

VALIDATED UPLC/Q-TOF-MS METHOD FOR SIMULTANEOUS DETERMINATION OF ANTI-HYPERTENSIVE AND DIURETIC DRUGS IN HUMAN PLASMA AND ITS APPLICATION TO PHARMACOKINETIC STUDY

Hamid Khan*

Chhatrapati Shivaji Institute of Pharmacy, Durg, CG, India-491001.

Article Received on
21 March 2018,

Revised on 11 April 2018,
Accepted on 02 May 2018

DOI: 10.20959/wjpps20186-11669

***Corresponding Author**

Dr. Hamid Khan

Chhatrapati Shivaji Institute
of Pharmacy, Durg, CG,
India-491001.

ABSTRACT

In the presented work the ultra-performance liquid chromatographic/quadrupole time-of-flight mass spectrometric (UPLC/Q-TOF-MS) method has been developed for simultaneous determination of telmisartan, hydrochlorothiazide and its major metabolite chlorothiazide in human plasma. For identification of drugs, the Q-TOF mass spectrometer was operated in negative ionization mode and quantification was done using the MS/MS transitions at m/z 513.18 to 469.13 for telmisartan, 295.80 to 204.94 for hydrochlorothiazide and 268.80 to 169.98 for chlorothiazide. The

chromatographic separation was achieved on Acquity UPLC™ BEH C₁₈ (100.0 × 2.1 mm, 1.7µm) column using isocratic mobile phase consisting of acetonitrile-2mM ammonium acetate (50:50, v/v) at a flow rate of 0.25 mL/min. The elution of telmisartan, hydrochlorothiazide and chlorothiazide was occurred at 2.25, 1.22, 1.50 min, respectively. The calibration curves were linear over the concentration range of 1-1000 ng/mL for all the compounds. The developed method was validated according to ICH guidelines. The method was applied for pharmacokinetic study of telmisartan and hydrochlorothiazide in human plasma.

KEYWORDS: UPLC/Q-TOF-MS, Telmisartan, Hydrochlorothiazide, Chlorothiazide, Pharmacokinetic Study.

INTRODUCTION

The UHPLC/Q-TOF-MS technique is the latest amongst all the chromatographic technique and has been used worldwide in drug discovery and development. It has been applied in pharmaceutical development particularly in the identification and quantitative analysis of drug products. The metabolite profiling has been investigated in various biological samples by applying UHPLC/Q-TOF coupled with MetaboLynx™ software. The technique is becoming very popular in the high-throughput screening of synthetic compounds rather than application of less sensitive techniques such as HPLC-UV, LC-MS, and LC-NMR. The use of conventional techniques, such as HPLC-UV and NMR, cannot address these high throughput analytical needs due to relatively less sensitivity, high sample purity requirement, necessity of operator expertise and the use of costly solvents. The Q-TOF mass spectrometry gives the accurate mass, reliable chemical fragmentation of synthetic compounds.^[1-5] Telmisartan (TEL), is chemically 4'-[[[4-Methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]]methyl] biphenyl-2-carboxylic acid and is an antihypertensive drug, belongs to a group of angiotensin converting enzyme (ACE) inhibitors. It is used for the treatment of hypertension.^[6] Hydrochlorothiazide (HCTZ) is chemically 6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7-sulfonamide. It is a diuretic drug used worldwide for lowering the blood pressure individually and in the combination of antihypertensive drugs. Chlorothiazide (CTZ) is a major metabolite of hydrochlorothiazide and also used as diuretic drug.^[7] Fixed dose combination tablets containing 80 mg of telmisartan and 25 mg of hydrochlorothiazide has been approved for the treatment of mild to moderate hypertension and widely available in the Indian market.

The literature survey revealed that few analytical methods have been reported for determination of telmisartan as an individual drug in formulations or its degradation products and/or in biological fluids such as HPLC,^[8-10] HPTLC^[11] and LC-MS.^[12-14] Determination of hydrochlorothiazide as an individual drug in formulations or its degradation products and/or in biological fluids has been reported by spectrophotometry,^[15] HPLC,^[16-18] and LC-MS.^[19-21] Simultaneous determination of telmisartan and hydrochlorothiazide in formulations and/or biological fluids has been reported by derivative spectrophotometry, TLC-densitometry and spectrofluorimetry,^[22] HPLC^[23,24] and HPTLC.^[25] However a HPLC-MS/MS method was developed for simultaneous determination of telmisartan and hydrochlorothiazide in human plasma but the developed method was found very complicated due to the use of internal standard along with main analytes and also gradient elution for chromatographic

separation.^[26] Comparatively the method which does not involve use of internal standard minimizes interference from the sample matrix and isocratic elution makes the method easy and fast.

Hence in the presented work an UPLC/Q-TOF-MS method is developed and validated for identification and quantitative determination of telmisartan, hydrochlorothiazide and chlorothiazide in human plasma using isocratic elution without use of internal standard.

EXPERIMENTAL

Chemicals and Reagents

Telmisartan ($C_{33}H_{30}N_4O_2$, Molecular weight 514.63, and purity 99.98%), Hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$, Molecular weight 297.72, and purity 99.99%) and Chlorothiazide ($C_7H_6ClN_3O_4S_2$, Molecular weight 295.72, and purity 99.98%) were kindly supplied as gift sample by Systopic Pharmaceuticals Ltd. (New Delhi, India). 4-Amino-6-Chloro-1,3-Benzene disulfonamide was supplied by Supra Chemicals Ltd. (Mumbai, India). Tablet formulations were obtained commercially with labeled amounts of 80 mg of telmisartan and 25 mg of hydrochlorothiazide. LC-MS grade water, acetonitrile, methanol, and ammonium acetate were purchased from Fluka analytical, Sigma-Aldrich Corporation, St. Louis, MO, USA. All other reagents used were of LC-MS grade.

Q-TOF-MS and UPLC Conditions

Mass spectrometry was performed on a Waters Synapt Q-TOF Premier (Micromass MS Technologies, Manchester, UK) mass spectrometer. Quantification was done by using MS/MS transitions, m/z 513.18 to 469.13 for telmisartan, 295.80 to 204.94 for hydrochlorothiazide and 268.80 to 169.98 for chlorothiazide. UPLC was performed with Waters Acquity UPLC system (Waters Corporation, MA, USA) equipped with a binary solvent manager, an auto-sampler, column manager and a tunable MS detector.

Preparation of Standard Solutions

Each of telmisartan, hydrochlorothiazide and chlorothiazide were weighed accurately and transfer to 50 mL volumetric flasks separately. The powders were then dissolved with approximately 25 mL of methanol and ultrasonicated for 5 min. The final volume was made up with methanol. The solutions were further diluted with methanol: water (50:50, v/v) to give a series of standard solutions containing required concentrations for each compound.

Preparation of sample solutions

500 μL of plasma sample was transferred to 10 mL glass tube. To this 5 mL of extraction solvent (diethyl ether: dichloromethane 70:30, v/v) was added. The sample was mixed by vortexer for 5 min. The organic layer was transferred to another glass tube. The solid residue was evaporated to dryness using evaporator at 40 $^{\circ}\text{C}$ under a stream of nitrogen. The dried extract was reconstituted in 200 μL of diluent (methanol: water, 50:50, v/v). This solution was filtered through 0.45 μm nylon membrane filter to remove all the particulate materials. 20 μL aliquot was injected in to UPLC system.

Validation of the Method

The developed method was validated according to ICH validation guidelines.^[27] The validation parameters addressed were linearity and range, limit of detection and quantitation, precision, accuracy, and specificity.

Linearity, Range, LOD and LOQ

Different standard concentrations each of the compound in the range of 1-1000 ng/mL (1, 10, 50, 100, 200, 500, and 1000 ng/mL) was spiked to 100 μL of blank human plasma separately in methanol: water (50:50, v/v). Similarly the low, medium and high concentration QC samples containing 160, 400, 800 ng/mL for telmisartan and 50, 100, 200 ng/mL for hydrochlorothiazide and chlorothiazide were prepared independently using the same procedure. The solutions were filtered through 0.20 μm nylon syringe filter and injected in to the UPLC/QTOF-MS system for analysis. Average peak area at each concentration level was subjected to linear regression analysis with the least squares method. Linearity was described by slope, intercept and correlation coefficient obtained from regression equations.

Accuracy and Precision

Intraday and interday accuracy and precision was evaluated by analyzing low, medium and high concentration QC samples containing 160, 400, 800 ng/mL for telmisartan and 50, 100, 200 ng/mL for hydrochlorothiazide and chlorothiazide, each concentration (n=6) on three consecutive days. The mean of percentage recoveries and the RSD (%) was calculated.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of sample matrix. The specificity of the method was examined by analyzing blank plasma extract. The chromatogram of drug free plasma was compared with the chromatograms

obtained from plasma spiked with both analytes.

Stability of samples

Sample stability was tested by analyzing QC samples containing 320 ng/mL of telmisartan, 100 ng/mL of hydrochlorothiazide and 100 ng/mL of chlorothiazide after short-term (6 h) storage at 25 °C, 12 h storage in an autosampler at 25 °C, after three freeze-thaw (-20 °C) cycles, and after long-term (15 days) storage at -20 °C. The results were compared with those QC samples freshly prepared and RSD (%) was calculated.

Pharmacokinetic Study

The method was applied to determine the plasma concentrations of telmisartan and hydrochlorothiazide from a clinical trial in which 3 healthy male volunteers received a FDC tablet containing 80 mg telmisartan and 25 mg hydrochlorothiazide. Blood samples were collected before and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h post-dosing. Plasma was separated by centrifugation of the heparinized samples at $2000 \times g$ for 10 min and was stored at -20 °C until analysis.

RESULTS AND DISCUSSION

All the compounds were dissolved in LC-MS grade water individually. Because the ionization in the water is highest to any other solvent. Each solution was injected to Q-TOF-MS/MS system to obtain their mass spectra. All the compounds have strong responses in the negative ionization mode. Therefore, the negative ions, $[M-H]^-$ at m/z 513.08 for telmisartan, m/z 295.86 for hydrochlorothiazide and m/z 268.80 for chlorothiazide were selected as the precursor ions. Under the selected MS/MS conditions the precursor ions were fragmented to major product ions at m/z 513.18 to 469.13 for telmisartan, 295.91 to 204.94 for hydrochlorothiazide, and 268.80 to 169.98 for chlorothiazide as shown in Figure 1, Figure 2, and Figure 3, respectively. The MS/MS product ion spectra of telmisartan was suggested that the fragmentation of molecules occurs from carboxylic group and loss of carbon dioxide results in the formation of one major product ion, at m/z 469.5. The proposed MS/MS fragmentation mechanism of telmisartan is shown in Figure 4. The product ion spectra of hydrochlorothiazide was occurred due to the fragmentation of compound via loss of neutral molecule namely HCN and degraded by hydrolysis, resulted in the formation of one intermediate product ion, at m/z 283.95 which was identified as 4-amino-6-chloro-1,3-benzenedisulfonamide, a major degradation product of hydrochlorothiazide. This is further fragmented in to another product ion with higher intensity at m/z 268.90 by the loss of NH_3

molecule. The product ion at intensity m/z 268.90 was identified as chlorothiazide. This product ion is further converted into major product ion with highest intensity at m/z 204.93 by the loss of SO_2 . This in turn fragmented into smallest intensity ion at m/z 169.00. The proposed MS/MS fragmentation mechanism of hydrochlorothiazide and chlorothiazide is shown in Figure 5. The fragmentation pattern of the compounds and obtained mass spectra in this study was found more authentic and accurate compared to those reported in previously published method.^[12-14, 19-21, 26] Quantification was done by taking the major product ions.

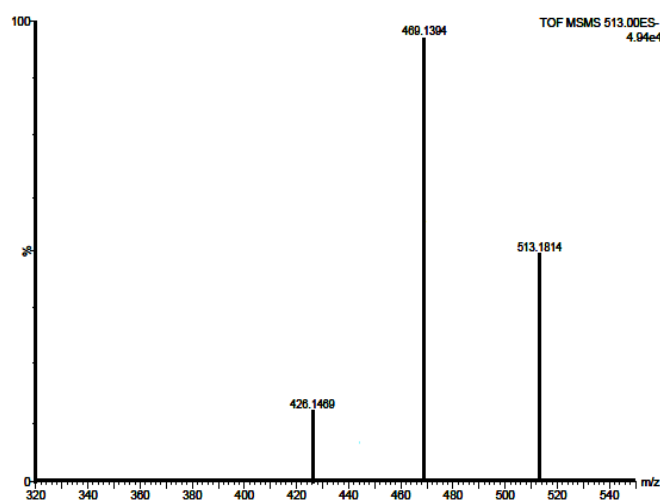


Figure 1: TOF-MS/MS spectra of Telmisartan.

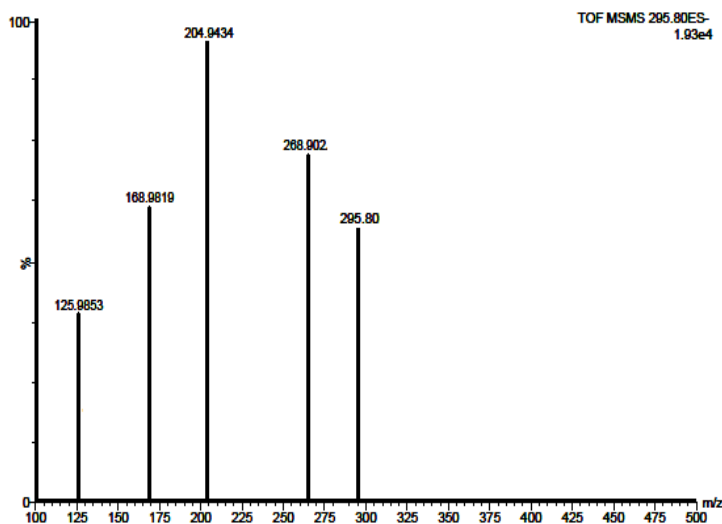


Figure 2: TOF-MS/MS spectra of Hydrochlorothiazide.

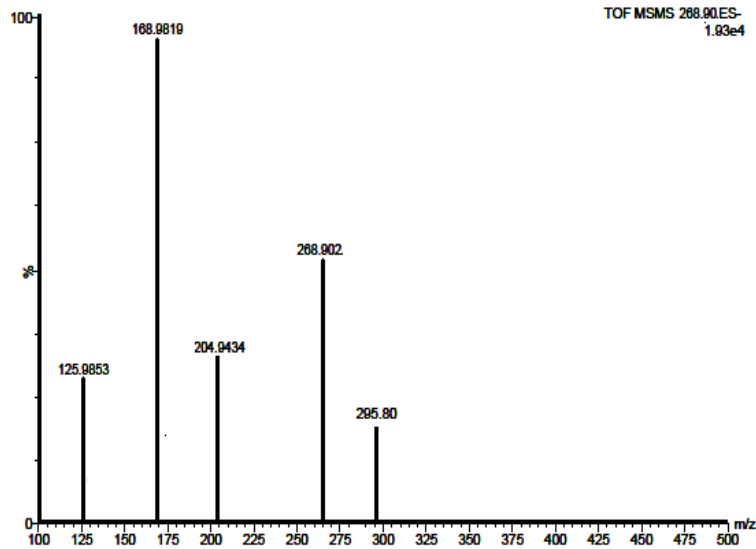


Figure 3: TOF-MS/MS spectra of Chlorothiazide.

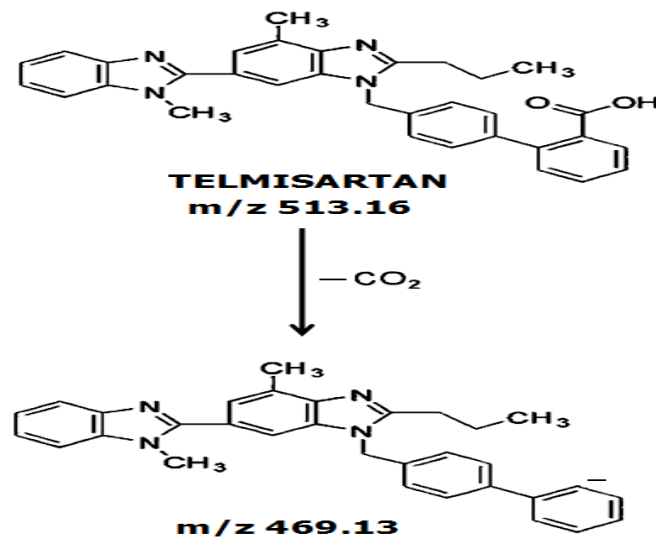


Figure 4: Proposed MS/MS fragmentation mechanism of Telmisartan.

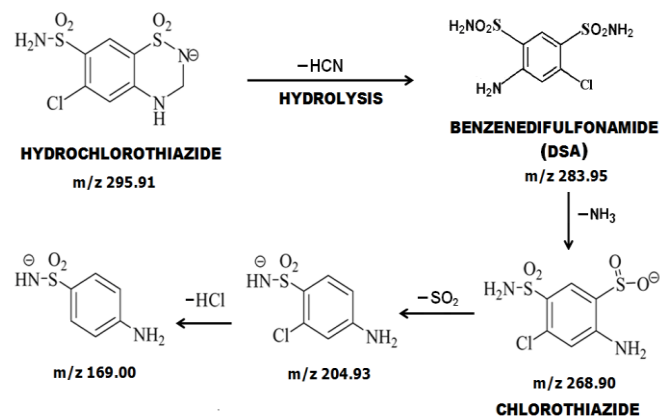


Figure 5: Proposed MS/MS fragmentation mechanism of Hydrochlorothiazide and Chlorothiazide.

Optimization of Q-TOF-MS and UPLC Conditions

The various parameters for Q-TOF-MS and UPLC conditions are presented in Table 1. The isocratic mobile phase containing acetonitrile-2mM ammonium acetate (50:50, v/v) at a flow rate of 0.25 mL/min provide peaks with short retention times. The retention time was found to be 2.25 min for telmisartan, 1.22 min for hydrochlorothiazide and 1.50 min for chlorothiazide with the total chromatographic run time of 2.50 min for each compound. UPLC-TOF-MS/MS chromatogram obtained from mixed standards (1ng/mL each) of telmisartan and hydrochlorothiazide is shown in Figure 6.

Table 1: Various Parameters for Q-TOF-MS and UPLC Conditions.

Q-TOF-MS Conditions		UPLC Conditions	
Capillary voltage	3.0 kV	Chromatography	Waters Acquity UPLC system
Sampling cone voltage	40 V	Column	Acquity UPLC BEH C ₁₈
Extraction cone voltage	4 V	Column dimension	100.0 × 2.1 mm, 1.7 μm
Source temperature	80°C	Mobile phase	Acetonitrile-2 mM ammonium acetate (50:50, v/v)
Cone gas flow	50 L/h	Mobile phase flow rate	0.25 mL/min
Source gas flow	0.50 mL/min	Elution mode	Isocratic
Collision gas (Argon)	2.5 × 10 ⁻⁴ mbar	Total run time	3 min
Collision energy	12 V	System pressure	2450 to 2500 psi

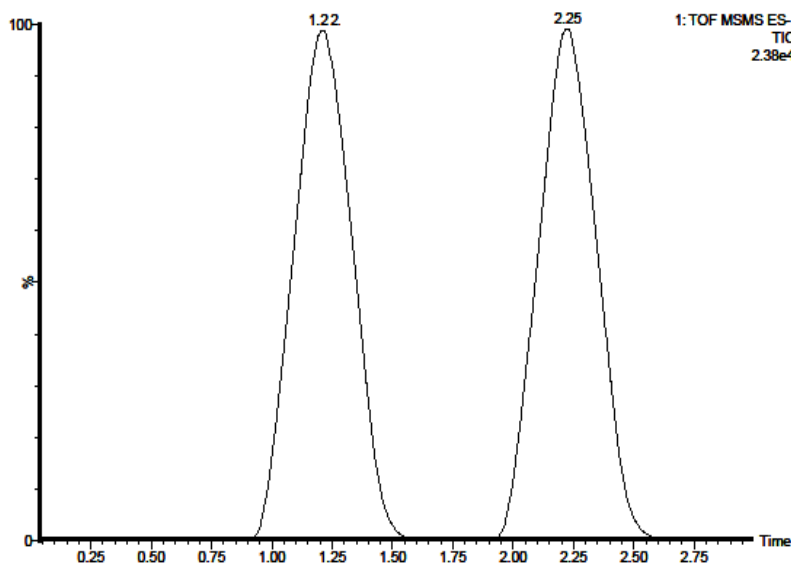


Figure 6: UPLC-TOF-MS/MS chromatogram obtained from mixed standards (1ng/mL each) of telmisartan (R_t 2.25 min) and hydrochlorothiazide (R_t 1.22 min).

Validation of the method

The results of linearity, LOD and LOQ are presented in Table 2. The obtained results indicated that higher sensitivity of the method. The RSD less than 2% were obtained for all the compounds by evaluation of intraday, interday, and different analysts precision suggested an acceptable precision of the method. The results of accuracy and precision are presented in Table 3. No significant interference in the blank plasma traces was seen from endogenous substances at the retention time of both the analytes.

Table 2: Results Obtained from Linearity, LOD, and LOQ.

Parameters	Telmisartan	Hydrochlorothiazide	Chlorothiazide
Linear range (ng/mL)	1-1000	1-1000	1-1000
Slope	12.102	12.145	15.125
Intercept	245.42	130.32	135.42
Correlation coefficient ^a	0.9997	0.9998	0.9998
LOD (ng/mL)	0.01	0.01	0.01
LOQ (ng/mL)	1	1	1

^aMean of six replicates (n = 6).

Table 3: Results Obtained from Recovery Studies and Precision.

Conc. Added (ng/mL)	Conc. Found (ng/mL)	Recovery (%) ^a	RSD (%)	
			Intraday	Interday
Telmisartan				
160	159.95	99.97	1.59	1.24
400	399.96	99.99	0.78	1.45
800	800.10	100.01	0.95	1.75
Hydrochlorothiazide				
50	49.92	99.84	1.10	1.35
100	99.02	99.02	1.55	1.45
200	200.15	100.07	1.71	1.28
Chlorothiazide				
50	49.85	99.70	1.78	1.52
100	100.02	100.02	1.94	1.78
200	199.98	99.99	1.64	1.82

^aMean of six replicates (n = 6)

Stability of samples

The stability of drugs in human plasma under various storage conditions and time period are presented in Table 4. The results indicated that no significant change in the concentration of drugs over the period of 12 h at room temperature which was covered the entire chromatographic procedure. There were no significant differences in the concentration of drugs when the samples were subjected to three freeze-thaw (-20 °C) cycles and after long-

term (15 days) storage at -20 °C ($p > 0.05$, ANOVA).

Table 4: Results Obtained from Stability Studies.

Storage conditions	Analyte	Conc. Added (ng/mL)	Conc. Found (ng/mL)	RSD (%)
Storage for 6 h at 25 °C	TEL	320	319.52	1.05
	HCTZ	100	99.02	1.22
	CTZ	100	99.97	1.57
Three freeze-thaw (-20 °C) cycles	TEL	320	320.15	1.27
	HCTZ	100	99.25	0.98
	CTZ	100	99.98	1.75
Storage for 15 days at -20 °C	TEL	320	319.07	0.85
	HCTZ	100	99.11	0.94
	CTZ	100	99.95	1.45

Pharmacokinetic Study

The method was applied to pharmacokinetic study of telmisartan and hydrochlorothiazide in human plasma. The results of pharmacokinetic parameters obtained from mean plasma concentration time curve after administration of single FDC tablet containing 80 mg telmisartan and 25 mg hydrochlorothiazide are presented in Table 5. The results obtained from pharmacokinetic parameters were not significantly different from the reported methods of each drug administered separately.^[13, 14, 20, 21] and also when both the drugs administered in combination tablets.^[26]

Table 5: Results Obtained from Pharmacokinetic Studies.

Pharmacokinetic Parameter	Telmisartan	Hydrochlorothiazide
T _{max} (h)	2.15 ± 0.25	1.10 ± 0.15
C _{max} (ng/mL)	525 ± 2.25	250 ± 1.24
AUC (ng·h/mL)	850 ± 1.12	350 ± 2.20
T _{1/2}	24.05	7.15

Data are mean ± S.D., n = 3.

CONCLUSION

The UPLC/Q-TOF-MS method was developed, validated and applied for identification and quantification of telmisartan, hydrochlorothiazide and chlorothiazide. The fragmentation mechanism of all the compounds was established on the basis of their m/z values of precursor and product ions. Such mechanism helps in the structural identification of these drugs in the human plasma. Established fragmentation mechanism of these compounds also helpful in the identification their major metabolites in the blood. The developed method has shown acceptable linearity, precision, accuracy and sensitivity of the drugs in human plasma. In

addition to this, use of isocratic chromatographic separation without any internal standard makes it an advantageous method for simultaneous determination of telmisartan, hydrochlorothiazide and chlorothiazide in human plasma.

ACKNOWLEDGEMENTS

The authors are grateful to Systopic Laboratories Ltd., Delhi, India, for providing gift samples of telmisartan and hydrochlorothiazide. The authors are also thankful to Dean and In-charge of Instrumentation Facilities, Faculty of Pharmacy, Jamia Hamdard, Hamdard University, New Delhi, India, for providing opportunities to work on UPLC/Q-TOF-MS system.

REFERENCES

1. Swartz ME. UPLC: An Introduction and Review. *Journal of Liquid Chromatography and Related Technology*, 2005; 28(1): 1253-1263.
2. Novakova L, Matysova L, Solich P. Advantages of Application of UPLC in Pharmaceutical Analysis. *Talanta.*, 2006; 68(3): 908-918.
3. Plumb R, Castro-Perez J, Granger J, Beattie I, Joncour K, Wright A. Ultra performance liquid chromatography coupled to quadrupole-orthogonal time-of-flight mass spectrometry. *Rapid Communication in Mass Spectrometry.*, 2004; 18(19): 2331-2337.
4. Khan H, Ali J. UHPLC/Q-TOF-MS: Introduction and Applications. *Letters in Organic Chemistry.*, 2015; 12(6): 371-378.
5. Khan H, Ali H, Ahmad S, Ahmad N, Ahuja A, Baboota S, Ali J. Validated UPLC/Q-TOF-MS method for simultaneous determination of aceclofenac, paracetamol, and their degradation products in tablets. *Journal of Liquid Chromatography and Related Technology.*, 2012; 35(1): 109-128.
6. Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 14th edition, Merck Research Laboratories, Merck and Co., Inc., White House Station, NJ, USA, 2006; 1569.
7. British Pharmacopoeia. HMSO: London, 2008; 1036-1037.
8. Torrealday N, Gonzalez L, Alonso RM, Jimenez RM, Lastra EO. Experimental design approach for the optimization of a HPLC-fluorimetric method for the quantitation of the angiotensin II receptor antagonist telmisartan in urine. *Journal of Pharmaceutical and Biomedical Analysis.*, 2003; 32(4/5): 847-857.
9. Palled MS, Rajesh PMN, Chatter M, Bhat AR. RP-HPLC determination of telmisartan in tablet dosage forms. *Indian Journal of Pharmaceutical Sciences.*, 2005; 67(1): 108-110.

10. Londhe SV, Kaul N, Agrawal H, Mahadik KR. Stability-indicating RP-HPLC method for analysis of telmisartan in the bulk drug and in formulations. *Acta Chromatographica.*, 2010; 22(4): 539-548.
11. Prabhu C, Subramanian GS, Karthik A, Kini S, Rajan MS, Udupa N. Determination of telmisartan by HPTLC- A stability-indicating assay. *Journal of Planar Chromatography.*, 2007; 20(6): 477-481.
12. Shah RP, Singh S. Identification and characterization of a photolytic degradation product of telmisartan using LC-MS/TOF, LC-MSⁿ, LC-NMR and on-line H/D exchange mass studies. *Journal of Pharmaceutical and Biomedical Analysis.*, 2010; 53(3): 755-761.
13. Chen B, Liang Y, Wang Y, Deng F, Zhou P, Guo, F, Huang L. Development and validation of liquid chromatography-mass spectrometry method for the determination of telmisartan in human plasma. *Analytica Chimica Acta.*, 2005; 540(2): 367-375.
14. Li P, Wang Y, Wang Y, Tang Y, Fawcett J P, Cui Y, Gu J. Determination of telmisartan in human plasma by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B.*, 2005; 828(1-2): 126-129.
15. Tamat S R, Moore D E. Photolytic decomposition of hydrochlorothiazide. *Journal of Pharmaceutical Sciences.*, 1982; 72(2): 180-183.
16. Daniels SL, Vanderwielen AJ. Stability-indicating assay for hydrochlorothiazide. *Journal of Pharmaceutical Sciences.*, 1981; 70(2): 211-215.
17. Tagliari MP, Stulzer HK, Murakami FS, Kuminek G, Valente B, Oliveira PR, Segatto SMA. Development and validation of a stability-indicating LC method to quantify hydrochlorothiazide in oral suspension for pediatric use. *Chromatographia.*, 2008; 67(7-8): 647-652.
18. Fang X, Bibart R, Mayr S, Yin Y, Harmon PA, Finnegan J, Tyrrell RJ, Reed RA. Purification and identification of an impurity in bulk hydrochlorothiazide. *Journal of Pharmaceutical Sciences.*, 2001; 90(11): 1800-1809.
19. Franolic JD, Lehr GJ, Barry TL, Petzinger G. Isolation of a 2:1 hydrochlorothiazide-formaldehyde adduct impurity in hydrochlorothiazide drug substance by preparative chromatography and characterization by electrospray ionization LC-MS. *Journal of Pharmaceutical and Biomedical Analysis.*, 2001; 26(4): 651-663.
20. Ramakrishna NVS, Vishwottam KN, Manoj S, Koteswara M, Wishu S, Varma DP. Sensitive liquid chromatography tandem mass spectrometry method for quantification of hydrochlorothiazide in human plasma. *Biomedical Chromatography.*, 2005; 19(10): 751-760.

21. Liu F, Xu Y, Gao S, Zhang J, Guo Q. Determination of hydrochlorothiazide in human plasma by liquid chromatography/tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis.*, 2007; 44(5): 1187-1191.
22. Bebawy LI, Abbas SS, Fattah LA, Refaat H H. Application of first derivative, ratio derivative spectrophotometry, TLC-densitometry and spectrofluorimetry for the simultaneous determination of telmisartan and hydrochlorothiazide in pharmaceutical dosage forms and plasma. *Farmaco.*, 2005; 60(10): 859-867.
23. Bhat LR, Godge RK, Vora AT, Damle MC. Validated RP-HPLC method for simultaneous determination of telmisartan and hydrochlorothiazide in pharmaceutical formulation. *Journal of Liquid Chromatography and Related Technology.*, 2007; 30(20): 3059-3067.
24. Rane VP, Sangshetti JN, Shinde DB. Simultaneous high-performance liquid chromatographic determination of telmisartan and hydrochlorothiazide in pharmaceutical preparation. *Journal of Chromatographic Science.*, 2008; 46(10): 887-891.
25. Shah NJ, Suhagia BN, Shah RR, Shah PB. Development and validation of a HPTLC method for the simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form. *Indian Journal of Pharmaceutical Sciences.*, 2007; 69(2): 202-205.
26. Yan T, Li H, Deng L, Guo Y, Yu W, Fawcett JP, Zhang D, Cui Y, Gu J. Liquid chromatographic-tandem mass spectrometric method for the simultaneous quantitation of telmisartan and hydrochlorothiazide in human plasma. *Journal Pharmaceutical and Biomedical Analysis.*, 2008; 48(4): 1225-1229.
27. International Conference on Harmonization, ICH Q2 (R1), Validation of Analytical Procedures: Text and methodology, Geneva, 2005.