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Original Research article

Development and Validation of Stability Indicating HPLC Method for the Determination of Process and Degradation Related Impurities in Telmisartan Drug Substance

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ABSTRACT

A new sensitive, specific, precise and accurate stability indicating reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of related substances of telmisartan drug substance. The method was developed to separate possible degradation and process related impurities. The method was developed on symmetry shield RP 8 (150 mm x 4.6 mm, 3.5 µm) column using 0.05% trifluoroacetic acid and acetonitrile as mobile phase in gradient elution. The eluents were monitored at 230 nm by UV-Visible detector. Telmisartan and its eleven impurities were well resolved by using these conditions. The limit of detection (LOD) for telmisartan and each of its impurities (Impurity-II, Impurity-III, Impurity-IV, Impurity-V, Impurity-VI, Impurity-VII, Impurity-IX, Impurity-X, Impurity-XI) were 0.01 (% w/w) and that of Impurity-I, Impurity-VIII were 0.02 (% w/w). The limit of quantitation (LOQ) for telmisartan and each of its impuries (Impurity-II, Impurity-III, Impurity-IV, Impurity-V, Impurity-VI, Impurity-VII, Impurity-IX, Impurity-X, Impurity-XI) were 0.03 (% w/w) and that of Impurity-I and Impurity-VIII peaks were 0.05 (% w/w). Forced degradation studies were performed and mass balance was established for acid, base, oxidative, photolytic, thermal and temperature and humidity degradation conditions. The method was validated as per international conference on harmonization of technical requirements for pharmaceuticals for human use (ICH) guidelines. The impurities (Impurity-VI to Impurity XI) were related to route of synthesis and the developed method was capable to quantify these impurities along with impurities (Impurity-I to Impurity-V) as per United States, European pharmacopeia.

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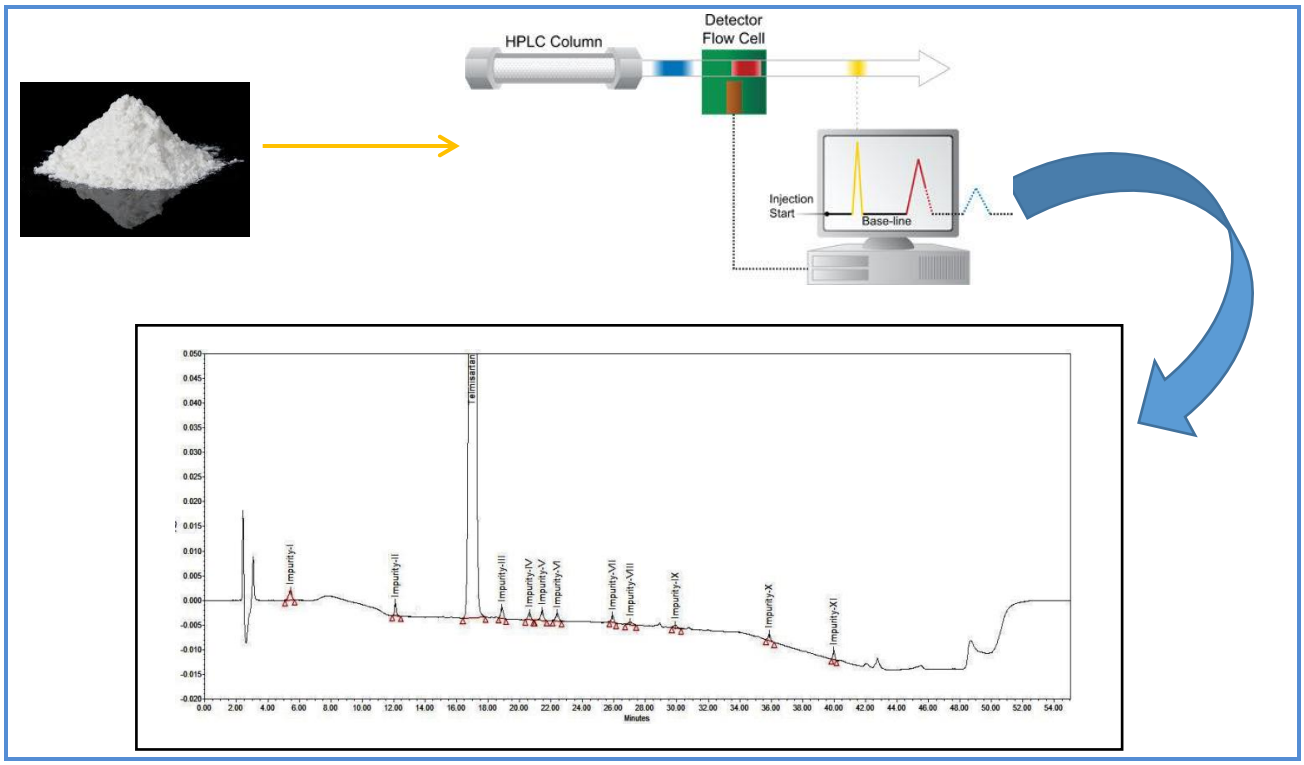
Graphical Abstract



Introduction

Telmisartan is chemically known as 4'-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl]biphenyl-2-carboxylic acid (Figure 1). It was developed by Boehringer Ingelheim as Micardis in 1999. It is an angiotensin receptor blocker (ARB) which shows more affinity for the angiotensin II type 1 (AT_1) receptor and has the longest half-life of about 24 hours [1]. The angiotensin (AT_1) receptor mediates virtually all of the known physical actions of angiotensin II in cardiovascular, renal, neuronal, endocrine, hepatic, and all other target cells [2]. Numerous studies have demonstrated that the peroxisome proliferator activated receptor-gama (PPARγ) plays an important role in regulating carbohydrate and lipid metabolism [3]. Telmisartan also functions as a partial agonist of PPARγ.

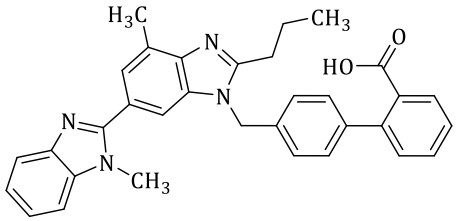


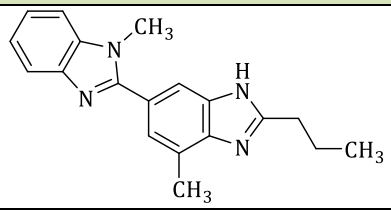
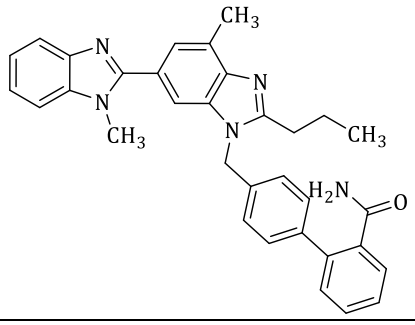
Figure 1. Telmisartan

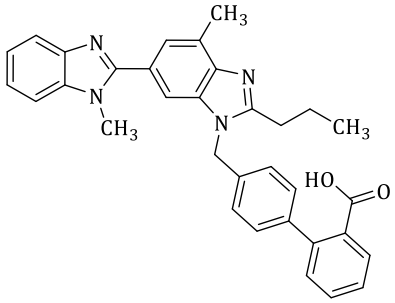
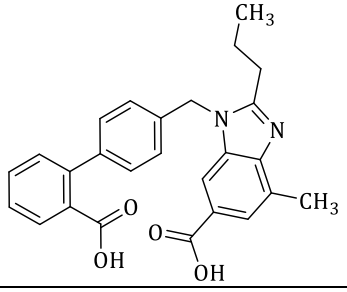
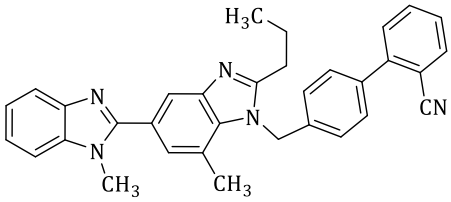
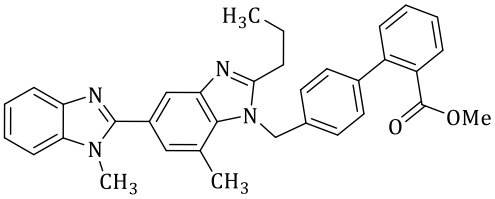
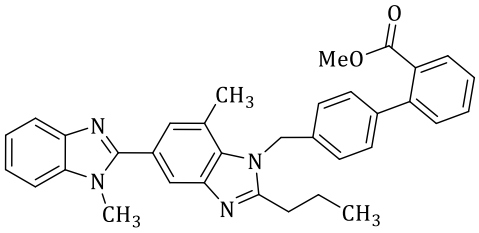
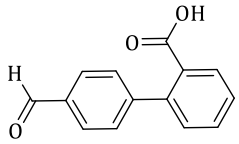
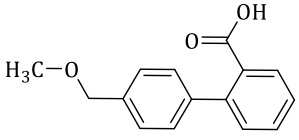
An impurity present in active pharmaceutical ingredient (API) affects quality and safety of drug product. Therefore, identification and control of an impurity is needed as per ICH guidelines [4]. An impurity may belong to an unreacted starting material, intermediates, a process and degradation substance. Starting materials are vital component to study an origin of a process, degradation and a carry-over impurity from intermediates to API molecule, which affects the quality and yield of the finished products. Therefore, a thorough knowledge of nature of starting material and all major related impurities present in them ought to be known to perform a successful synthesis of API. In case of a situation where impurities are present in the starting materials either by choice or by compulsion, a versatile and sensitive analytical method to identify and control these impurities in intermediate and API stages are always in demand [5-7].

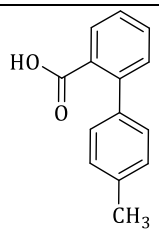
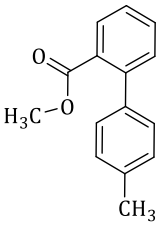
Available literature reveals that there are only few methods to quantify the related substances of telmisartan. For example, R. Nageswara Rao et al., [8] used a HPLC method to quantify seven intermediates of their route of synthesis of telmisartan, except EP and USP pharmacopeia impurities. Some ion pair methods [9-12] efficiently resolve pharmacopeia impurities I-V, but failed to incorporate process related impurities VI-XI (Table 1). Also, these ion pair methods face disadvantages in terms of column life, long time for equilibration and reproducibility. Further, the use of ion pair reagent in a gradient elution mode is not preferred because of system artifacts [13].

We found that an UPLC method [11] developed by V. Bhavani et al., could not resolve all the process related impurities of telmisartan. This limitation of aforesaid method led us to develop a simple and stability indicating method to resolve all the eleven impurities.

Table 1. List of Ph. Eur./USP, process and degradation related impurities of telmisartan

S. No.	Impurity code	Structure	Origin of impurity
1	Impurity-I		Ph. Eur. Impurity-A & USP related compound-A
2	Impurity-II		Ph. Eur. Impurity-F & USP telmisartan amide

3	Impurity-III		Ph. Eur. Impurity-B & USP related compound-B
4	Impurity-IV		Ph. Eur. Impurity-E & USP telmisartan acid
5	Impurity-V		Ph. Eur. Impurity-G
6	Impurity-VI		Process related / degradation impurity
7	Impurity-VII		Process related impurity
8	Impurity-VIII		Process related impurity
9	Impurity-IX		Process related impurity

10	Impurity-X		Process related impurity
11	Impurity-XI		Process related impurity

The developed method is suitable for determination of a large number of impurities in telmisartan together, which arise particularly due to synthetic process along with consecutive *in situ* steps (Figure 2).

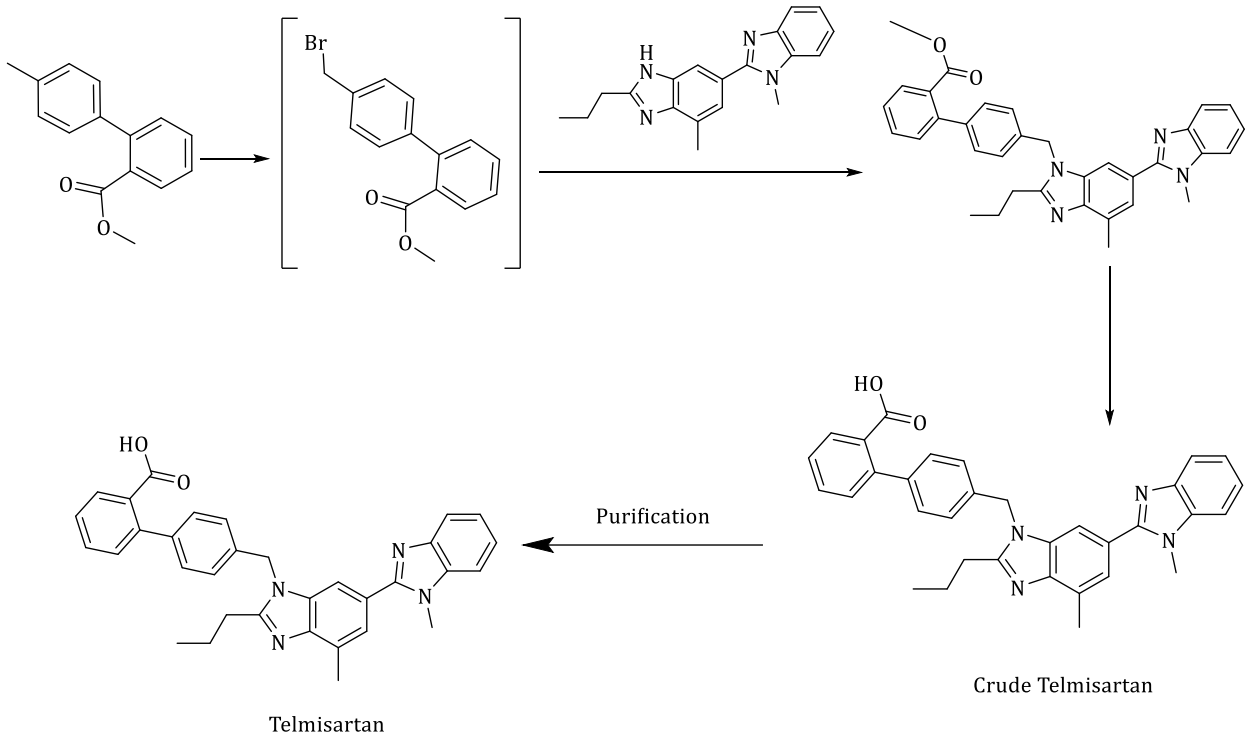
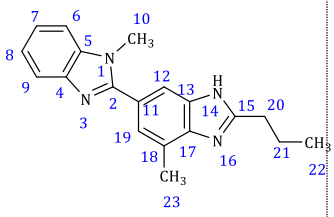
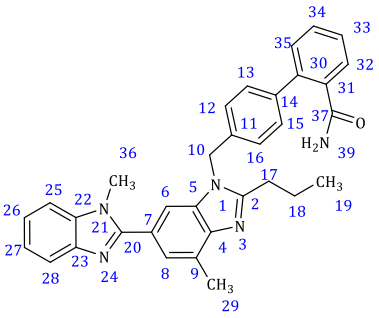
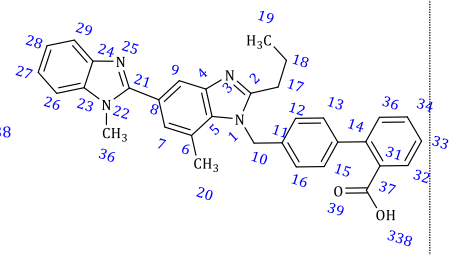


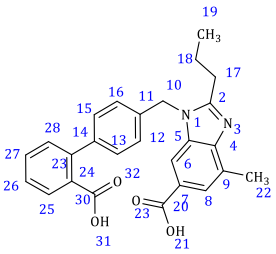
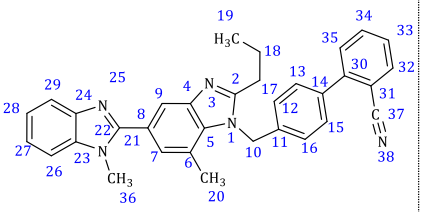
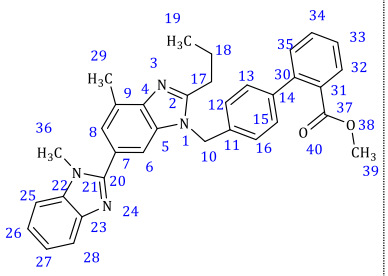
Figure 2. Route of synthesis route of telmisartan

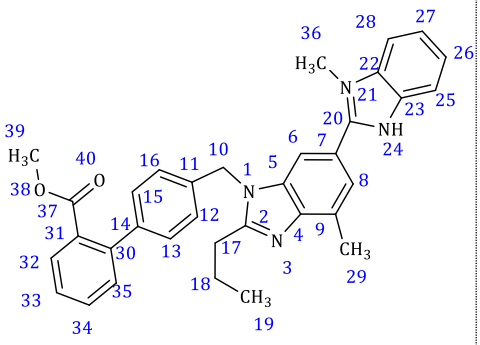
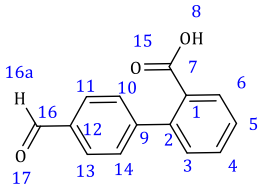
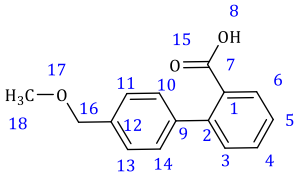
The impurities (Impurity-VI to Impurity-XI) were related to the route of synthesis and the developed method was capable to quantify these impurities along with impurities as per USP and EP (Impurity-I to Impurity-V) and the characterization data were given in supplementary (Table

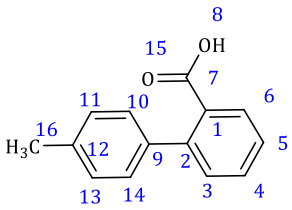
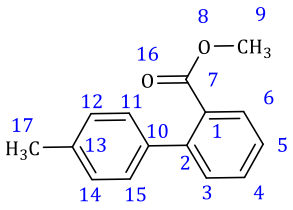
S1). The present method employed C8 column with mobile phase-A consists of 0.05% trifluoroacetic acid and mobile phase-B as acetonitrile. This method also having LCMS compatibility for impurity profiling. The forced degradation studies [15-18] were carried out to prove the method is stability indicating and was thoroughly validated according to ICH guidelines [19]. The method was found to be specific, sensitive, precise, reproducible, which can be used as quality control tool.

Table S1. Chracterization data of related substance (impurities) of telmisartan

Batch No.	**Impurity-I**	**Impurity-II**	**Impurity-III**
Structure			
Mass	304.39 (305.18-[M+H]⁺)	513.63 (514.28 [M+H]⁺)	514.62 (515.25 [M+H]⁺)
Proton Interpretation	**0.95-1.00 [22-CH_3, 3p, t (J=6 Hz)], 1.78-1.87 [21-CH_2, 2p, m], 2.58 [23-CH_3, 3p, s)], 2.82-2.87 [20-CH_2, 2p, t(J=6 Hz)], 3.90 [10-CH_2, 3p, s], 7.21-7.30 [7 & 8-CH , 2p, m], 7.43 [19-CH (1p,s], 7.58-7.75 [6, 9 & 12-CH (3p, m], 12.46 [14-NH, 1p,bs]**	0.99-1.03 **[19-CH_3, 3p, t (J=7.6 Hz)]**, 1.79-1.89 **[18-CH_2 , 2p, m)]**, 2.67 **[20-CH_3, 3p, s)]**, 2.89-2.93 **[17-CH_2, 2p, t (J=7.6 Hz)]**, 3.76 **[36-CH_3, 3p, s]**, 5.26 **[39'-NH_2, 1p, bs)]**, 7.04-7.06 **[12 & 16-CH , 2p, d(J=7.6 Hz)]**, 7.19-7.40 **[6, 8, 13, 15, 25, 26, 27, 34, 33 & 35-CH, 10p, m)]**, 7.60-7.62 **[32-CH, 1p, d (J= 7.6 Hz)]**, 7.80-7.82 **[28-CH, 2p,m)].**	0.77-0.81 **[19-CH_3, 3p, t(J= 7.6 Hz))]**, 1.62-1.68 **[18-CH_2 , 2p, q(J=7.2 Hz)]**, 2.32**[20-CH_3, 3p, s)]**, 2.63-2.67 **[17-CH_2, 2p, t (J= 7.6 Hz)]**, 3.73 **[36-CH_3, 3p, s)]**, 5.48 **[10-CH_2 , 2p, s)]**, 6.77-6.79 **[12 & 16-CH, 2p, d(J=8.0 Hz)]**, 7.25-7.43, **[7, 13, 15, 26, 27, 28, 33, 34 & 35-CH (9p,m)]**, 7.80-7.86 **[9, 29 & 32-CH (3p, m)].**
Carbon Interpretation	13.72 **[22-CH_3]**, 16.79 **[23-CH_3]**, 21.03 **[21-CH_2]**, 30.57 **[20-CH_2]**, 31.72 **[10-CH_3]**, 110.30 **[6-CH]**, 118.62 **[12-CH]**, 121.67 **[9-CH]**, 121.87 **[8-CH]**, 122.87 **[7-CH]**, 123.10 **[18 & 19-CH, 11-C]**, 136.60 **[5 & 17-C]**, 142.52 **[4 & 13-C]**, 154.31 **[2-C]**, 156.15 **[15-C]**.	13.99 **[19-CH_3]**, 16.82 **[29-CH_3]**, 21.87 **[18-CH_2]**, 29.69 **[17- CH_2]**, 31.84 **[36- CH_3]**, 47.21 **[10- CH_2]**, 109.55 **[25- CH]**, 109.70 **[6- CH]**, 118.99 **[28- CH]**, 122.90 **[26 & 27-CH]**, 123.41 **[8- CH]**, 126.55 **[12 & 16- CH]**, 127.64 **[33- CH]**, 128.70 **[32- CH]**, 129.27 **[5-C]**, 129.32 **[13 &15- CH]**, 129.74 **[35- CH]**, 130.15 **[34- CH]**, 134.87 **[9-C]**, 135.09 **[7 & 14-C]**, 135.12 **[31-C]**, 135.98 **[11-C]**, 138.83 **[4 & 22-C]**, 140.09 **[30-C]**, 143.17 **[23-C]**, 153.90 **[20-C]**, 156.55 **[2-C]**, 171.03 **[37-C].**	13.68 **[19-CH_3]**, 18.09 **[20-CH_3]**, 20.75 **[18-CH_2]**, 28.75 **[17-CH_2]**, 31.81**[36-CH_3]**, 47.81 **[10-CH_2]**, 109.80 **[26-CH]**, 117.56 **[9-CH]**, 118.72 **[29-CH]**, 122.23 **[4-C]**, 123.18 **[27 & 28-CH]**, 124.51 **[12 & 16-CH]**, 127.28 **[7 & 33-CH]**, 129.37 **[13 & 15-CH]**, 129.74 **[32-CH]**, 130.13 **[35-CH]**, 130.44 **[34-CH]**, 132.99 **[6 & 8-C]**, 134.52 **[14-C]**, 135.29 **[5 & 31-C]**, 135.67 **[23-C]**, 140.83 **[24-C]**, 141.20**[30-C]**, 141.76 **[11-C]**, 153.16 **[21-C]**, 156.94 **[2-C]**, 171.27 **[37-C].**

Batch No.	**Impurity-IV**	**Impurity-V**	**Impurity-VI (TEL-3)**
Structure			
Mass	428.48 (429.16 $[M+H]^+$)	495.61 (496.33 $[M+H]^+$)	528.64 (529.31-$[M+H]^+$)
Proton Interpretation	0.94-0.97 **[19-CH_3 (3p,t (*J* =7.2 Hz)]**, 1.73-1.82 **[18-CH_2 (2p,m)]**, 3.15-3.19 **[17-CH_2 (2p,t (*J* =8.0 Hz)]**, 5.85 **[10-CH_2 (2p,s)]**, 7.22-7.25 **[12 & 16-CH (2p,d (*J* =4.0 Hz)]**, 7.31-7.33 **[13, 15, & 28-CH (3p,m)]**, 7.42-7.45 **[26-CH (1p, t (*J* =7.6 Hz)]**, 7.52-7.56 **[27-CH (1p, t (*J* =7.6 Hz)]**, 7.70-7.72 **[25-CH (1p,d (*J* =7.6 Hz)]**, 7.86 **[8-CH (1p,s)]**, 8.16 **[6-CH (1p,s)]**, 12.89 **[-COOH (2p, brs)]**.	0.97-1.01 **[19-CH_3, 3p, t (J= 7.2 Hz)]**, 1.79-1.84 **[18-CH_2, 2p, m]**, 2.64 **[20-CH_3, 3p, s]**, 2.91-2.94 **[17-CH_2, 2p, t (J= 7.2 Hz)]**, 3.82 **[36-CH_2, 3p, s)]**, 5.69 **[10-CH_2, 2p, s]**, 7.20-7.29 **[12, 16, 27 & 28-CH, 4p, m]**, 7.49 **[7-CH, 1p, s]**, 7.54-7.58 **[13, 15, 26, 33 & 34-CH, 5p, m]**, 7.63-7.65**[29-CH, 1p, d (J= 7.6 Hz)]**, 7.73-7.77 **[9 &35-CH, 2p, m]**, 7.91-7.93 **[32-CH, 1p, d (J= 7.6 Hz]**.	1.06-1.10**[19-CH_3, 3p, t(J= 7.2 Hz)]**, 1.85-1.94 **[18-CH_2, 2p, m)]**, 2.80 **[29-CH_3, 3p, s)]**, 2.94-2.98 **[17-CH_2, 2p, t (J= 7.6 Hz)]**, 3.59 **[36-CH_3, 3p, s)]**, 3.82 **[39-CH_3, 3p, s)]**, 5.47 **[10-CH_2, 2p, s)]**, 7.11-7.13 **[12 &16-CH (2p,d (*J* =8.0 Hz)]**, 7.25-7.28 **[13 & 15-CH (2p, m)]**, 7.30-7.32 **[26, 27& 33-CH (3p,m)]**, 7.37-7.39 **[34-CH (1p, m)]**, 7.41-7.43 **[8-CH (1p, m]**, 7.46 **[6-CH (1p, s)]**, 7.50-7.54 **[32 & 35-CH (2p, m)]**, 7.82-7.84 **[25 & 28-CH (2p, d (*J* =6.8 Hz)]**.
Carbon Interpretation	13.59 **[19-CH_3]**, 16.67 **[22-CH_3]**, 20.76 **[18-CH_2]**, 27.28 **[17-CH_2]**, 46.96 **[10-CH_2]**, 111.45 **[27-CH]**, 125.92 **[14-C]**, 126.08 **[26-CH]**, 126.39 **[12 & 16-CH]**, 127.12 **[23-C]**, 127.46 **[28-CH]**, 128.89 **[13 & 15-CH]**, 129.21 **[25-CH]**, 130.50 **[8-CH]**, 130.95 **[6-CH]**, 132.14 **[9 & 11-C]**, 132.59 **[4-C]**, 134.25 **[5-C]**, 140.34 **[7-C]**, 140.60 **[24-C]**, 156.94 **[2-C]**, 167.00 **[20-C]**, 169.42 **[30-C]**.	14.30 **[19-CH_3]**, 16.94 **[20-CH_3]**, 21.20 **[18-CH_2]**, 29.19 **[17-CH_2]**, 32.19 **[36-CH_3]**, 46.42 **[10-CH_2]**, 109.68 **[7-CH]**, 110.62 **[9-CH]**, 110.82 **[26-CH]**, 118.94 **[37-C]**, 119.17 **[6-C]**, 122.24 **[27-CH]**, 122.50 **[28-CH]**, 123.75 **[33-CH]**, 123.86 **[34-CH]**, 127.33 **[12-CH & 16-CH]**, 128.72 **[35-CH]**, 128.76 **[23-C]**, 129.61 **[13-CH & 15-CH]**, 130.54 **[29-CH]**, 133.98 **[32-CH]**, 134.29 **[5-C]**, 135.22 **[4-C]**, 137.13 **[14-C]**, 137.49 **[30-C]**, 138.27 **[31-C]**, 142.99 **[8-C]**, 143.15 **[11-C]**, 144.48 **[24-C]**, 154.49 **[21-C]**, 156.67 **[2-C]**.	14.12-**[19-CH_3]**, 16.93**[29-CH_3]**, 21.89 **[18-CH_3]**, 29.89 **[17-CH_2]**, 31.89 **[39-CH_3]**, 47.15 **[10-CH_2]**, 51.90 **[36-CH_3]**, 109.09 **[35-CH]**, 109.55 **[34-CH]**, 119.47 **[25-CH]**, 122.45 **[26-CH]**, 122.61 **[27-CH]**, 123.67 **[31-C]**, 123.83 **[6-CH]**, 125.95 **[12 & 16-CH]**, 127.41 **[8-CH]**, 129.03 **[13 & 15-CH]**, 129.50 **[9-C]**, 129.94 **[28-CH]**, 130.58 **[11-C]**, 130.72 **[33-CH]**, 131.40 **[32-CH]**, 134.75 **[7-C]**, 135.10 **[5-C]**, 136.58 **[23-C]**, 141.16 **[14-C]**, 141.75 **[30-C]**, 142.60 **[4-C]**, 143.21 **[22-C]**, 154.60 **[2-C]**, 156.55 **[20-C]**, 168.69 **[37-C]**

Batch No.	**Impurity-VII**	**Impurity-VIII**	**Impurity-IX**
Structure			
Mass	528.64 (529.33[M+H]$^+$)	226.23 (225.08[M-H]$^-$)	242.27 (241.13 [M-H]$^-$)
Proton Interpretation	0.97-1.00 **[19-CH_3 (3p,t (*J* =7.2 Hz)]**, 1.79-1.91 **[18-CH_2, 2p, m]**, 2.50 **[29-CH_3, 3p, s]**, 2.76-2.80 **[17-CH_2 (2p,d (*J* =8 Hz)]**, 3.55 **[36-CH_3, 3p, s]**, 3.89 **[39-CH, 1p,s]**, 5.58 **[10-CH 2p, s]**, 6.88-6.90 **[12 & 16-CH (2p,d (*J* =7.6 Hz)]**, 7.19-7.26 **[13, 15, 32, 33 & 35-CH 5p,m]**, 7.31-7.35 **[25 & 28-CH, 2p, m]**, 7.43-7.48 **[34-CH 1p, m]**, 7.52 **[8-CH, 1p, m]**, 7.75-7.78 **[26 & 27-CH 2p, s]**, 7.86 **[6-CH, 1p, m]**,	7.27-7.29 **[3-CH, 1p, d (J= 7.6 Hz)]**, 7.40-7.43 **[5, 10 & 14-CH, 3p, m]**, 7.52-7.56 **[4-CH, 1p, t (J= 7.6 Hz)]**, 7.81-7.83 **[11 & 13-CH, 2p, d (J=7.6Hz)]**, 7.94-7.96 **[6-CH, 1p, d (J= 8.0 Hz)]**, 9.98**[17-CHO]**.	3.44 **[18-CH_3 3p, s]**, 4.55 **[16-CH_2 2p, s]**, 7.36-7.45 **[[3, 5, 10, 11, 13 & 14-CH, 6p, m]**, 7.55-7.58 **[4-CH (1p, t (*J* =7.6 Hz)]**, 7.97-7.99 **[6-CH (1p, d (*J* =7.6 Hz)]**, 11.09 **[8-OH, 1p, bs]**.
Carbon Interpretation	14.07 **[19-CH_3]**, 18.29 **[29-CH_3]**, 20.94 **[18-CH_2]**, 29.55 **[17-CH_2]**, 32.03 **[36-CH_3]**, 47.94 **[10-CH_2]**, 51.91 **[39-CH_3]**, 109.68 **[25-CH]**, 118.09 **[6-CH]**, 119.42 **[27-CH]**, 121.87 **[7-C]**, 122.53 **[32-CH]**, 122.69 **[33-CH]**, 123.47 **[9-C]**, 124.87 **[12 & 16-CH]**, 127.08 **[8-CH]**, 127.46 **[28-CH]**, 129.22 **[13 & 15-CH]**, 129.99 **[26-CH]**, 130.60 **[31-C]**, 130.72 **[35-CH]**, 131.44 **[34-CH]**, 135.03 **[11-C]**, 136.19 **[22-C]**, 136.51 **[5-C]**, 141.12 **[4-C]**, 141.71 **[14-C]**, 142.34 **[23-C]**, 142.82 **[30-C]**, 154.20 **[20-C]**, 157.12 **[2-C]**, 168.78 **[37-C]**.	128.13 **[5-CH]**, 128.79 **[1-C]**, 129.25 **[10 & 14-CH]**, 129.50 **[11 & 13-CH]**, 130.98 **[3-CH]**, 131.18 **[6-CH]**, 132.54 **[4-CH]**, 135.21 **[12-C]**, 142.43 **[2-C]**, 147.66 **[9-C]**, 172.57 **[7-C]**, 192.20 **[16-C]**.	57.99 **[18-CH_3]**, 74.40 **[16-CH_2]**, 127.25 **[5-CH]**, 127.56 **[11 & 13-CH]**, 128.62 **[10 & 14-CH]**, 129.73 **[1-C]**, 130.66 **[3-CH]**, 131.20 **[6-CH]**, 132.00 **[4-CH]**, 136.99 **[9-C]**, 140.63 **[2-C]**, 142.99 **[12-C]**, 173.14 **[7-C]**.

Batch No.	**Impurity-X**	**Impurity-XI**
Structure		
Mass	212.34 (211.10 $[M-H]^-$)	226.27 (227.10 $[M+H]^+$)
Proton Interpretation	2.35 **[16-CH_3, 3p, s]**, 7.20-7.25 **[10, 11, 13 & 14-CH, 4p, m]**, 7.35-7.38 **[3-CH, 1p, dd (J= 0.8 & 7.6 Hz)]**, 7.41-7.45 **[4-CH, 1p, m]**, 7.53-7.58 **[5-CH, 1p, m]**, 7.69-7.71 **[6-CH, 1p, dd(J= 0.8 & 7.6 Hz)]**, 12.75 **[8-OH, 1p, bs]**,	2.43 **[17-CH_3, 3p, s)]**, 3.69 **[9-CH_3, 3p, s)]**, 7.24 **[11, 12, 14 & 15-CH, 4p, s]**, 7.39-7.44 **[5 & 6-CH, 2p, m)]**, 7.52-7.56 **[4-CH, 1p, m)]**, 7.82-7.84 **[3-CH, 1p, m)]**.
Carbon Interpretation	20.71 **[16-CH_3],** 127.01 **[4-CH],** 128.17 **[11 & 13-CH],** 128.73 **[10 & 14-CH],** 128.96 **[6-CH],** 130.36 **[3-CH],** 130.74 **[5-CH],** 132.39 **[9-C],** 136.38 **[12-C],** 137.90 **[2-C],** 140.75 **[1-C],** 169.82 **[7-C].**	21.19 **[17-CH_3],** 51.94 **[9-CH_3],** 126.90 **[5-CH],** 128.16 **[11-CH & 15-CH],** 128.79 **[12-CH & 14-CH],** 129.68 **[3-CH],** 130.72 **[6-CH],** 130.79 **[2-C],** 131.18 **[4-CH],** 136.90 **[10-C],** 138.29 **[13-C],** 142.41 **[1-C],** 169.23 **[7-C]**.

Experimental

Material and methods

Standards, samples and reagents

Telmisartan, Impurity-I {4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazole}, Impurity-II {4'-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl]biphenyl-2-carboxamide}, Impurity-III {4'-[[7-methyl-5-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl]biphenyl-2-carboxylic acid}, Impurity-IV {1-[(2'-carboxybiphenyl-4-yl)methyl]-4-methyl-2-propyl-1*H*-benzimidazol-6-carboxylic acid}, Impurity-V {4'-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl]biphenyl-2-carbonitrile{4'-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl]biphenyl-2-carbonitrile}, Impurity-VI {4'-[[methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl]biphenyl-2-carboxylic acid methyl ester}, Impurity-VII {4'-{[7-methyl-5-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-*1H*-benzimidazol-1-yl]methyl}biphenyl-2-carboxylic acid methyl ester}, Impurity-VIII {4'-formylbiphenyl-2-carboxylic acid}, Impurity-IX {4'-(methoxymethyl)biphenyl-2-carboxylic acid}, Impurity-X {4'-methyl biphenyl-2-carboxylic acid}, Impurity-XI {4'-methyl biphenyl-2-carboxylic acid methyl ester.} reference standards and telmisartan samples (Table 1) were obtained

from micro labs API limited (Bangalore, India). Trifluoroacetic acid, methanol, acetonitrile, sodium hydroxide, hydrochloric acid and hydrogen peroxide HPLC grades were purchased from Merck life science private limited (Mumbai, India). Milli-Q pure water was obtained from a Millipore Elix water purification system purchased from Millipore India Pvt. Ltd. (New Delhi, India).

Instrumentation and chromatographic conditions

The HPLC system used for method development, forced degradation and validation study was Shimadzu (Model: LC2010 C_{HT}) with quaternary gradient and UV/Photo diode array detector (Shimadzu, Japan) with Empower 3 software (Waters Chromatography Division, Milford, USA). The separation was achieved on symmetry shield RP 8 (150 mm × 4.6 mm, 3.5 µm) column with 0.05% trifluoroacetic acid as mobile phase (A) and acetonitrile as mobile phase (B) in gradient mode. The HPLC linear gradient program was set as (T_{min} A:B): $T_0$87:13, $T_3$87:13, $T_7$75:25, T_{20}70:30, T_{30}60:40, T_{40}20:80, T_{46}87:13, T_{55}87:13 with the flow rate of 0.8 mL/min. The injection volume was 10 µL, column temperature was maintained at 25 °C, sample cooler temperature was maintained at 5 °C and detection wavelength was 230 nm throughout the analysis.

System suitability, sensitivity, standard and sample solutions preparation

To establish the system suitability criteria, solution was prepared by spiking the impurities (I–XI) at 0.1% level with respect to test concentration in telmisartan drug substance. The standard solution was prepared at a concentration of 0.3 µg/mL, sensitivity solution was prepared at 0.09 µg/mL and sample was prepared at 300 µg/mL in methanol as a diluent.

Method validation

The developed method was validated as per the ICH [19] guidelines with respect to system suitability, solution stability, specificity, precision, linearity, accuracy, limit of detection and limit of quantitation for all the impurities along with telmisartan drug substance. The system precision was carried out by injecting a standard solution containing 0.3 µg/mL of telmisartan in methanol and calculated the %RSD. The stability of prepared solutions was established by injecting spiked sample at different time intervals. The specificity of the method was established to evaluate interference and peak homogeneity, and it was established by injecting blank, sample solution, spiked sample solution, individual impurity solutions. The peak homogeneity was also evaluated by comparing purity angle and purity threshold for all impurities. The LOD and LOQ values for all the eleven impurities and temisartan were predicted by injecting known concentration solutions and calculating signal to noise ratio of 3:1 for LOD and 10:1 for LOQ respectively (Figure S6). The

linearity of the method was established by injecting six concentration levels (LOQ, 50%, 80%, 100%, 120% and 150% respectively) with respect to specification limit as given in [Figure 4]. The slope, regression coefficient, y-intercept values were predicted by plotting a graph with concentration versus peak responses and relative response factors were derived by comparing slope of individual impurity with slope of telmisartan standard peak. The recovery studies were evaluated in accuracy parameter by injecting triplicate preparations at three levels (LOQ and 100%, 150% of specification level) as given in (Figure 5). The accuracy of the method was predicted by calculating % recovery and %RSD for all the 11 impurities. The method precision and intermediate precision were evaluated by injecting six spiked sample preparations at 100% specification level and the %RSD, cumulative %RSD of each impurity content was calculated. Intermediate precision of analytical method was evaluated by different analyst by injecting six spiked sample preparations using different instrument in different day and the above results were tabulated in (Table 2). Small deliberate changes were made in chromatographic conditions to study the robustness of the method. The flow rate of mobile phase was altered by ±0.1 mL/minute from ideal condition and the ideal condition was 0.8 mL/minute and it was altered to 0.7 and 0.9 mL/minute. The column oven temperature was altered by ±2 °C from ideal condition, Ideal condition was 25 °C and it was altered to 27 °C and 23 °C. The robustness study results were tabulated in (Table 3).

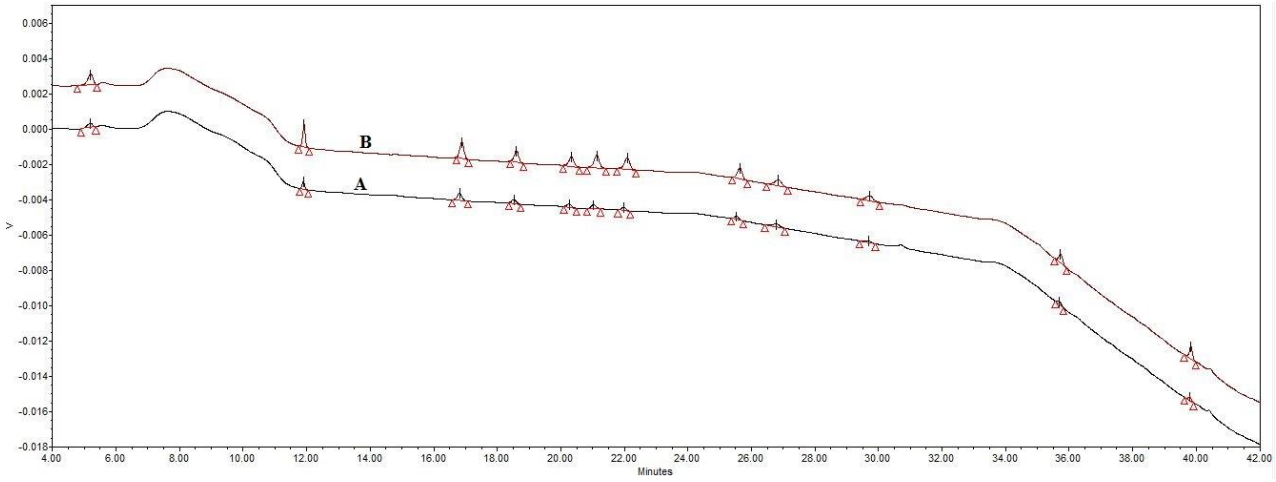


Figure S6. Zoomed chromatogram of telmisartan and its related impurities at (A) LOD and (B) LOQ

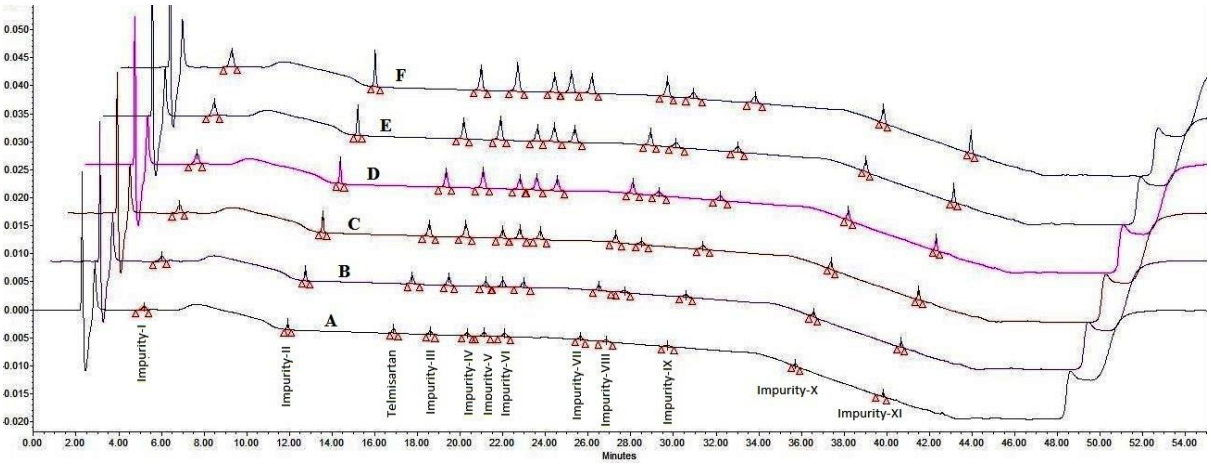


Figure 4. Chromatogram of Telmisartan and its related impurities at linearity (A) Linearity at LOQ level (B) Linearity at 50% level (C) Linearity at 80% level (D) Linearity at 100% level (E) Linearity at120% level (F) Linearity at 150% level

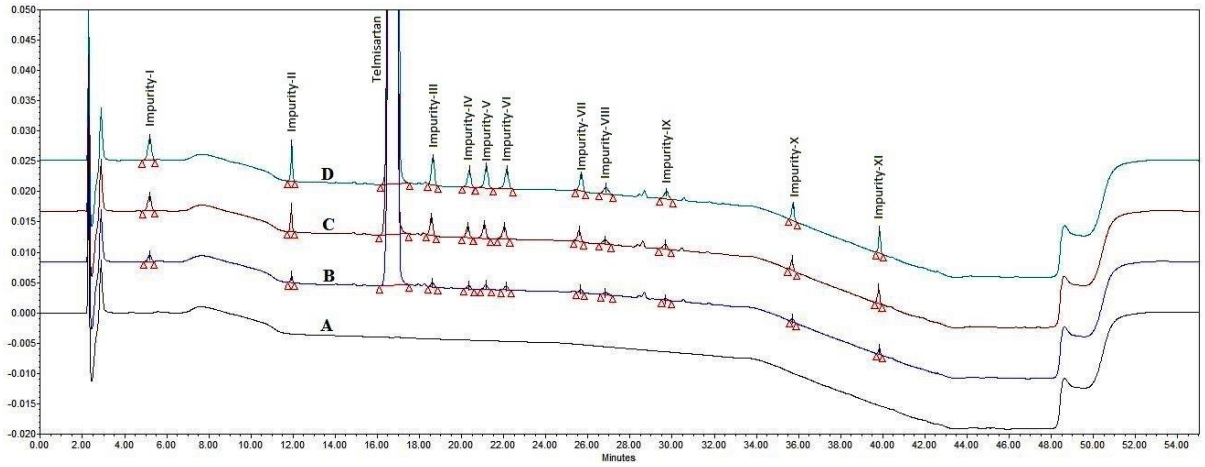


Figure 5. Chromatogram of Telmisartan and its related impurities at Accuracy (A) Blank (B) Accuracy at LOQ level (C) Accuracy at 100% level (D) Accuracy at 150% level

Table 2. Validation summary report of analytical method

Test parameter	Component name	Impurity-I	Impurity-II	Telmisartan	Impurity-III	Impurity-IV	Impurity-V
System suitability	Retention time (min)	5.44	12.11	16.97	18.89	20.63	21.44
	Resolution	-	23.27	15.94	5.32	5.43	2.53
	Sensitivity (EP s/n)	-	-	101	-	-	-
	Standard deviation	-	-	335.420	-	-	-
	% RSD	-	-	1.19	-	-	-
Specificity	Purity angle	9.237	7.912	9.615	6.938	12.565	10.277
	Purity threshold	16.272	13.162	14.855	12.629	22.946	16.586
DL, QL & Linearity	Correlation co-efficient	0.999	1.000	0.999	1.000	1.000	1.000

	Slope	163632	223812	242377	224593	217638	264152
	% y-intercept	-4.36	1.65	4.74	-2.70	-0.36	1.87
	Detection limit (% w/w)	0.017	0.010	0.010	0.010	0.010	0.010
	Quantitation limit (% w/w)	0.050	0.030	0.031	0.030	0.029	0.030
	Standard deviation at quantitation level	293.241	16.342	328.279	150.096	161.320	211.790
	Precision % RSD at Quantitation level (n=6)	4.20	0.22	3.84	2.27	2.66	2.45
	Standard deviation at 150 % level	1002.725	304.075	467.130	566.253	599.921	245.820
	Precision % RSD at 150 % level (n=6)	2.79	0.89	1.23	1.14	1.87	0.60
	RRF	0.68	0.92	1.00	0.93	0.90	1.09
Accuracy at LOQ level	Amount added (% w/w)	0.049	0.029	-	0.029	0.029	0.030
	Amount recovered (% w/w)	0.056	0.033	-	0.029	0.031	0.034
	% Recovery	112.60	113.14	-	98.34	107.61	114.18
Accuracy at 100% level	Amount added (% w/w)	0.147	0.098	-	0.147	0.096	0.099
	Amount recovered (% w/w)	0.156	0.104	-	0.133	0.099	0.107
	% Recovery	105.88	105.79	-	90.90	103.33	108.24
Accuracy at 150% level	Amount added (% w/w)	0.220	0.146	-	0.218	0.143	0.148
	Amount recovered (% w/w)	0.236	0.156	-	0.204	0.153	0.161
	% Recovery	107.42	106.82	-	93.55	106.86	109.36
Precision	Method precision (n=6) standard deviation	0.00546	0.0081	-	0.00105	0.00197	0.00128
	Method precision (n=6) % RSD	3.02	0.78	-	0.75	1.95	1.17
	Intermediate precision (n=6) standard deviation	0.00532	0.00064	-	0.00129	0.00123	0.00444
	Intermediate precision (n=6) % RSD	2.73	0.60	-	0.89	1.24	4.35
	Ruggedness standard deviation	0.00914	0.00167	-	0.00270	0.00170	0.00490
	Ruggedness % RSD	4.86	1.58	-	1.90	1.68	4.67

Table 2. Validation summary report of analytical method (Continued)

Test parameter	Component name	Impurity-VI	Impurity-VII	Impurity-VIII	Impurity-IX	Impurity-X	Impurity-XI
System suitability	Retention time (min)	22.40	25.92	27.05	29.90	35.88	39.99
	Resolution	2.81	11.13	3.30	7.91	18.01	17.36
	Sensitivity (EP s/n)	-	-	-	-	-	-
	% RSD	-	-	-	-	-	-
Specificity	Purity angle	10.082	10.689	12.192	23.278	10.394	8.190
	Purity threshold	18.431	19.424	22.207	44.082	18.373	14.047
DL, QL & Linearity	Correlation co-efficient	0.999	0.999	0.995	0.999	1.000	1.000
	Slope	246701	207249	107568	121029	157647	151909
	% y-intercept	1.39	-0.57	-2.52	1.05	-1.44	-0.46
	Detection limit (% w/w)	0.010	0.010	0.017	0.010	0.010	0.010
	Quantitation limit (% w/w)	0.029	0.031	0.051	0.031	0.030	0.031
	Standard deviation at quantitation level	293.241	216.234	208.444	274.600	62.217	158.268
	Precision % RSD at quantitation level (n=6)	4.30	3.18	3.78	6.84	1.37	3.25
	Standard deviation at 150 % level	445.864	197.989	155.779	249.313	201.769	171.487
	Precision % RSD at 150 % level (n=6)	1.21	0.62	0.94	1.30	0.86	0.74
	RRF	1.02	0.86	0.44	0.50	0.65	0.63
Accuracy at LOQ level	Amount added (% w/w)	0.029	0.030	0.051	0.030	0.029	0.030
	Amount recovered (% w/w)	0.031	0.026	0.052	0.029	0.030	0.033
	% Recovery	107.72	86.64	102.30	93.88	103.20	110.18
Accuracy at 100% level	Amount added (% w/w)	0.096	0.101	0.101	0.101	0.097	0.100
	Amount recovered (% w/w)	0.099	0.091	0.099	0.103	0.102	0.105
	% Recovery	102.92	90.01	98.57	101.37	105.56	105.01
Accuracy at 150% level	Amount added (% w/w)	0.143	0.151	0.150	0.150	0.144	0.148
	Amount recovered (% w/w)	0.156	0.145	0.146	0.151	0.158	0.161
	% Recovery	108.66	96.25	97.88	100.10	109.55	108.29

Precision	Method precision (n=6) standard deviation	0.00141	0.00081	0.00227	0.00222	0.00084	0.00067
	Method precision (n=6) % RSD	1.41	0.88	2.06	2.16	0.82	0.58
	Intermediate precision (n=6) standard deviation	0.00142	0.00091	0.00161	0.00161	0.00111	0.00301
	Intermediate precision (n=6) % RSD	1.43	0.97	1.56	1.66	1.05	2.55
	Ruggedness standard deviation	0.00144	0.00129	0.00441	0.00368	0.00203	0.00259
	Ruggedness % RSD	1.44	1.39	4.13	3.68	1.93	2.23

Table 3. Summary report of robustness data

Resolution						
Component name	**Flow increase (0.9 mL/minute)**	**Flow decrease (0.7 mL/minute)**	**Column oven temperature increase (27°C)**	**Column oven temperature decrease (23°C)**	**Column Lot-1**	**Column Lot-2**
Impurity-I	NA	NA	NA	NA	NA	NA
Impurity-II	25.28	22.45	21.61	19.62	23.97	20.43
Telmisartan	18.74	19.40	18.9	18.88	18.63	18.66
Impurity-III	5.89	6.06	5.77	5.8	5.94	5.75
Impurity-IV	5.77	6.11	6.43	7.6	6.01	7.05
Impurity-V	2.84	2.59	2.92	1.55	2.57	2.18
Impurity-VI	3.07	3.08	3.65	3.41	3.00	3.52
Impurity-VII	11.08	10.61	11.58	11.51	11.62	11.87
Impurity-VIII	1.27	4.55	3.15	5.56	2.76	4.37
Impurity-IX	7.63	7.33	8.59	8.14	6.88	8.11
Impurity-X	20.02	17.82	21.53	22.01	21.53	21.7
Impurity-XI	19.37	17.49	22.59	22.25	19.35	22.36

Forced degradation studies were performed to prove the method is stability indicating. The solid state degradation was performed as per ICH guidelines [17, 18]. The photolytic degradation study was carried out by exposing telmisartan sample to 1.2 million lux hours and 200 Wh/m2, thermal degradation sample was exposed at 70 °C for 24 hours. The temperature and humidity degradation study was carried out by exposing telmisartan at 60 °C and 80% RH for 48 hours. The degraded samples were analysed for related substances, assay and found that telmisartan was not showing significant degradation in above stated conditions.

The liquid state degradation was also performed under acidic, alkaline and oxidative conditions. The acidic degradation of telmisartan was studied in hydrochloric acid (0.1 N, methanolic) at 40 °C for 6 hours and alkaline degradation was studied in sodium hydroxide (0.1 N, methanolic) at 40 °C for 6 hours and the samples were cooled to room temperature and, neutralized and dilute to volume with methanol. Oxidation degradation was studied in 30% H_2O_2 for 24 hours at room temperature and then diluted to volume with methanol. The samples were analyzed for related substances, assay and found that no significant degradation observed under acidic, alkaline and oxidative degradation conditions. Mass balance of the degradation conditions were established and tabulated in (Table 4).

Table 4. Summary report of forced degradation and mass balance

Condition	Total impurities (% w/w) in degradation sample	Assay (% w/w) of degradation sample	Mass balance
Acid degradation, 0.1 N methanolic HCl, at 40°C for 6 hours	3.75	95.8	99.55
Base degradation, 0.1 N methanolic NaOH, at 40°C for 6 hours	0.19	99.2	99.39
Oxidative degradation, 3%v/v H_2O_2, at room temperature for 24 hours	0.45	98.2	98.65
Photolytic degradation, 1.2 million lux hours and 200 WH/m^2	0.22	98.7	98.92
Thermal degradation, at 70°C for 24 hours	0.20	99.0	99.20
Temperature & humidity degradation, at 60°C and 80% RH	0.19	98.9	99.09

Result and Discussion

Optimization of chromatographic conditions

Telmisartan and related impurities are basic as well as polar in nature. As the part of method development, our main goal was to achieve symmetrical peak shapes and baseline separation amongst telmisartan and the related impurities.

Initial trials were conducted with process related and pharmacopieal impurities by using United States and European pharmacopieal HPLC methods on Kromasil 100-5 C18 with dimension (125×4.0) mm, 5 µm [8, 9]. We observed the co-elution of Impurity-III with Impurity-VII, and Impurity-II with Impurity-VIII (Figure S1. and Figure S2). In order to get rid of the co-elutions, the pharmacopeial methods were modified by changing gradient as well as composition of mobile phase. Nevertheless separations of above impurity peaks were not obtained. The pharmacopeial HPLC methods rely on ion pair reagents which suffers from short life time of column and extra time

for column equilibration. In order to avoid ion pair reagents in mobile phase, trials using phosphate buffer were performed. However, separation between co-eluted impurity peaks were not found.

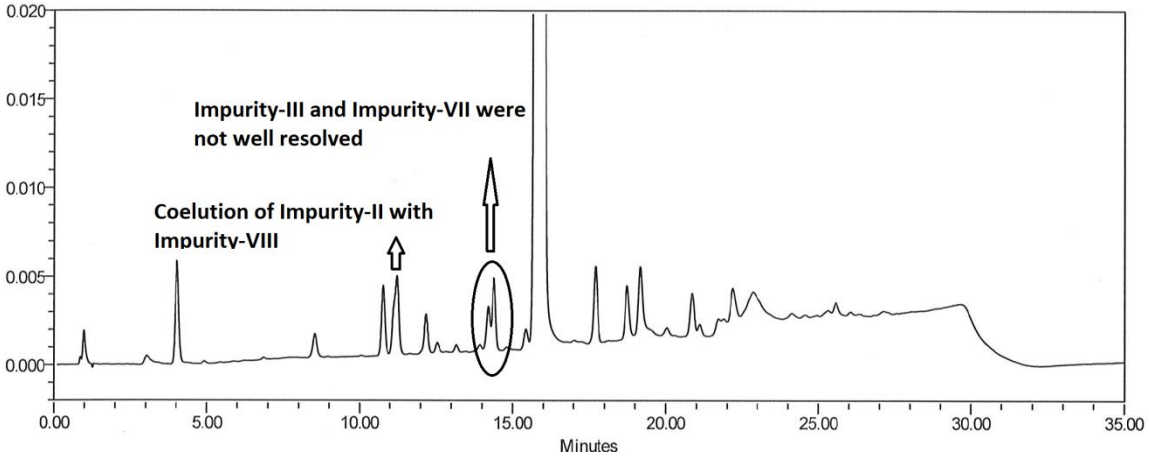


Figure S1. Spiked sample solution as per European pharmacopoeia method

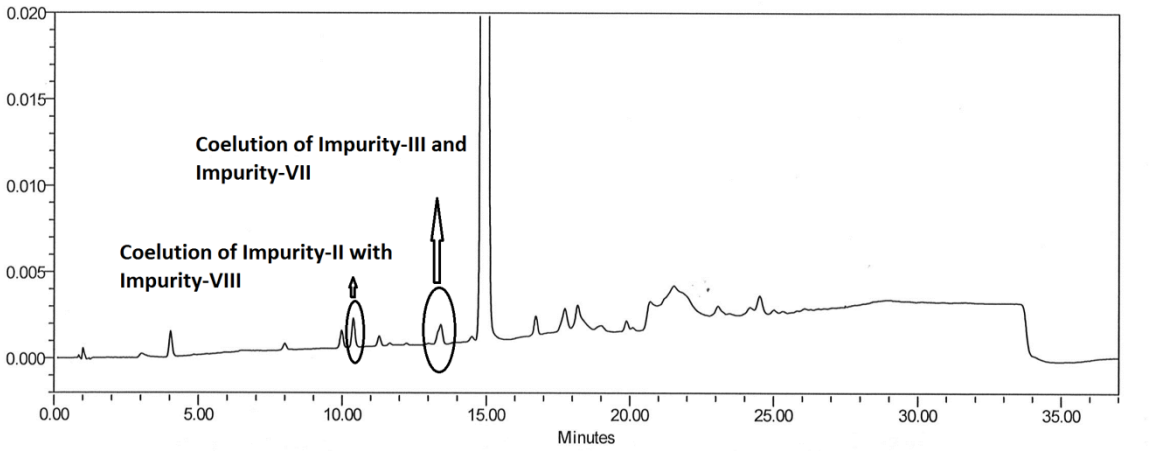


Figure S2. Spiked sample solution as per United States pharmacopoeia method

Further trials were conducted based on chromatographic conditions reported by R. Nageswara Rao et al. [8]. The spiked sample solution was injected on Lichrosphere RP-18 column (250×4.6) mm, 5 µm using a solution containing 0.1% (v/v) triethylamine and 20 mM ammonium acetate adjusted to pH 3.0 with trifluoroacetic acid as mobile phase-A and acetonitrile as mobile phase-B. The co-elution of Impurity-II with Impurity-VIII, Impurity-VI with Impurity-VII and Impurity-X with telmisartan was observed (Figure S3). Interference of blank peaks with known peak was also observed. It may be accounted on the basis of blank interferences of ammoniuam acetate at lower wavelengths <260 nm. Therefore, ammonium acetate buffer was also excluded from mobile phase.

After unsuccessful attempts to achieve the separation among the process related and pharmacopeial impurities by using reported HPLC methods, well separation was attained with core

shell column (Poroshell EC 18 with dimension (150×4.6) mm, 2.7 µm). Gradient programme using mobile phase-A as 0.1% (v/v) triethylamine and 10 mM sodium hydrogen phosphate mono hydrate adjusted to pH 3.0 with orthophosphoric acid, and mobile phase-B as acetonitrile. It was found that peak shape of telmisartan was poor, however, resolution between all the impurities and telmisartan were satisfactory [Figure S4]. To improve the peak shape, mobile phase-A was replaced with 0.05% (v/v) trifluoroacetic acid. Pleasantly, symmetric peak shape of telmisartan was achieved but unknown peak was observed in tailing (Figure S5). In continuation with the above trial, symmetry shield RP 8 (150×4.6) mm, 3.5 µm column was employed due to polar embedded group (i.e. amide group). This column has a tendency to improve peak shapes by reducing silanol activity due to the embedded polar group close to the silica surface. This imparted the required selectivity for the separation of all the impurities and with acceptable resolution (Figure 3).

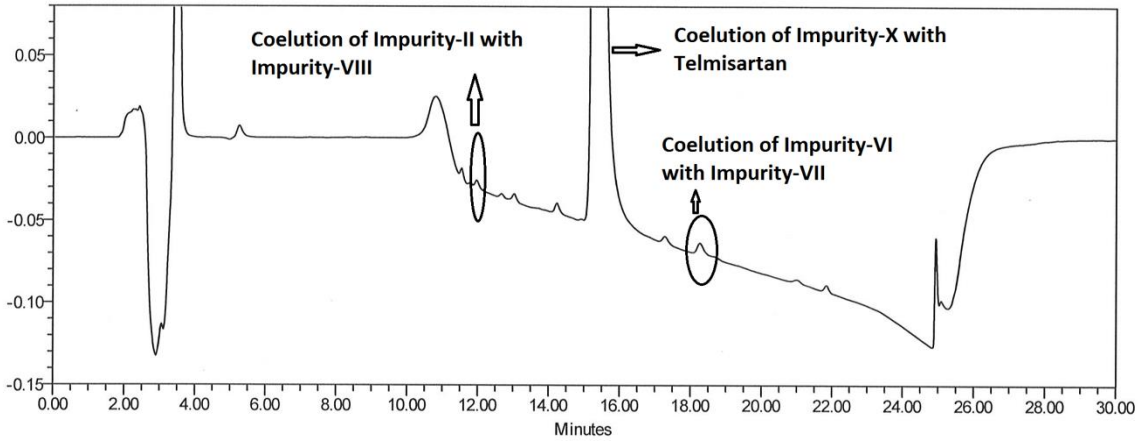


Figure S3. Spiked sample solution as per R. Nageswara Rao et al., method

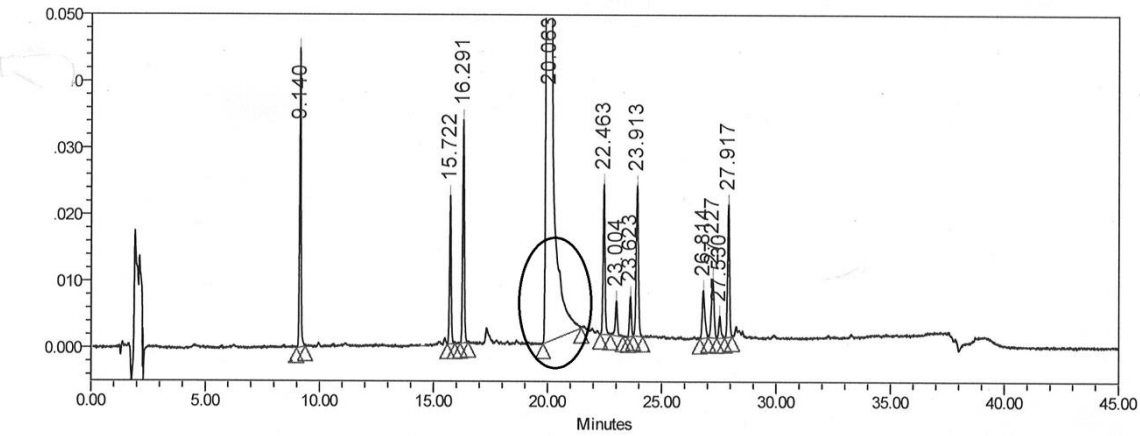


Figure S4. Mobile Phase-A: 10mM in Sodium dihydrogen phosphate monohydrate consisting 0.1% (v/v) triethylamine in water. Adjusted pH 3.5 with orthophosphoric acid. Mobile phase-B: Acetonitrile, Column: Poroshell EC 18 (150*4.6) mm, 2.7 µm, Elution mode: Gradient

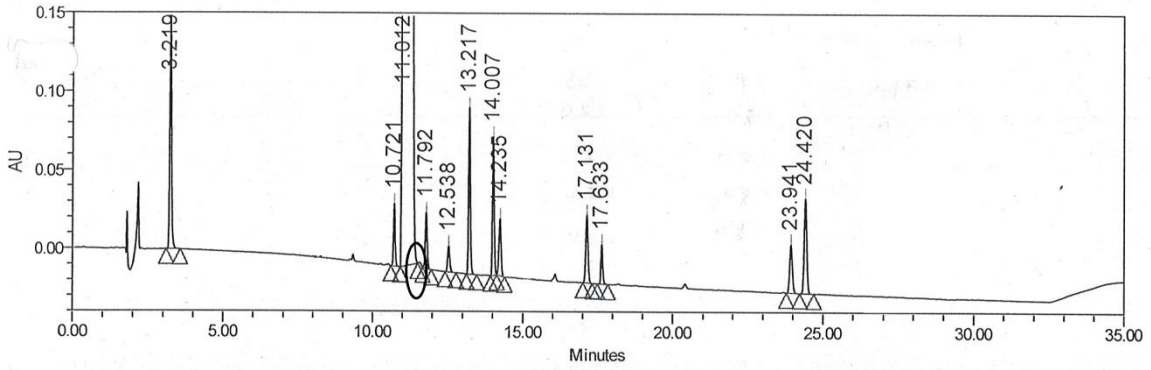


Figure S5. Mobile Phase-A: 0.05% (v/v) trifluoroacetic acid. Mobile phase-B: Acetonitrile, Column: Poroshell EC 18 (150*4.6) mm, 2.7 µm, Elution mode: Gradient

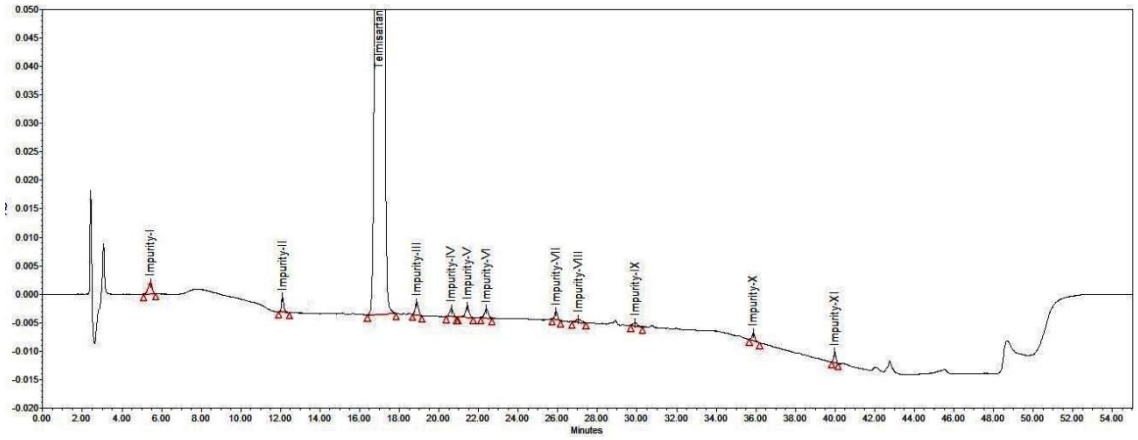


Figure 3. Spiked sample solution chromatogram in optimized method

The method was finalized on Symmetry shield RP 8 (150×4.6) mm, 3.5 µm column in binary gradient mode by using 0.05% (v/v) trifluoroacetic acid as mobile phase-A and acetonitrile as mobile phase-B. The separation was achieved with flow rate of 0.8 mL/minute, 10 µL of injection volume, the column oven temperature was maintained at 25 °C and the eluents were monitored at 230 nm.

Conclusion

A simple gradient RP-HPLC method was developed and validated to resolve all the eleven impurities from telmisartan drug substance. The method was found to be precise, specific, accurate, linear, robust and stability indicating, and it was found to be suitable for its intended purpose. The method can be used for the determination of related substances in telmisartan drug substance and to assure the quality of telmisartan.

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