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Low Level Quantification of Potential Genotoxic Impurities In Telmisartan Drug Substance by HPLC

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ABSTRACT

A sensitive and rapid HPLC method was developed and validated for the determination of potential genotoxic impurities i.e (Bromomethyl)biphenyl methyl ester and (Dibromomethyl)biphenyl methylester at trace level in Telmisartan drug substance by applying the concept of threshold of toxicological concern (TTC). The HPLC method was developed and optimized on Symmetry Shield RP18, 3.5 µ (150mm × 4.6mm) column with oven temperature maintaining at 40°C and 0.02M Phosphate buffer pH 2.5 was chosen as mobile phase A and mixture of acetonitrile and Phosphate buffer (55:45) was selected as mobile phase B in gradient reverse phase mode in isocratic mode of composition. Chromatographic parameters i.e flow rate: 1.0 ml/min, wavelength detection: 205 nm, injection volume: 20µl and run time: 25 min were applied in this methodology. Based on validation data, the method is found to be specific, sensitive, accurate and precise. The established limits of Limit of detection and Limit of quantification for subjected impurities are found to be 2.4 µg/g and 4.7 µg/g respectively for each impurity. The recovery at LOQ level obtained was 98.2% for (Bromomethyl) biphenyl methyl ester and 99.2% for (Dibromomethyl) biphenyl methyl ester. This method can be used as good quality control tool for quantization of these impurities at low level. The experimental results are discussed in detail in this research paper.

Keywords: Telmisartan, Genotoxicity, Validation, HPLC.

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INTRODUCTION

Telmisartan is chemically known as 4'-[[4-methyl-6-(1-methyl-1H-benzimidazol-2-YL)-2-propyl-1H-benzimidazol-1-YL] methyl] biphenyl-2-carboxylic acid its molecular formula is $C_{33}H_{30}N_4O_2$ .and molecular weight is 514.6.

Telmisartan is an antihypertensive drug of the Angiotensin-II receptor blockers category[1]. It is an efficient antagonist to angiotensin-II, [2] and this drug h is a potent vasoconstrictor for both arteries and veins. The results proven that, the arteries and veins enlarge and blood pressure falls. It is also used for reducing the risk of heart attack, stroke, or death from cardiovascular causes [3]. It is also producing a beneficial protective effect against vascular and renal damage instigated in diabetes and cardiovascular diseases, due to its arteriolar and venous dilation capability [4]. Typically, Telmisartan given in combination with hydrochlorothiazide and available in fixed-dose combination with hydrochlorothiazide doses of 40 mg/12.5 mg and 80 mg/12.5 mg. The combination is useful in the treatment of mild to moderate hypertension, well tolerated with a lower incidence of cough than ACE inhibitors [5].

Few analytical methods for the determination of the impurities either in bulk drugs or in pharmaceuticals have been reported. In the last few years, it was observed that an interest was increased for the identification and quantification of impurities in bulk drugs using new methodologies. For the determination of Telmisartan and its related substances and genotoxic impurities, many methods are available in literature [6-8]. Chemical structure of Telmisartan is shown in Figure 1.

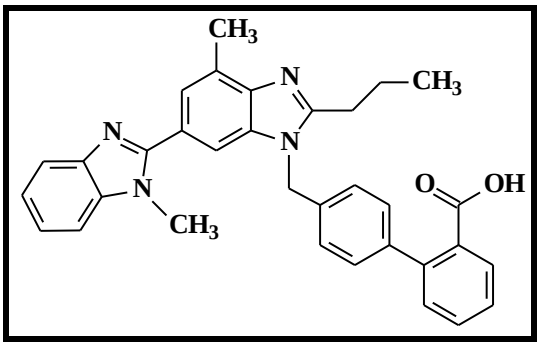


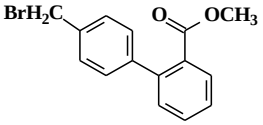
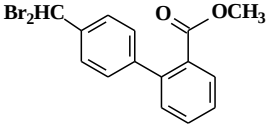
Figure 1: Chemical structure of Telmisartan

Since past decade of years, impurities, especially genotoxic impurities' analysis and control strategies development in the final products became an epicenter in the pharmaceuticals and regulatory authorities. Active pharmaceutical Ingredients susceptible to comprise different impurities that may arise from starting materials, reagents employed for the synthesis and by products in the synthetic process. Different reactants will be meticulously selected based on their reactivity in the synthesis of the drugs to achieve the optimum yields of end products. However,

this same reactivity of the reactants could result in genotoxicity if any unreacted material left with the final product as an impurity, which makes these impurities to consider critically eliminating them from the final drug product[9]. The risk of carryover into the drug substance should be assessed for identified impurities that are present in starting materials / intermediates and impurities that are reasonably expected by products in the route of synthesis from the starting material to the drug substance.

(Bromomethyl) biphenyl methylester is one of the key raw materials in the preparation of Telmisartan, further (Dibromomethyl)biphenyl methylester is possible impurity of (Bromomethyl)biphenyl methylester, and these two impurities are structurally alert genotoxic impurities as per silico toxicity assessment, impurities structures and toxicity information given in Table.1. The acceptable limit of 18.75µg/g calculated based on the maximum daily dose 80mg and lifetime duration of treatment considered for Telmisartan drug by TTC approach as per ICH M7 [10].

Table 1: Impurities chemical structures and Toxicity information

Impurity	Chemical structure	Category	In-silico toxicity information	
			Derek & sarah	Leadscope
(Bromomethyl) Biphenyl methyl ester		Raw material	**Derek:** Plausible **Sarah:** Positive	Consensus: Positive Positive Probability: 0.760
(Dibromomethyl) Biphenyl methyl ester		Possible impurity of Raw material	**Derek:** Plausible **Sarah:** Positive	Consensus: Positive Positive Probability:0.776

The goal of this research study is to develop a sensitive, selective, accurate, reproducible and simple method to analyze these genotoxic impurities in Telmisartan drug substance. For the sensitivity of impurity level, we have chosen HPLC technique. To the best of our knowledge, determination of (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester by HPLC in Telmisartan drug substance has not been reported in literature till date. This paper describes the development, optimization of HPLC method for the determination of subjected impurities and method validated accordance with ICH guidelines [11].

MATERIALS AND METHOD

Chemicals, reagents and samples

Telmisartan drug substance, subjected analytes (i.e (Bromomethyl) biphenyl methylester, (Dibromomethyl)biphenyl methylester) were gifted from APL Research Centre-II (A division of Aurobindo Pharma Ltd., Hyderabad). Potassium dihydrogen ortho phosphate (Analytical grade), Orthophosphoric acid (~88%) (GR grade), Sodium hydroxide (AR grade) Methanol (HPLC grade),

Dichloromethane (AR grade), Acetonitrie (HPLC grade) were procured from Merck and highly pure milli-Q water was obtained by using millipore purification system.

Instrumentation and Chromatographic conditions

Chromatographic separations were performed on HPLC (High Performance Liquid Chromatograph) system with Alliance –waters e2695 separation module with 2998 PDA detector and 2489 UV detector using Empower software. The mobile phase is consisting acetonitrile and buffer in the ratio of 55:45 v/v, where buffer was prepared by dissolving 2.72 g of Potassium dihydrogen orthophosphate in 1000 ml of water. Adjust to pH 2.5±0.05 with orthophosphoric acid. The analysis was carried out on Symmetry Shield RP18 (150mm × 4.6mm),3.5 µm µm particle diameter column (Make: Waters), maintained at temperature 40°C. Mobile phase was flushed through the column at a flow rate of 1.0 ml/min and pump was in isocratic mode. The injection volume was 20 µl and the analyte was monitored at 205 nm and running time is 25 min. (Bromomethyl) biphenyl methylester peak retention time is 9.6 min and (Dibromomethyl)biphenyl methylester retention time is 14.0 min.

Preparation of Solutions:

Standard solution A (0.4 mg/ml)

Accurately weigh and transfer about 40 mg of (Bromomethyl)biphenyl methylester reference standard into a 100 ml clean, dry volumetric flask, add 70 ml of methanol and sonicate to dissolve. Make up to volume with methanol.

Standard solution B: (0.4 mg/ml)

Accurately weigh and transfer about 40 mg of (Dibromomethyl)biphenyl methylester reference standard into a 100 ml clean, dry volumetric flask, add 50 ml of Dichloromethane and sonicate to dissolve. Make up to volume with acetonitrile.

Standard solution

Transfer each 5 ml of standard solution A and standard solution B into a 100 ml clean, dry volumetric flask and make up to volume with methanol. Dilute 5 ml of this solution to 100 ml with methanol. Further dilute 5 ml of this solution to 50 ml with methanol. Filter through 0.45µ or finer porosity membrane filter.

Blank solution

Transfer 100 µl of sodium hydroxide* solution into a 10 ml clean, dry volumetric flask containing 5 ml of methanol and make up to volume with methanol.

**Preparation of 1N Sodium hydroxide solution:* Dissolve 4.0 g of sodium hydroxide in 100 ml of water.

Sample solution

Accurately weigh and transfer about 50 mg of sample into a 10 ml clean, dry volumetric flask, add 2 ml of methanol and 100 µl 1N sodium hydroxide solution and sonicate to dissolve. Make up to volume with methanol. Filter through 0.45 µ or finer porosity membrane filter.

RESULTS AND DISCUSSION

Method validation

The developed and optimized method was then validated for the following parameters.

- Specificity
- Limit of Detection (LOD) and Limit of Quantitation (LOQ)
- Linearity
- Precision (System Precision)
- Accuracy
- Stability of Sample Solution
- Robustness
- System suitability.

The results of each of the above validation experiments indicated the conformity to the stipulated acceptance criteria and these experimental results have been discussed in this research article.

Specificity

Specificity of the method is its ability to detect and separate all the impurities present in the drug substance. Specificity of the method is demonstrated in terms of spectral as well as peak purity data of the drug and its impurities present in the drug. Peak passed the peak purity test. (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester solution, all known related substances were prepared individually and injected to confirm retention time. Solution of Telmisartan drug substance, Telmisartan drug substance spiked with (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester (Control Sample) and Telmisartan drug substance spiked with all known related substances including (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester (Spiked Sample) were injected to confirm any co-elution with (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester peak from any known related substances. Peak purity for (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester were established by using LC solution software and found to be passed (Purity angle should be less than purity threshold). Retention Times (Bromomethyl) biphenyl methylester and (Dibromomethyl)biphenyl methylester obtained with Standard and Test sample spiked with (Bromomethyl)biphenyl

methylester and (Dibromomethyl)biphenyl methylester were comparable and the peak purity data of (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester in Control Sample and Spiked Sample indicates that the peaks are homogeneous and have no co-eluting peaks indicating specificity of the method. Specificity experiments are shown in Table 2 and typical HPLC chromatograms of specificity experiments are shown in in Figure 2.

Table 2: Specificity experiment results

For RT confirmation				
Name	**Retention Time (min.)**			
	Standard		**Sample**	
(Bromomethyl)biphenyl methylester	10.109		10.127	
(Dibromomethyl)biphenyl methylester	15.029		15.054	
For Peak Purity				
Name	**RT (min.)**	**Peak Purity index**	**Single point Threshold**	**Minimum Peak Purity Index**
Control Sample				
(Bromomethyl)biphenyl methylester	10.127	0.999981	0.995984	3997
(Dibromomethyl)biphenyl methylester	15.054	0.999449	0.987660	11789
Spiked Sample				
(Bromomethyl)biphenyl methyl ester	10.132	0.999892	0.994608	5284
(Dibromomethyl)biphenyl methylester	15.067	0.999744	0.986188	13556

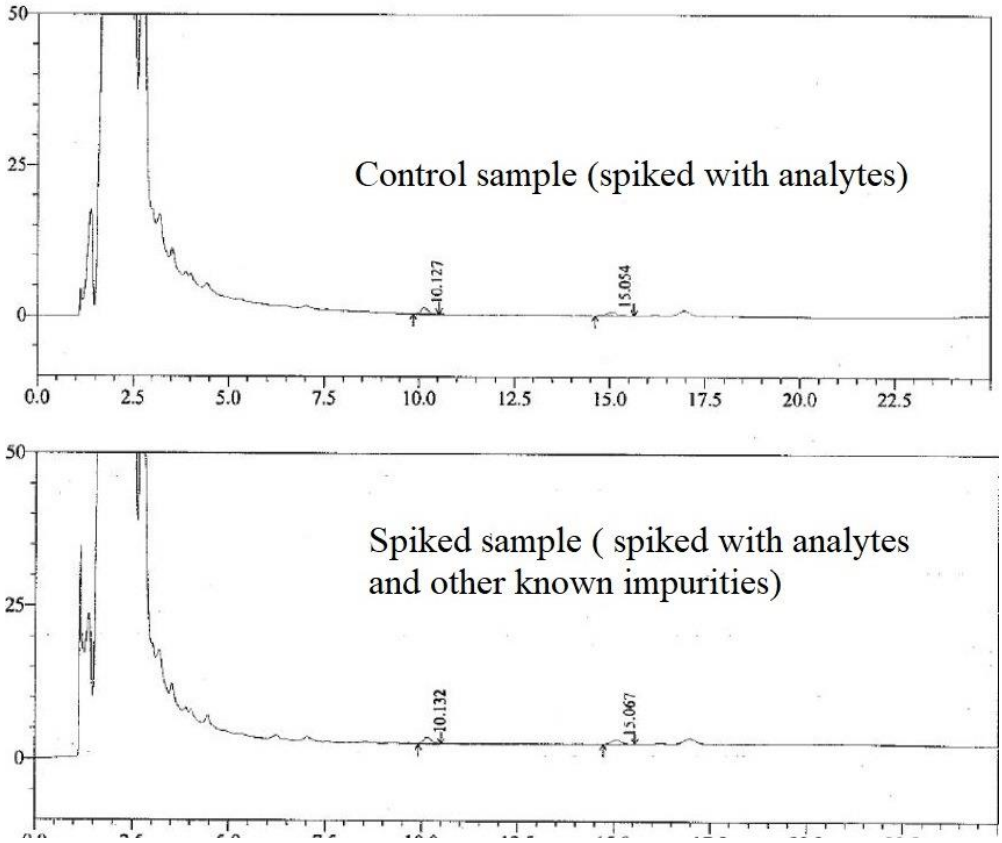


Figure 2: A typical HPLC chromatograms of Specificity experiments

LOD and LOQ

The sensitivity for detection can be demonstrated by determining the limit of detection (LOD) and limit of quantitition (LOQ). LOD/LOQ values of desired analytes were determined from based on response of analytes. The predicted concentrations of LOD and LOQ for these contents were verified for precision by preparing the solutions containing impurities at about predicted concentrations. Each of these solutions six times injected into the HPLC.

Linearity

Linearity of the method was checked by preparing solutions at nine concentration levels from LOQ to 150% of specification level by prepared using (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester standard solutions and each solution was injected into HPLC. Linearity was established by using concentration (µg/ml) on X-axis, area on Y-axis and calculated statistical values like slope, intercept, residual sum of squares and correlation coefficient. The linearity, LOD and LOQ experiments data is shown in Table 3.

Table 3: Linearity, LOD & LOQ experiments data

(Bromomethyl)biphenyl methylester		
Concentration (µg/mL)	Area	Statistical analysis

4.712	3147	Slope	692
9.424	6553	Intercept	-57
14.136	9632	Residual Sum of Squares	97
18.848	13113	Correlation Coefficient	0.9999
23.560	16256	Limit of Detection (ppm)	2.4
28.272	19465	Limit of Quantification (ppm)	4.7
(Dibromomethyl)biphenyl methylester			
Concentration (µg/mL)	Area	Statistical analysis	
4.690	2714	Slope	562
9.380	5473	Intercept	155
14.070	8173	Residual Sum of Squares	77
18.760	10676	Correlation Coefficient	0.9999
23.450	13282	Limit of Detection (ppm)	2.4
28.140	15987	Limit of Quantification (ppm)	4.7

Accuracy

Accuracy of the method was performed by recovery experiments using standard addition technique. Sample solutions were prepared in triplicate by spiking two analytes at levels of LOQ & 100% of specification limit as per test method and injected each solution into HPLC as per methodology and the percentage recoveries were calculated. The fully validated recovery results are shown in Table 4.

Table 4a: Accuracy data of (Bromomethyl)biphenyl methylester

(Bromomethyl)biphenyl methylester				
LOQ level				
% Level / Sample ID	Amount Added (µg/g)	Amount Found (µg/g)	% Recovery	
LOQ Level Sample - 1	4.857	4.725	97.3	
LOQ Level Sample - 2	4.866	4.798	98.6	
LOQ Level Sample - 3	4.837	4.778	98.8	
Statistical Analysis				
Mean 98.2	SD	0.81	% RSD	0.8
(100% level)				
Concentration / Sample ID	Amount Added (µg/g)	Amount Found (µg/g)	% Recovery	
100% Level Sample 1	18.78	18.71	99.6	
100% Level Sample 2	18.82	18.74	99.5	
100% Level Sample 3	18.42	18.53	100.6	
Overall Statistical Analysis				
Mean 99.9	SD	0.61	% RSD	0.6

Table 4b: Accuracy data of (Dibromomethyl)biphenyl methylester

(Dibromomethyl)biphenyl methylester			
LOQ level			
% Level	Amount Added	Amount	% Recovery

/Sample ID	(µg/g)	Found	
LOQ Level	4.835	4.755	98.3
LOQ Level	4.844	4.853	100.2
LOQ Level	4.815	4.765	99.0
Statistical Analysis			
Mean 9.2	SD 0.96	% RSD	1.0
(100% level)			
Concentration / Sample ID	Amount Added (µg/g)	Amount Found (µg/g)	% Recovery
100% Level Sample 1	18.42	18.53	100.6
100% Level Sample 2	18.80	18.72	99.4
100% Level Sample 3	18.81	18.74	99.5
Overall Statistical Analysis			
Mean 99.8	SD 0.66	% RSD	0.6

Precision

System precision was demonstrated by preparing the standard solutions of (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester as per methodology and analyzed by injecting six replicates. For Method precision experiments, six sample solutions were prepared individually using single batch of Telmisartan drug substance spiked with (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester at specification level and injected each solution into HPLC as per methodology. For intermediate precision sample (same batch used in method precision) solutions of same sets were prepared individually as described under method precision spiked with (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester at specification level and injected each solution into HPLC as per the methodology by another analyst, on a different day using different system and different column. achieved results like %RSD and 95% confidence interval for six determinations are summarized in Table 5.

Name	**System precision (area)**					
	Inj-1	**Inj-2**	**Inj-3**	**Inj-4**	**Inj-5**	**Inj-6**
(Bromomethyl) biphenyl methylester	13724	13621	13751	13863	13774	13672
(Dibromomethyl) biphenyl methylester	11635	11740	11420	11287	11529	11250
Statistical analysis						
	Mean		SD	% RSD	95% confidence interval (±)	
(Bromomethyl) biphenyl methylester	13734		84	0.6	88	
(Dibromomethyl) biphenyl methylester	11477		194	1.7	204	

Table 5: Precision experiments data

Name	Method precision (µg/g)					
	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6
(Bromomethyl) biphenyl methylester	17.89	18.34	17.64	17.51	17.37	17.64
(Dibromomethyl) biphenyl methylester	18.73	18.42	18.63	18.47	18.49	18.68
Statistical analysis						
	Mean	SD	% RSD	95% confidence interval (±)		
(Bromomethyl) biphenyl methylester	17.73	0.34	1.9	0.36		
(Dibromomethyl) biphenyl methylester	18.57	0.13	0.7	0.14		
	Intermediate precision (Ruggedness) (µg/g)					
	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6
(Bromomethyl) biphenyl methylester	17.97	18.87	18.22	18.27	17.51	19.01
(Dibromomethyl) biphenyl methylester	19.63	20.89	20.73	21.21	19.50	18.21
Statistical analysis						
	Mean	SD	% RSD	95% confidence interval (±)		
(Bromomethyl) biphenyl methylester	18.31	0.56	3.1	0.59		
(Dibromomethyl) biphenyl methylester	20.03	1.13	5.6	1.19		

Solution stability

For the determination of stability of the standard and sample solutions, Telmisartan drug substance spiked with (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester at specification level was prepared as per methodology and analyzed initially and at different time intervals by keeping the solutions at room temperature (~ 25°C) and refrigerator (~6°C) conditions. to determine stability acceptance criteria, the following difference has been considered, "Percentage difference between the area obtained at initial and different time interval should be not more than 10.0" for (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester. From the experimental, it can be observed that in sample solution there is gradually decreases in the peak area of (Bromomethyl) Biphenyl methyl ester. Therefore, the stability of sample solution at room temperature (~ 25°C) can be considered stable for at least 5 hours and is stable for at least 48 hours at refrigerator condition (~6°C). The experimental data is tabulated in Table 6.

Room temperature (~ 25°C)				
Time (in Hours)	**(Bromomethyl) biphenyl methylester**		**(Dibromomethyl) biphenyl methylester**	
	Area	% Difference	Area	% Difference
Initial	13507	-	10185	-
After 1 hr	13237	2.0	10151	0.3

After 15 hrs	10944	20.0	10161	0.1

Refrigerator condition (~ 6°C)				
Time (in Hours)	**(Bromomethyl) biphenyl methylester**		**(Dibromomethyl) biphenyl methylester**	
	Area	% Difference	Area	% Difference
Initial	13353	-	10383	-
After 1 hr	13359	0.0	10375	0.1
After48 hrs	13213	1.1	10592	2.0

CONCLUSION

The HPLC chromatography method was developed, optimized and validated for the determination of (Bromomethyl)biphenyl methylester and (Dibromomethyl)biphenyl methylester contents at trace level in Telmisartan drug substance and the results of various validation parameters proved that the method is specific, sensitive, precise and accurate and the method can be introduced into routine testing.

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