026 for HCTZ and 1. 094-601. 86 for telmisartan. The acceptance limit of accuracy for each of the back-calculated concentrations is ±15% except LLOQ, where it is ±20%. Nine replicate analyses were performed on each calibration standard. The samples were run in the order from low to high concentration.

Carry over effect in matrix: The carry over effect in matrix was determined to ensure the area of the analyte and internal standards. The area of the LOQ, ULOQ samples and the blank matrix samples were estimated and the acceptance criteria include the carry over effect should be less than 20% for the analyte and 5% for the internal standard.

Recovery: Recovery of the analytes and the internal standards from the extraction procedure was determined by comparing the peak areas of the analytes in plasma quality control samples (six determinations each of low, middle, and high QC samples) with that of the peak areas of the un extracted aqueous standard samples containing the same concentration of drug and internal standard. The % recoveries was calculated using the equation

$$\% \text{ Recovery} = \frac{\text{Mean peak response of extracted samples} \times 100}{\text{Mean peak response of un extracted samples} \times \text{C. F}}$$

Sensitivity: Sensitivity was established from the background noise or response from four replicate of pooled blank matrix samples and spiked LOQ samples. The mean value of the signal to noise ratio was calculated for both samples and compared. The value should be >5% for all LOQ samples

Stability: Stability tests were conducted to evaluate the analyte stability in stock solutions and in plasma samples under different conditions. The stability of the stock solution was determined using fresh samples and IS at room temperature and refrigerated conditions (2–8°C) for a specified time period. Six replicates of each of the samples were injected and the response were taken. The stability studies include short term stability for 24hrs, Bench top stability for 24 hrs), auto sampler stability for 48hrs, freeze–thaw stability four cycles and long-term stability (30 days) were performed at LQC and HQC levels using four replicates at each level. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15% SD) and precision (≤15% RSD). [10-15]

Result and Discussion

Mass conditions: The method development for the estimation of telmisartan and hydrochlorthiazide was carried out using the LC/MS in human plasma. The mass parameters for the analyte samples and internal standard were tuned in positive ionization mode (telmisartan and telmisartan d3) and negative ionization mode (HCTZ, HCTZ C13 d2). The most sensitive mass transition was determined using m/z values. The m/z value of telmisartan was found to be 515. 3-276. 2 and for HCTZ m/z value was 296-269.1. The m/z values of the internal standards telmisartan d3 and HCTZC13d2 was respectively 518. 5-279. 5 and 299-270. 1. The source parameters and compound parameters of the drug and IS were also determined. All the parameters were found optimum for the validation studies [8, 9]

HPLC conditions: The liquid chromatographic conditions like the selection of column, mobile phase, buffer system etc were optimized to achieve

good resolution and symmetric peak shape within a short run time. The reverse phase chromatographic mode was selected as the best and the various C18 columns of different manufacturers like nova-pake, zorbax and ascentic18 were tried and was found unsatisfactory due to peak tailing. Finally the chromatographic column was selected as Ascentis C18 (150╳4. 6MM, 5µm) since it showed good peak shape and response. The internal standards were selected based on the fact that they belong to same class of compounds as the analyte. Several class of compounds were investigated and finally HCTZ C13d2 and telmisartan d3 were found appropriate since they have no significant effect on analyte recovery and sensitivity with negligible ion suppression and identical chromatographic behavior.

The composition of mobile phase were optimized, initially the mobile phase composition was selected as acetonitrile: Buffer at different ratios having different PH. Due to peak tailing P^H was changed to acidic side using formic acid and the optimized composition was found to be 80:20. The column oven temperature was set as 35± 1^0C at a flow rate of 0. 7ml/min and observed a good peak shape and response. The retention time of the analyte and standards was found as; telmisartan and telmisartand3 (3-4 min), HCTZ and HCTZC13d2 (1-2 min) which is small enough to provide a short run time. Acetonitrile: HPLC grade water was used as the rinsing solution [6, 7]

Selectivity and chromatography: The degree of interference of plasma constituents with the analyte and the IS was assessed by using the blank plasma sample chromatograms. No significant interference was observed in the results.

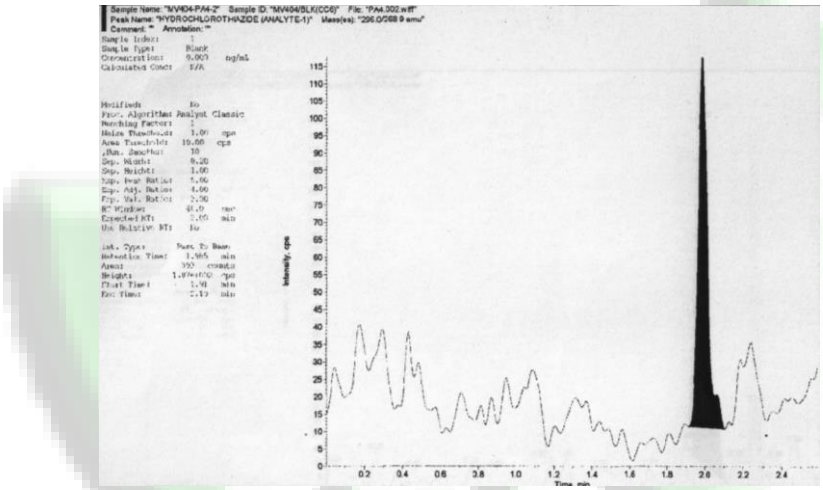


Figure I: HPLC Chromatogram of hydrochlorthiazide

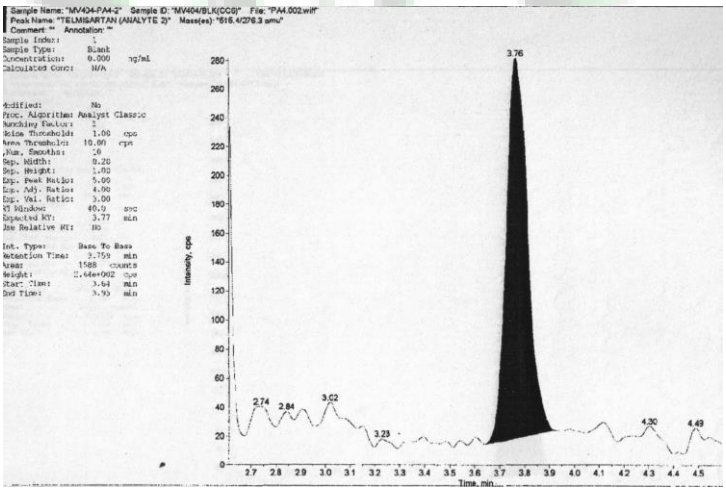


Figure II: HPLC Chromatogram of telmisartan

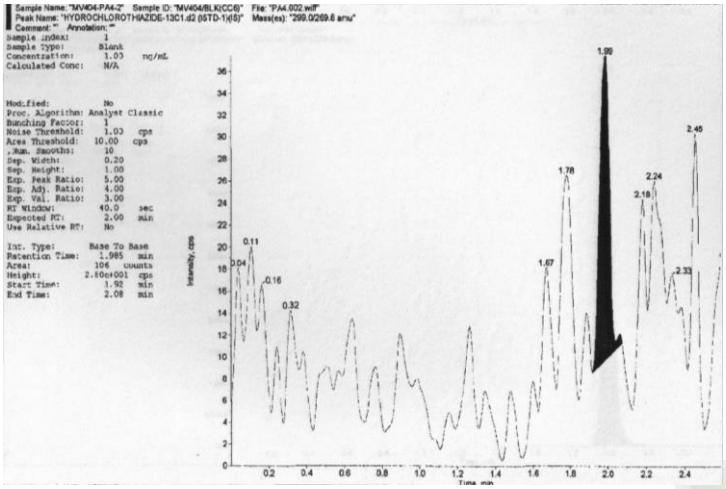


Figure III: HPLC Chromatogram of hydrochlorthiazide 13cd2

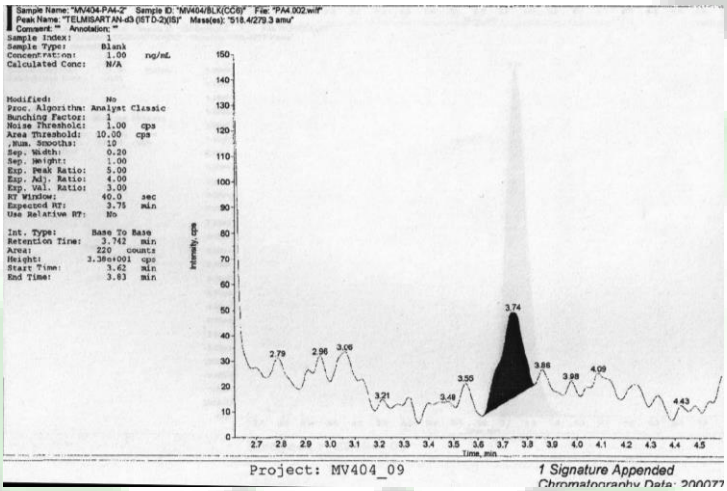


Figure IV: HPLC Chromatogram of telmisartan d3

Validation Studies [16-24]

Selectivity: The plasma blank was evaluated six times and the interfering peak was observed at the retention time of the drugs and the retention time of the internal standards. The peaks were of good shapes and the retention time was found to be 3-4 and 1-2 min for HCTZ and telmisartan.

Table 1: Blank plasma screening selectivity for telmisartan and HCTZ

Blank Samples (6 times)	Telmisartan		Hydrochlorthiazide	
	LOQ Area (Mean±S. D)	IS Area (Mean±S. D)	LOQ Area	IS Area
	15074.5±786.2	385997±10752.55	2324.5±142.42	81974±3020.7

Precision and accuracy: Precision and accuracy of the method was characterized by running three analytical batches containing the following samples. The precision and accuracy data for the interday and intraday plasma sample s of telmisartan and hydrochlorthiazide are shown below

Table 2: Intraday (within batch) and inter day (between batch) precision and accuracy for HCTZ and Telmisartan

S. No.	Samples	Concentration		% Accuracy		% Precision	
			Mean±S. D	Interday	Intraday	Interday	Intraday
1	Telmisartan	LLOQ	1. 036±0. 025	97. 69	92. 91	1. 480	2. 39
		LQC	3. 271±0. 09	97. 88	106. 16	1. 64	2. 65
		MQC	231. 864±3. 92	99. 51	101. 59	1. 068	1. 69
		HQC	428. 05±6. 85	96. 32	93. 78	1. 106	1. 60
2	Hydrochlorthiazide	LLOQ	0. 765±0. 05	104. 42	94. 16	1. 67	6. 26
		LQC	2. 255±0. 05	101. 38	95. 63	3. 76	2. 20
		MQC	132. 948±4. 1	99. 54	98. 68	1. 41	3. 08
		HQC	266. 752±16. 6	96. 62	99	1. 90	6. 22

Intraday and Interday precision and accuracy for hydrochlorthiazide: Within batch or intraday precision and accuracy for hydrochlorthiazide was found to be 6. 26-6. 22% and 94. 16-99% respectively. Between Batch/ Interday precision and accuracy for hydrochlorthiazide was found to be 1. 67-1. 90% and 104. 42-96. 62%. respectively.

Intraday and Interday precision and accuracy for telmisartan: The intraday precision and accuracy for telmisartan was found to be 2. 39-1. 60% and 92. 91-93. 78% respectively.

The interday precision and accuracy for telmisartan was found to be1. 48-1. 10% and 97. 69-96. 32% respectively. It revealed that the developed method was accurate and precise for quantification of telmisartan and hydrochlorthiazide in plasma sample.

Linearity and range: The linearity of the method was determined by a weighed least square regression analysis of standard plot associated with nine-point standard curve. The calibration curve was plotted against concentration ratio v/s area ratio and was found to be linear over the concentration range of 0. 809-350. 026 ng/ml for HCTZ and 1. 094-601. 861ng/ml for telmisartan. The mean regression equation for HCTZ are y=0. 035+0. 074

Table 3: Concentration vs Area ratio for HCTZ and Telmisartan standards

S.No.	Hydrochlorthiazide		Telmisartan	
	Conc.	Area ratio	Conc.	Area ratio
1	350. 026	12. 2533	601. 861	13. 2294
2	273. 020	9. 8229	451. 396	9. 8784
3	136. 510	5. 1324	306. 949	6. 9647
4	68. 2554	2. 552	208. 725	4. 6869
5	30. 7154	1. 1604	73. 054	1. 7219
6	15. 357	0. 5864	21. 916	0. 4979
7	7. 6799	0. 2919	7. 89	0. 1823
8	2. 380	0. 0903	2. 998	0. 0733
9	0. 809	0. 0302	1. 094	0. 0282

Carry over effect in matrix: The LOQ, ULOQ and blank matrix samples without the addition of internal standard was injected and the carry over effect was found to be less than 20% for the analyte and less than 5% for the internal standard.

Table 4: Carry over effect of the blank matrix samples

	HCTZ			Telmisartan	
	Analyte area	ISTD AREA		Analyte area	ISTD AREA
LOQ	8100	237622	LOQ	15178	275194
ULOQ	8022	240418	ULOQ	14394	270301
N	2	2	N	2	2
Carry over effect in matrix (%)	1. 848	0. 018		0. 379	0. 004

Recovery: The percentage recoveries for the drugs and the internal standards were compared by comparing the peak area responses.

Table 5: The percentage recovery of samples and internal standard

	% Total recovery of HCTZ	% Total recovery of Telmisartan	% Total recovery of internal standards	
			HCTZ-13C1d2	Telmisartan d3
% Recovery	83. 88	82. 39	75. 7	81. 7
S. D	10. 291	5. 794	9. 06	5. 4
C. V (%)	12. 3	7. 0	11. 2	6. 5

Sensitivity: The lowest limit of reliable quantification for the analytes was set at the concentration of the LLOQ. The sensitivity was analysed using 4 replicates of spiked LLOQ samples and 4 replicate of pooled blank matrix sample and the signal to noise ratio was calculated and compared it with the blank matrix and it should not be greater than 5% for all LLOQ samples.

Table 6: Sensitivity for Telmisartan andHCTZ

Samples	Mean value	S/N Ratio
Telmisartan	14. 65	27. 2-31. 9
Hydrochlorthiazide	127. 03	9. 1-16. 6

Stability studies

Stock solution stability: The analyte and the stock solution were refrigerated at specified time period and compared with the fresh analyte and internal standard stock solution and the % stability was determined using the formula

$$\% \text{ stability} = \frac{\text{Mean peak area response of stability samples} \times 100}{\text{Mean peak area response of comparison samples} \times \text{C. F}}$$

Short term stability studies: Dilutions were made from the standard stock solution for the analyte and IS. The stability dilutions (after storing for 6-24 hrs at room temperature) and comparison dilutions (freshly prepared) were taken for the HCTZ and telmisartan and their internal standards.

Table 7: Stock solution stability and short term stability for HCTZ, Telmisartan and their standards

Stability parameters	Samples (at refrigerated temperature)	Stock conc.	Mean±S. D	% stability
Stock Solution Stability	HCTZ	202. 9	759843. 7±47326. 5	106. 1
	HCTZ-13C1, d2 (IS)	217. 72	434988. 8±7144. 04	103. 6
	TELMISARTAN	207. 54	3712467. 5±26962. 02	107. 0
	TELMISARTAN d3	210. 38	2102592. 3±17597. 89	117. 9
Short Term Stability (at 37^{0}C for 6-24 hrs)	HCTZ	202. 9	946874. 2±18858. 12	98. 6
	HCTZ-13C1, d2 (IS)	217. 72	522680. 2±10951. 02	102. 5
	TELMISARTAN	207. 54	2681929. 2±6970. 87	98. 0
	TELMISARTAN d3	210. 38	1185672. 3±20651. 37	98. 0

Post processing or auto sampler stability studies: This stability study indicates that the drug can remain stable in the auto injector (below 10^0c) for longer time periods which exceeds the time period from the injection of first sample upto the inject6ion of final sample in a batch

Bench top stability studies
The drug was found to be stable for 4-24 hours at bench top (room temperature)

Bench top stability in matrix: The bench top result indicate that the drug is stable for a sufficient time period at room temperature so that it can withstand the time period between the retrieval of the samples from the cold room upto its final processing which is done at room temperature. It indicates that the drug can remain stable from the time of withdrawal of the blood sample from the subject upto the time of analysis of the final sample.

Bench top stability during extraction: It indicates that the drug will be stable during extraction using the same parameters as that of the stability in the matrix. It indicates that the temperature variations during the extraction was overcome by the samples.

Freeze thaw stability: The % degradation was determined by back calculating their concentration against freshly spiked calibration curve samples.

Long term stability: This indicates that the HCTZ and Telmisartan can withstand when stored under temperature conditions of -15^0C and -50^0C under the long term stability studies.

Table 8: Comparison of the various stability parameters

Stability Test	Telmisartan			Hydrochlorthiazide		
	QC (spiked concentration, ng/mL)	Mean±SD (ng/mL)	Stability %	QC (spiked concentration, ng/mL)	Mean±SD (ng/mL)	Stability %
Auto sampler stability (at 10^0C for 48 hrs)	LQC 3. 08	3. 06±0. 03	100. 2	2. 36	2. 46±0. 07	100. 5
	HQC 456. 44	452. 7±1. 56	102	269. 44	275. 2±5. 08	102. 5
Bench top stability (at 37^{0}C for 24 hrs) (in matrix)	3. 08	3. 27±0. 05	102. 4	2. 36	2. 35±0. 08	98. 1
	456. 08	421. 9±6. 45	103. 6	269. 44	269. 09±7. 05	102. 3
Bench top stability (at 37^{0}C for 24 hrs) (during extraction)	3. 08	3. 34±0. 08	99. 0	2. 36	2. 26±0. 08	96. 3
	456. 08	425. 7±4. 48	102. 6	269. 44	274. 98±9. 54	102. 8
Freeze–thaw stability	3. 08	3. 14±0. 03	98. 7	2. 36	2. 31±0. 11	101. 7
	456. 44	406. 29±6. 05	99. 3	269. 44	270. 03±10. 12	103. 4
Long-term stability (at −15 °C for 30 days)	3. 08	3. 01±0. 03	101. 2	2. 36	2. 31±0. 04	97. 5
	456. 44	446. 01±2. 4	102. 4	269. 44	247. 3±5. 17	98. 7
Long-term stability (at −50 °C for 30 days)	3. 08	3. 06±0. 02	102. 7	2. 36	2. 28±0. 11	96. 0
	456. 44	450. 6±4. 75	103. 5	269. 44	250. 3±5. 9	99. 9

Ruggedness
It is done to evaluate the ruggedness of the method. It is done using a different column by a different analyst employing the same or another instrument. The precision and accuracy of the determinations were used for evaluating the parameter.

Table 9: Percentage stability of the quality control samples

Samples	% Stability Q. C			
	LOQQC (ng/ml)	LQC (ng/ml)	MQC (ng/ml)	HQC (ng/ml)
HCTZ	100. 2	102. 1	107. 4	95. 6
TELMISARTAN	98	99. 9	90.5	94. 1

Conclusion

A highly selective, sensitive and rapid method was developed for the determination of telmisartanandhydrochlorthiazide in human plasma and was reported using high performance liquid chromatography with detection tandem mass spectrometry. The method involves relatively simple solid phase extraction process using HLB catridge and showed high recovery for the drugs and the internal standards. The method required only 500µl of plasma and allow high sample throughput due to simple sample preparation and short run time. The acceptable precision and accuracy is obtained within standard curve range 0. 809-350. 026ng/ml for hydrochlorthiazide and 1. 094-601. 861 ng/ml for telmisartan. The simultaneous detection and quantitation of both drugs by LC-MS/MS is advantageous compared to other methods of detection. The method proved to besensitive, accurate, precise, reproducible and the sample preparation showed high recovery for the quantitative determination of telmisartan and hydrochlorthiazide in human plasma. The method has been successfully applied to the bioequivalence and pgarmacokinetic study involving a single oral dose of combination tablet containing telmisartan and hydrochlorthiazide I human plasma. Thebioanalytical work supports the regulatory submission and meet a level of confidence in

terms of various parameters like accuracy, precision, reproducibility and stability.

Discussion

The simultaneous quantification of telmisartan and hydrochlorthiazide using their own standars in human plasma was determined using liquid chromatography mass spectroscopic technique and validated using various parameters for assessment of confidence in the study. All the parameters showed consistent results and a new method was developed for this simultaneous analysis and was simple and rapid with a short run time and effective characterization using solid phase extraction procedures.

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