

Sensitive and selective analytical method for the quantification of two potential Genotoxic impurities in Azilsartan drug substance by using LC-MS/MS with Multiple Reaction Monitoring(MRM mode)

Abstract

The main aim of the present study to Synthesize, method development and method validation for quantification of two potential genotoxic impurities i.e., Methyl(Z)-2-ethoxy-1-((2'-(N'-hydroxycarbamimidoyl)-[1,1'-biphenyl]-4-yl) methyl)-1H-benzo[d]imidazole-7-carboxylate (Impurity-A) and 2-ethoxy-1-((2'-(N'-hydroxycarbamimidoyl)-[1,1'-biphenyl]-4-yl)methyl)-1H-benzo[d]imidazole-7-carboxylic acid (Impurity-B) by using LC-MS/MS MRM mode at trace level determination in Azilsartan drug substance. The new LC-MS/MS MRM mode method was developed by using Inertsil ODS-3V 150x4.6mm, 5 μ m column as stationary phase. The mobile phase used is the composition of 10Mm Ammonium formate pH3.00 buffer: Acetonitrile (9:1) % v/v as mobile phase-A and 10Mm Ammonium formate pH3.00 buffer: Acetonitrile (2:8) % v/v as mobile phase-B, isocratic elution of mobile phase-A and Mobile phase-B (60:40) v/v at flow rate of 0.8 mL/min. The concentration limits of the both genotoxic impurities were calculated a limit of 37.5 ppm based on the concept of TTC (threshold of toxicological concern) and MDD (maximum daily dosage which is 40 mg/day for Azilsartan drug substance). The limit of detection (LOD) was found to be 1.4 ppm for both Impurity-A and Impurity-B. The limit of quantification (LOQ) for Impurity-A was 4.7 ppm and Impurity-B was 4.5 ppm respectively. The method was found to be linear from 4.7 ppm to 78.4 ppm for Impurity-A (correlation coefficient: 1.000) and 4.5 ppm to 75.7 ppm for Impurity-B (correlation coefficient: 0.999). The method was precise and found percentage of relative standard deviation for six replicate sample preparations of Impurity-A and Impurity-B was below 5.0%. The method accuracy was confirmed based on the recovery studies. Based on the method validation study method was sensitive and selective for quantification both genotoxic impurities.

Keywords: LC-MS/MS, MRM (Multiple reaction monitoring, TTC (Threshold of toxicological concern), MDD (Maximum daily dosage), Azilsartan, validation

1. Introduction

Azilsartan, Chemical name 2-ethoxy-1-((2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-[1,1'-biphenyl]-4-yl)methyl)-1H-benzo[d]imidazole-7-carboxylic acid, which is used to treat high blood pressure (hypertension). Azilsartan belongs to a class of drugs called angiotensin receptor blockers (ARBs).

Impurity-A used as a key starting material for synthesis of Azilsartan drug substance. Impurity-B will arise during synthesis of Azilsartan drug substance. ROS of Azilsartan shown in figure-1. Structure and chemical name of Impurity-A and Impurity-B shown in figure-2 and figure-3 respectively. Impurity-A and Impurity-B confirmed as a potential genotoxic impurities based on the available literature [1-7]. The presence of trace level impurities present in the drug substance or drug product may potentially cause severe harmful effects on human health. The concentration limit of genotoxic impurities, Impurity-A and Impurity-B has been calculated based on TTC [1-7] and maximum daily dose [1-7]. So Impurity-A and Impurity-B each must to be controlled at below 37.5ppm.

Literature survey show some work related to Azilsartan and Azilsartanmedoxomil [8-15] related substances by using high performance liquid chromatographic methods. So the accurate quantification of Impurity-A and Impurity-B at ppm levels the above Literature methods are inadequate. Literature survey reveals that there was no sensitive and selective method available for the quantification of Impurity-A and Impurity-B by using LC-MS/MS Multiple reaction monitoring (MRM) mode. . So, the objective of this work is to develop and validate a highly Sensitive, accurate and selective LC-MS/MS MRM mode method developed and validated for the determination of trace level quantification of Impurity-A and Impurity-B in Azilsartan drug substance.

Quantitative structure-activity relationship (QSAR) analysis carried out for all the raw materials, reagents, intermediate, impurities and reagents used in the process of Azilsartan drug substance to identify the Mutagenic impurities. We have found the Impurity A and Impurity B are mutagenic due to certain electrophilic moieties within a chemical structure. Both compounds are mutagenic as well as DNA-reactive. i.e. Impurity-A is intermediate of Azilsartan and Impurity-B is process impurity due to hydrolysis of intermediate. Following illustration below shows.

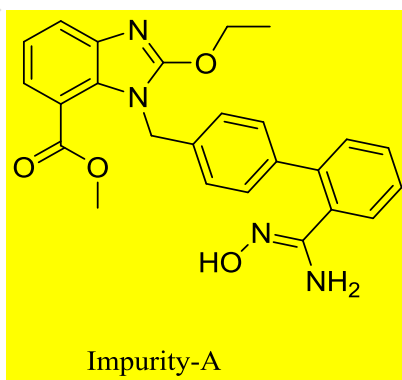


Figure 1 : Structure of Methyl(Z)-2-ethoxy-1-((2'-(N'-hydroxycarbamimidoyl)-[1,1'-biphenyl]-4-yl)methyl)-1H-benzo[d]imidazole-7-carboxylate (Impurity-A)

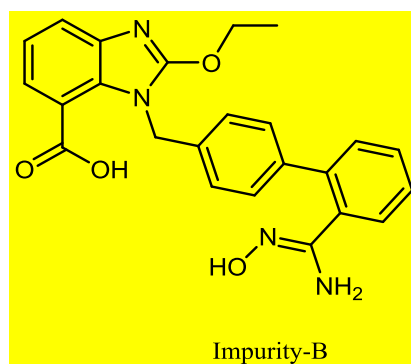


Figure 2 : Structures of 2-ethoxy-1-((2'-(N'-hydroxycarbamimidoyl)-[1,1'-biphenyl]-4-yl) methyl)-1H-benzo[d]imidazole-7-carboxylic acid (Impurity-B)

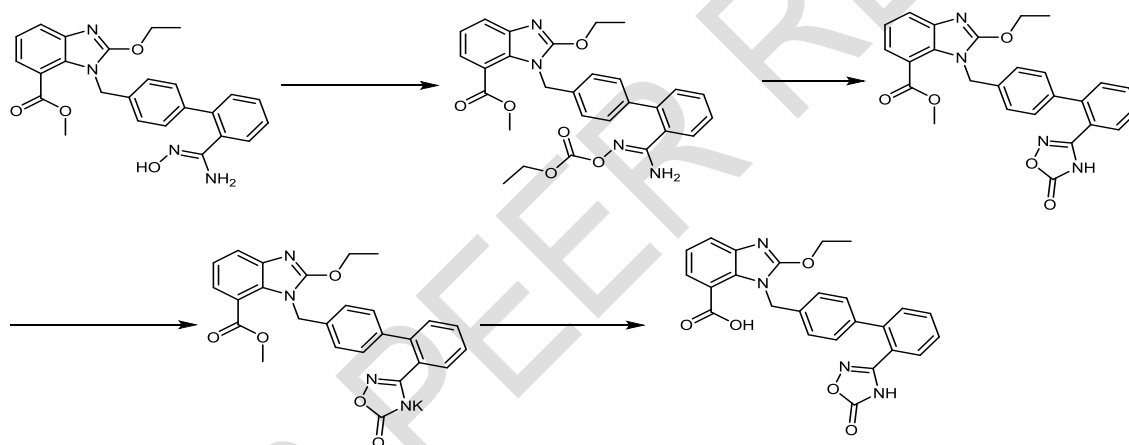


Figure 3 : synthetic route of Azilsartan drug substance

2. Materials and methods

Azilsartan samples were obtained as a gift samples from reputed pharma company upon request. All chemicals were purchased from Sigma Aldrich and used directly. Reaction progress was monitored by thin-layer chromatography (TLC) using silica gel/aluminum sheets (60F-254) and UV light. ¹H nuclear magnetic resonance (NMR) spectra were recorded on BrukerAvance 400 MHz spectrometer with tetramethylsilane (TMS) as an internal standard. Splitting patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), or doublet of doublet (dd) and multiplet (m). The broad (br) signals were also indicated. The value of chemical shifts (δ) is given in ppm and coupling constants (J) in Hertz (Hz). Mass spectra were obtained using waters XEVO TQ LCMS instrument was used with an electrospray(ESI) positive and negative ionization modes.

2.1 Synthesis of Methyl(Z)-2-ethoxy-1-((2'-(N'-hydroxycarbamimidoyl)-[1,1'-biphenyl]-4-yl)methyl)-1H-benzo[d]imidazole-7-carboxylate (Impurity-A): To the stirred solution of Hydroxylamine hydrochloride (16.8g ,0.24mmol) and Sodium bicarbonate (30.6g ,0.36mmol) in Dimethyl sulfoxide (100 mL) heated to 50°C for 1-2h, Methyl 1-((2'-cyano-[1,1'-biphenyl]-4-yl)methyl)-2-ethoxy-1H-benzo[d]imidazole-7-carboxylate (10g, 0.024mmol) was added slowly at 50°C. The reaction mixture was stirred for 12h at 80°C and then further stirred at 40 °C. The reaction progress was monitored by TLC. The inorganic solids were filtered, and the obtained filtrate was poured into water (100 mL) and then stirred for 1 h. The obtained solid was filtered, and washed with water (50 mL) and dried under reduced pressure. The purified compound was then obtained by crystallization in Methanol to afford compound (A). White colour solid, yield:70%, ¹H NMR (400 MHz, DMSO-d₆) δ 9.16 (s, 1H, OH), 7.69 (dd J=8 Hz and 1Hz, 1H ArH), 7.38 (m, 6H, ArH), 7.29 (dd, J=8 Hz and 1Hz, 1H ArH), 7.19 (t, 1H, ArH), 6.94 (d, J=8 Hz ,2H, ArH), 5.52 (m, 2H, CH₂), 5.51 (m, 2H, NH₂), 4.62 (q, 2H, CH₂), 3.72 (s, 3H, OCH₃), 1.42 (t, 3H, CH₃). MS (m/z): Calculated mass for C₂₅H₂₄N₄O₄ and measured mass for Impurity-A [M+H]⁺:445.21.

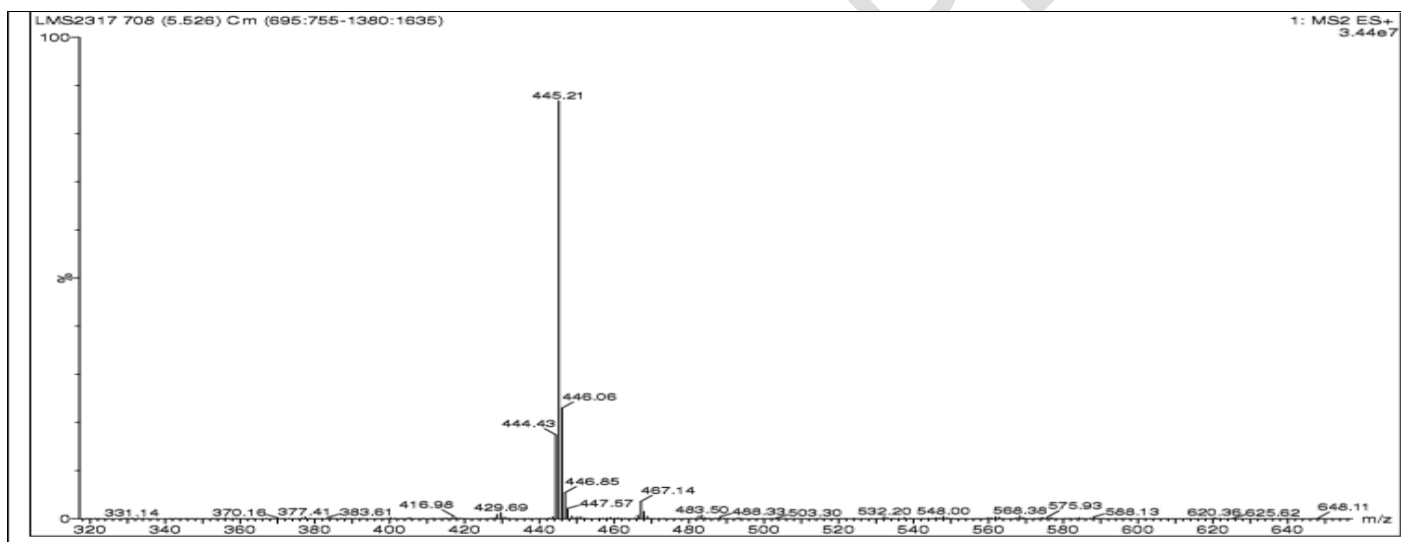


Figure 4 Mass spectra of Impurity-A: [M+H]=445.21

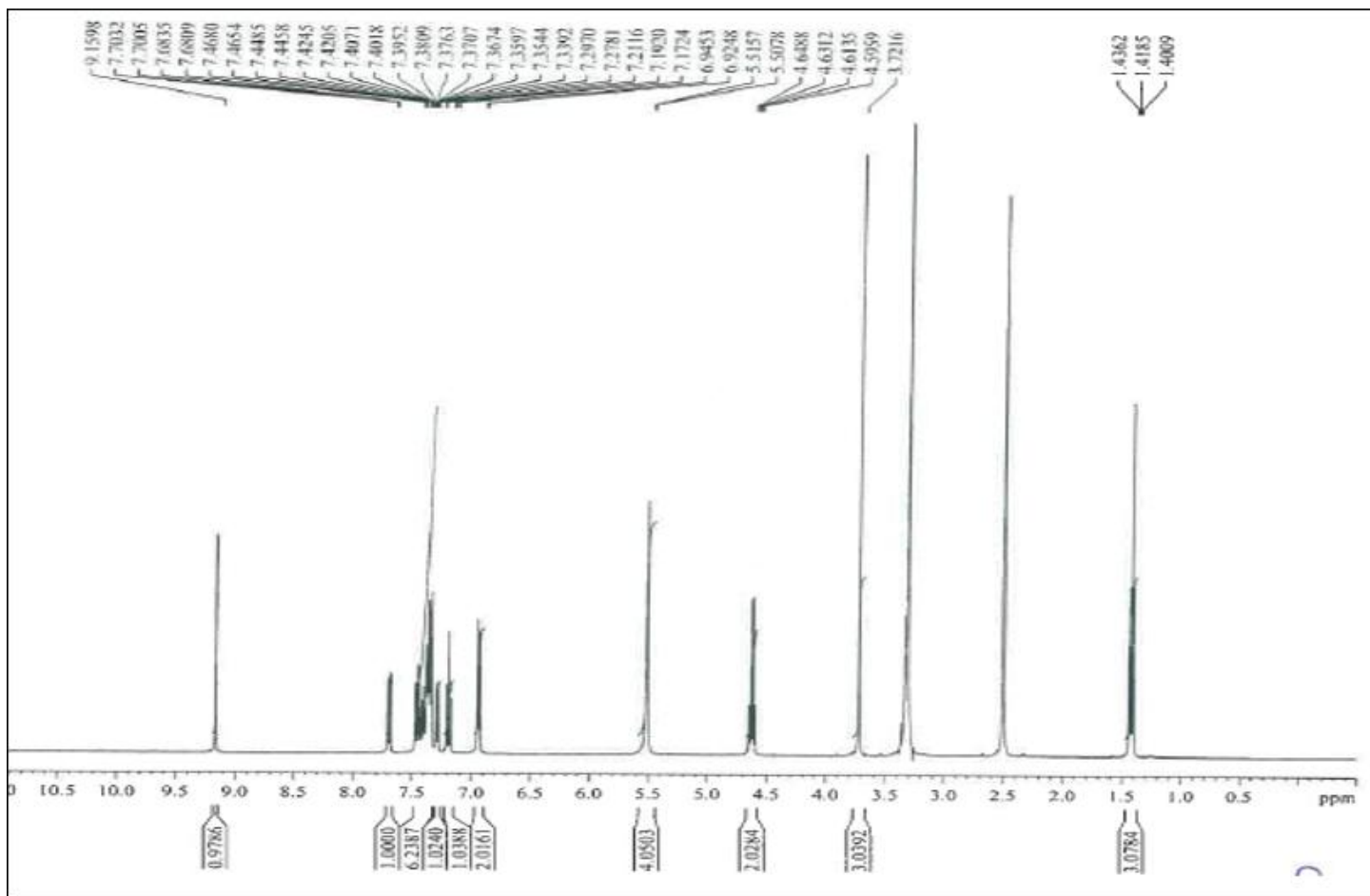


Figure 5: ^1H NMR Spectrum of Impurity-A

2.2 Synthesis of 2-ethoxy-1-((2'-(N'-hydroxycarbamimidoyl)-[1,1'-biphenyl]-4-yl)methyl)-1H-benzo[d]imidazole-7-carboxylic acid (Impurity-B): To the stirred solution of Impurity-A (10g ,0.22mmol) and Sodium hydroxide (2.7g ,0.067 mmol) in Methanol (50 mL) and water (50 mL) mixture , The reaction mixture was stirred for 5h at 60°C. The reaction progress was monitored by TLC then cooled to 30 °C. The obtained reaction mass was poured into water (100 mL) and Hydrochloric acid (15mL) then stirred for 1 h , the obtained solids were filtered, and dried under reduced pressure to to afford compound (B). White colour solid, yield:85%, ^1H NMR (400 MHz, DMSO- d_6) δ 9.17 (s, 1H, COOH), 7.65 (d, 1H, ArH), 7.53(d, 1H, ArH), 7.38 (m, 6H, ArH), 7.17 (t, 1H ArH), 7.01 (d, 2H, ArH), 5.66 (s, 2H, CH₂), 5.55 (s, 2H, NH₂) 4.61 (q, 2H, CH₂), 1.42 (t, 3H, CH₃). MS (m/z): Calculated mass for C₂₄H₂₂N₄O₄ and measured mass for Impurtiy-B [M+H]⁺:431.07.

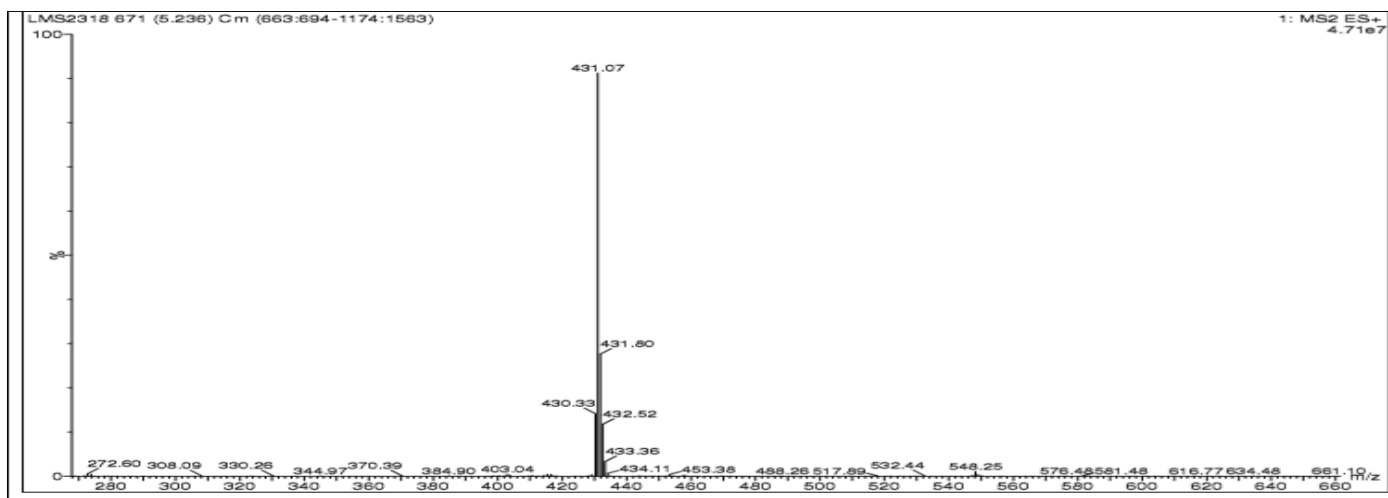


Figure 6 Mass spectra of Impurity-B: $[M+H]^+ = 431.07$

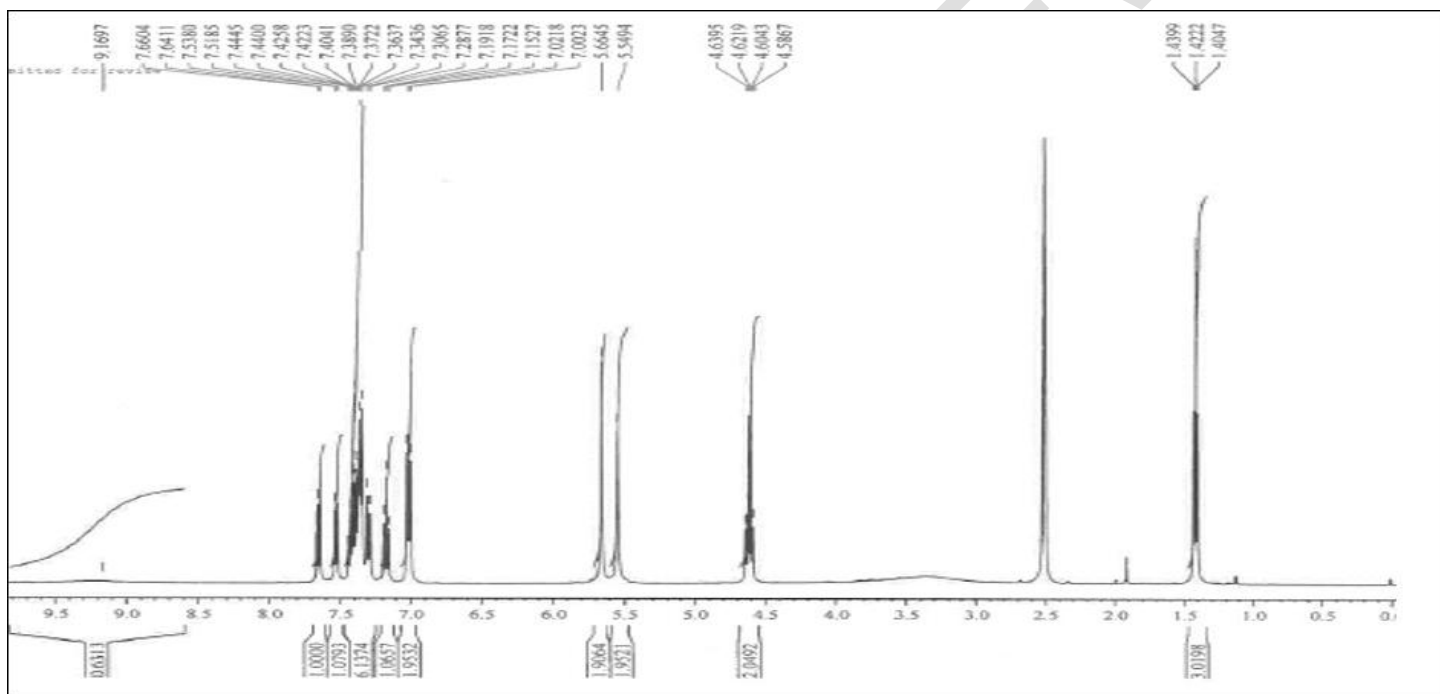


Figure 7 1H NMR Spectrum of Impurity-B

2.3 Method development and optimization

The objective of LC-MS/MS in this study to develop a sensitive, selective and Accurate method for quantification of Impurity-A and Impurity-B in Azilsartan drug substance. Different acidic mobile phases such as formic acid, trifluoroacetic acid, difluoroacetic acid mix with organic modifiers such as Acetonitrile and methanol isocratic mode elution have been tested. Different stationary phases like C18, C8 and Phenyl HPLC columns has been tested and found Inertsil ODS-3V(150x4.6mm), 5 μ m has been (Make: GL Sciences, Japan)

separation of Impurity- A, Impurity-B and Azilsartan drug substance. Gaussian curve peak shapes observed in Ammonium formate mobile phase pH=3.00, pre-mix with Acetonitrile in the isocratic mode elution with flow rate 0.8mL/min. Finalised mobile phase conditions pre mix of 10Mm Ammonium formate buffer pH=3.00:Acetonitrile(90:10)% v/v as Mobile phase-A and pre mix of 10Mm Ammonium formate buffer pH=3.00:Acetonitrile(20:80)% v/v as Mobile phase-B. Isocratic elution of Mobile phase-A: Mobile phase-B(60:40)% v/v found good sensitivity and separation of Analytes.

MS/MS Conditions optimization started with electron spray ionization(ESI) source in positive mode. MRM (multiple reaction monitoring) mode showed higher sensitivity than SIR(Single ion recording) mode. MRM (multiple reaction monitoring) mode had greater advantage to improve sensitivity due to both parent ion and daughter ions are monitored at a time when compared to SIR (single ion recording), here only parent ion only studied. Injected standard solution of Impurity-A, observed its $[M+H]^+$ at m/z 445.35 and further MS/MS fragmentation with collision energy 30eV found stable daughter ions with higher sensitivity at m/z 207.15 and 225.18 respectively. Similarly injected standard solution of Impurity-B observed its $[M+H]^+$ at m/z of 431.33 and further MS/MS fragmentation with collision energy 20eV found stable daughter ions with higher sensitivity at m/z values 207.24 and 225.16 respectively.

2.4 Methodology

LC-MS grade of Ammonium formate and formic acid from sigma-Aldrich. LC-MS grade Acetonitrile from Fisher chemicals. Purified water collected from Mill-Q plus water purification system. The method development and method validation was performed in Water's Acquity UPLC H-Class connected to Xevo TQ MS/MS detector. The data were collected and processed using Mass lynx software.

2.4.1 Mobile phase preparation: Preparation of Buffer solution: Weighed about 0.63g of Ammonium formate salt and dissolved in 1000mL of water and adjusted pH 3.00 ± 0.05 with formic acid. Preparation of Mobile phase-A: Buffer: Acetonitrile (90:10) % v/v, Preparation of Mobile phase-B: Buffer: Acetonitrile (20:80) % v/v
Diluent for samples and standard preparation: Acetonitrile: Water (50:50) % v/v

2.4.2 Preparation of standard solution: Weighed about each 20mg of Impurity-A and Impurity-B and transferred in 100mL volumetric flask and dissolved with diluents (Stock-1). Pipette out 1.0mL from Stock-1 into 100mL volumetric flask and make up to the mark with diluents (Stock-2). Further transferred 1.0mL of stock-2 solution into 100mL volumetric flask and make up to the mark with diluent (Standard solution).

Preparation of sample solution: Weighed about 50mg of Azilsartan drug substance sample transferred into 100mL volumetric flask, dissolved and make up to the mark with diluent.

2.4.3 LC-MS/MS Operating Conditions: Experimentation performed using Water's Acquity UPLC H-Class connected to Xevo-TQ MS/MS detector with ESI Source (Electron spray ionization). Inertsil ODS-3V (150x4.6mm), 5 μ m column used to separate the Impurity-A, Impurity-B and Azilsartan. Chromatographic method developed using isocratic mode of elution with Mobile phase-A and Mobile phase-B (60:40)% v/v with a flow rate of 0.8mL/min and a runtime of 10minutes for standards solution and 25mins runtime for samples solution. Column oven temperature maintained at 30°C and auto sampler temperature maintained at 10°C with an injection volume of 5 μ L.

A triple quadrupole MS equipped with a positive electron spray ionization (ESI) source was used in the MRM mode. The equipment was set with a Capillary voltage 3.2kV, Cone voltage 20V, Source temperature 150°C, Desolvation temperature 600°C, Desolvation Gas flow 850L/hr.

Table 1. Impurity-A and Impurity-B MRM transitions

S.No	Analyte	Parent(m/z)	Daughter (m/z)	Dwell(s)	Collision Energy(eV)
1	Impurity-A	445.21	207.12	0.078	30
			225.14	0.078	30
2	Impurity-B	431.07	207.12	0.078	20
			225.10	0.078	20

2.5 Method validation:

2.5.1 Specificity:

The specificity of the method was verified by injecting the individual impurity standards Impurity-A and Impurity-B each at about 37.5ppm level with respect to 0.5mg/mL analyte concentration, azilsartan drug substance at 0.5mg/mL, Spiked sample solution of Azilsartan drug substance containing Impurity-A and Impurity-B.

2.5.2 Sensitivity:

The Limit of detection (LOD) and Limit of quantification (LOQ) was determined from Signal to noise ratio (S/N) method. Prepared and injected a series of diluted solutions from individual standard solutions. Based on S/N ratios of diluted solutions reported LOD and LOQ concentrations of Impurity-A and Impurity-B

reported. Injected LOQ solution of Impurity-A and Impurity-B standards six replicates to conform the precision at LOQ.

2.5.3 Linearity:

Linearity studies were performed for Impurity-A and Impurity-B at different concentrations from QL to 200% (QL, 25, 50, 100, 150 and 200%) of the specification level with respect to analyte concentration. Plotted a linear graph by taking the MRM peak areas on Y-axis and corresponding concentration on X-axis. Reported the values of correlation co-efficient, slope, y-intercept and residual sum of squares from linearity study.

2.5.4 Precision:

Prepared the spiked sample solution in six times containing each Impurity-A and Impurity-B at specification level at each preparation and injected each once. Calculated the content of each Impurity-A and Impurity-B and reported % RSD for Impurity-A, Impurity-B content from six spiked sample preparations.

2.5.5 Accuracy:

The accuracy of the test method was demonstrated by prepared the un spiked sample solutions and spiked sample solution with known concentration of Impurity-A, Impurity-B at LOQ level, 50%, 100% and 150% of the specification limit. Calculated the % recovery of Impurity-A and Impurity-B at each level.

3. Results and discussion

3.1 Validation results of the method:

The developed method for the quantification of trace level determination of Impurity-A and Impurity-B in Azilsartan drug substance was validated as per ICH guidelines. The method was evaluated for its specificity, LOD (limit of detection), LOQ (Limit of quantification), Linearity, Accuracy and Precision.

Table 2. Specificity results for Impurity-A, Impurity-B and Azilsartan drug substance

S.No	Component name	Retention time(min)	
		Data from Individual standards	Data from Spiked sample standards
1	Impurity-A	6.74(From TIC)	6.75(From TIC)

2	Impurity-B	3.22(From TIC)	3.22(From TIC)
3	Azilsartan	21.18(From PDA detector)	21.19(From PDA detector)

TIC: Total ion Chromatogram, PDA: Photodiode array detector

Table 3.Sensitivity: Limit of detection (LOD) results

S. No	Component name	S/N Ratio	LOD Concentration
1	Impurity-A	3.2	1.4ppm
2	Impurity-B	3.4	1.4ppm

Table 4.Sensitivity: Limit of quantification (LOQ) results

S. No	Component name	S/N Ratio	LOQ Concentration
1	Impurity-A	10.4	4.7ppm
2	Impurity-B	10.2	4.5ppm

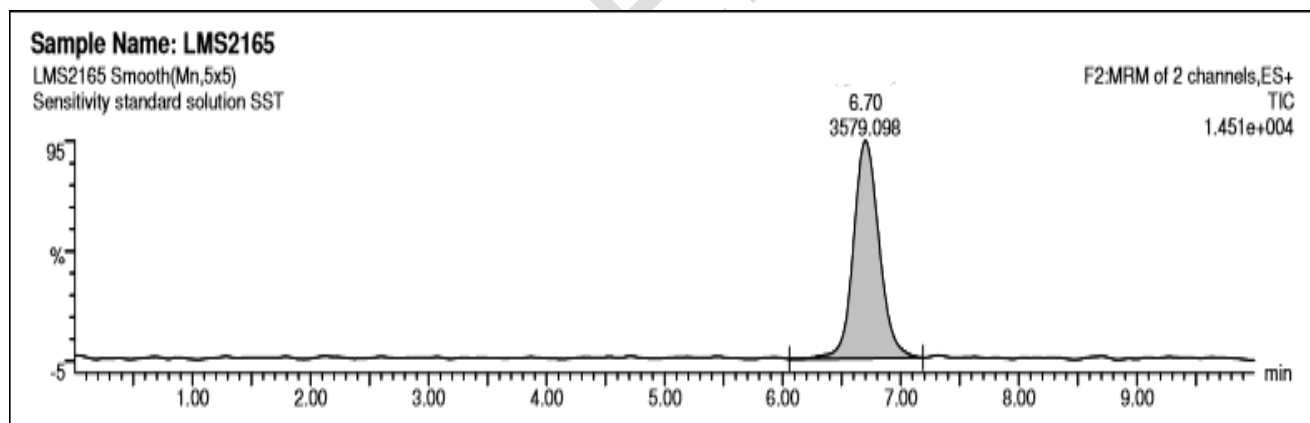


Figure 8: LOQ (Limit of quantification)MRM Chromatogram of Impurity-A

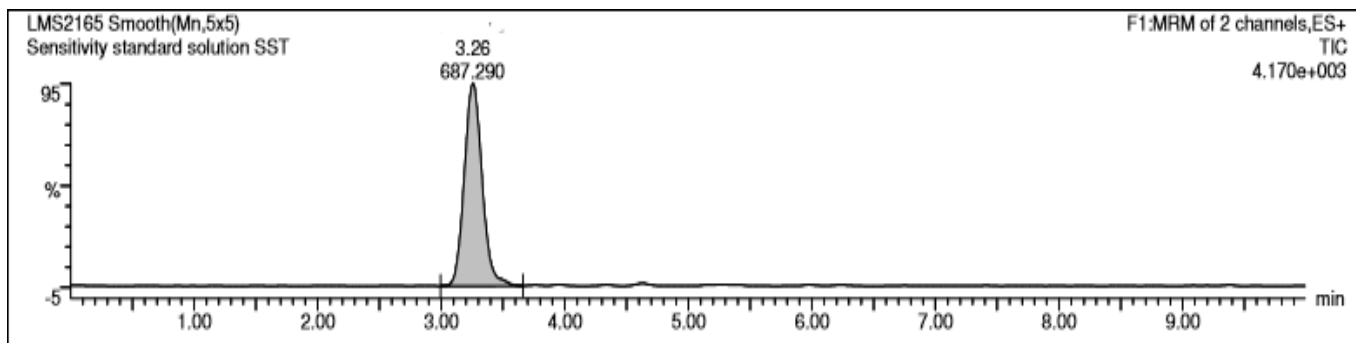


Figure 9: LOQ (Limit of quantification)MRM Chromatogram of Impurity-B

Table 5.LOQ precision results

S.No	Name of the solution	Impurity-A area	Impurity-B area
1	LOQ Standard solution injection-1	3712.226	732.009
2	LOQ Standard solution injection-2	3909.835	721.173
3	LOQ Standard solution injection-3	3821.25	731.111
4	LOQ Standard solution injection-4	3636.271	722.679
5	LOQ Standard solution injection-5	3666.535	768.314
6	LOQ Standard solution injection-6	3649.165	663.937
7	Mean	3732.5	723.2
8	Standard deviation	109.8102	33.7400
9	%RSD	2.9	4.7

Table 6.Linearity:Impurity-A Linearityresults

Level	Concentration(ppm)	MRM Peak Area
LOQ	4.7066688	3717.891
25%	9.80556	7535.895
50%	19.61112	15223.849

100%	39.22224	30506.014
150%	58.83336	45692.574
200%	78.44448	60327.133
Correlation co-efficient		1.0000
Slope		770.6755555
Y-Intercept		113.5046674
Residual sum of square		160669.0451

Table 7.Linearity: Impurity-B Linearity results

Level	Concentration(ppm)	MRM Peak Area
LOQ	4.54477272	675.027
25%	9.4682765	1510.123
50%	18.936553	3205.101
100%	37.873106	6368.086
150%	56.809659	9875.648
Correlation co-efficient		0.9997
Slope		171.0879622
Y-Intercept		-70.94994526
Residual sum of square		80280.73968

Table 8.Precision: Method precision results

Level	Impurity-A(ppm)	Impurity-B(ppm)
Method Precision Preparation-1	39.227	42.528
Method Precision Preparation-2	38.73	43.066
Method Precision Preparation-3	40.229	43.884
Method Precision Preparation-4	38.987	43.044
Method Precision Preparation-5	39.789	42.806
Method Precision Preparation-6	39.339	42.608

Mean	39.4	43.0
Standard deviation	0.5459	0.4901
%RSD	1.39	1.14

Table 9. Accuracy: %Recovery of Impurity-A and Impurity-B

	LOQ	50%(18.8ppm)	100%(37.5ppm)	150%(56.3ppm)
Mean % recovery for Impurity-A (n=3)	99	99	100	102
Mean % recovery for Impurity-B(n=3)	93	101	103	103

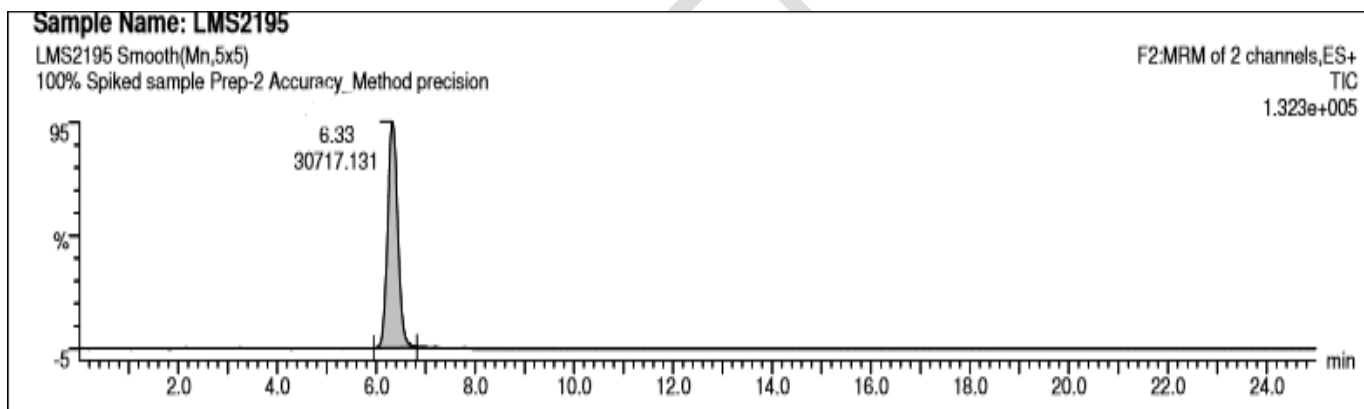


Figure 10: 100% Spiked sample MRM Chromatogram of Impurity-A

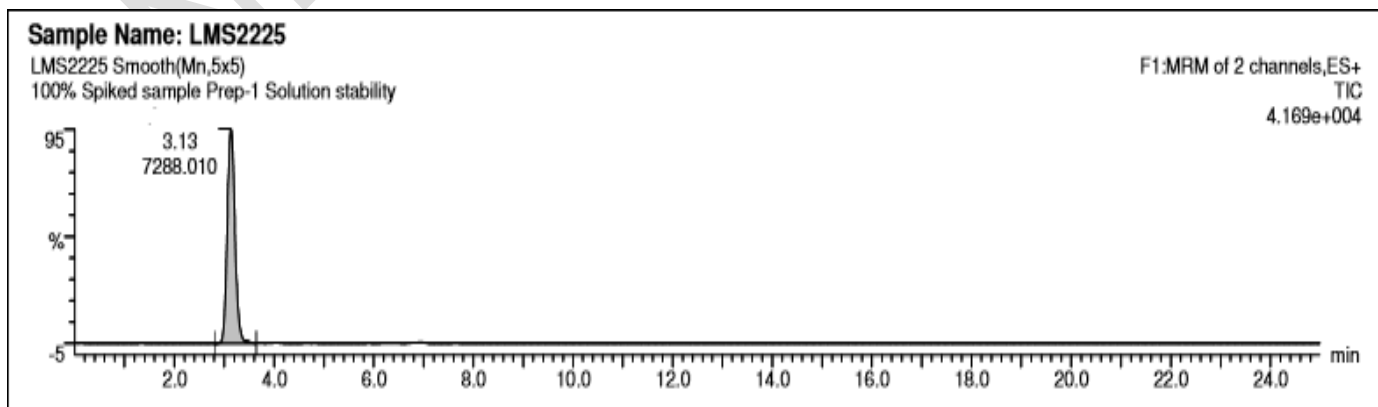


Figure 11: 100% Spiked sample MRM Chromatogram of Impurity-B

3.2 Specificity:

From specificity results of Table:2, it was observed that Impurity-A, Impurity-B and Azilsartan peaks were well resolved from each other. No blank interference was not observed at retention times of impurity-A and Impurity-B. So the method was specific for quantification of Impurity-A and Impurity-B in Azilsartan drug substance.

3.3 Sensitivity:

Limit of detection (LOD) concentration for Impurity-A observed as 1.4ppm(signal to noise ratio:3.2) and Impurity-B observed as 1.4ppm (signal to noise ratio:3.4). Limit of quantification(LOQ) concentration for Impurity-A observed as 4.7ppm (signal to noise ratio:10.4) and Impurity-B observed as 4.5ppm ((signal to noise ratio:10.2). %Relative standard deviation was observed from LOQ precision experiment for Impurity-A and Impurity-B was 2.9 and 4.7 respectively. From these results, current method was sensitive for quantification of Impurity-A and Impurity-B in Azilsartan drug substance.

3.4 Linearity:

From Linearity study, it was found that analyte response directly proportional to the concentration of analyte, it clearly indicates that linear relationship between analyte response and analyte concentration. Correlation coefficient for Impurity-A and Impurity-B observed as 1.0000 and 0.9997 respectively (refer table no-6&table no-7). Method was found linear from LOQ(4.7ppm) to 200%(78.4ppm) for Impurity-A and LOQ(4.5ppm) to 150%(56.8ppm) for Impurity-B.

3.5 Precision:

Repeatability expresses the closeness of agreement between the series of measurements. From method precision study of 100% Spiked sample solution 6 preparations found that the %RSD(relative standard deviation) for Impurity-A and Impurity-B was 1.39 and 1.14 respectively(refer table no-8). LC-MS/MS MRM mode method was precise for trace level quantification of Impurity-A and Impurity-B in Azilsartan drug substance.

3.6 Accuracy:

Accuracy expresses the closeness of agreement between the true value and measured value. % Recovery for Impurity-A at LOQ,50%,100% and 150% was 99%,99%,100% and 102% respectively. Similarly, %Recovery for Impurity-B at LOQ,50%,100% and 150% was 93%,101%,103% and 103% respectively. From accuracy study method was found accurate from LOQ(4.7ppm) to 150%(56.3ppm) for Impurity-A and LOQ(4.5ppm) to 150%(56.3ppm) for Impurity-B. Current method was accurate for quantification of Impurity-A and Impurity-B in Azilsartan drug substance.

4. Conclusion

From method validation study method was found that current LC-MS/MS MRM mode method was Specific, Sensitive, Precise, Accurate and Linear for trace level quantification of genotoxic impurities i.e; Methyl(Z)-2-ethoxy-1-((2'-(N'-hydroxycarbamimidoyl)-[1,1'-biphenyl]-4-yl) methyl)-1H-benzo[d]imidazole-7-carboxylate (Impurity-A) and 2-ethoxy-1-((2'-(N'-hydroxycarbamimidoyl)-[1,1'-biphenyl]-4-yl)methyl)-1H-benzo[d]imidazole-7-carboxylic acid (Impurity-B) in Azilsartan drug substance.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

References

1. ICH harmonized guideline: assessment and control of dna reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. Ich m7 (r1).
2. David j. Snodin, genotoxic impurities: from structural alerts to qualification, organic process research & development 2010, 14, 960–976.
3. David j. Snodin, genotoxic impurities: from structural alerts to qualification, organic process research & development 2010, 14, 960–976.
4. Guideline on the limits of genotoxic impurities, committee for medicinal products (chmp), european medicines agency, london, 28 june 2006 (cpmp/swp/5199/02, emea/chmp/qwp/251344/2006); questions and answers on the 'guideline on the limits of genotoxic impurities' emea/chmp/swp/431994/2007 rev 3.
5. Center for drug evaluation and research, food and drug administration, guidance (draft) for industry genotoxic and carcinogenic impurities in drug substances and products: recommended approaches, center for drug evaluation and research, food and drug administration, 2008.
6. Lutz Müller, robert j. Mauthe, christopher m. Riley, marta m. Andino, david de antonis, chris beels, joseph degeorge, alfonso m. de knaep, dean ellison, jane a. Fagerland, rebecca frank, betsy fritschel, sheila galloway, ernie harpur, charles d. n. humfrey, alexander s. Jacki, nirdosh jagota, john mackinnon, ganapathymohan, daniel k. Ness, michael r. O'donovan, mark d. Smith, gopivudathala, larry yotti, a rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity regulatory toxicology and pharmacology 44 (2006) 198–211.
7. Andrew teasdale, john wiley & sons ;genotoxic impurities: strategies for identification and control, inc. 2010. Pp. 97.
8. N. V. D. Harikiran Maddi, Srinivas, Garaga, Ambati, V. Raghava Reddy, Paul Douglas Sanasi and Raghubabu Korupolu; Synthesis and characterization of related substances of Azilsartan Kamedoxomil; *Journal of current issues in pharmacy and medical sciences*, volume 30, No. 1, Pages 31–35; DOI: <https://doi.org/10.1515/cipms-2017-0007>.

9. Wentao Zhou, Yuxia Zhou, Lili Sun, Qiaogen Zou, Ping Wei, Pingkai Ouyang ;Structural Elucidation of Potential Impurities in Azilsartan Bulk Drug by HPLC;*Journal of AOAC INTERNATIONAL*, Volume 97, Published: 27 November 2019 , Pages 1552–1562; <https://doi.org/10.5740/jaoacint.14-022>.
10. J. Sreenivasulu, P. Venkata Ramana, G. Sampath Kumar Reddy, CH.V.S. Nagaraju, S. Thirumalai Rajan, S. Eswaraiyah; A Rapid Novel HPLC Method for Estimation of Eight Related Compounds in Azilsartan Kamedoxomil and Identification of Degradation Compounds by Using LC–MS ;*Journal of Chromatographic Science*, Volume 53, Issue 9, October 2015, Pages 1463–1474, <https://doi.org/10.1093/chromsci/bmv039>
Published: 29 April 2015.
11. Stanislav Rádl, Josef Černý, Jan Stach, Jan Holec, Ondřej Piša, Zuzana Gablíková ; Synthesis of Azilsartan and Its Selected Potential Impurities, *Journal of Heterocyclic Chemistry*, 50(4), 2013, 929-936 First published: 10 July 2013 <https://doi.org/10.1002/jhet.1783>.
12. Stanislav Rádl, Josef Černý, Jan Stach, Jan Holec, Ondřej Piša, Zuzana Gablíková ; Synthesis and Characterization of Impurities of a Common and Advanced Intermediate of Candesartan and Azilsartan Antihypertensive ; *International Research Journal of Pure and Applied Chemistry*, Page 140-149 DOI: 10.9734/IRJPAC/2015/13162.
13. Takahiro Yoshikawa 1, Naoto Hayashi 2, Naoya Hatta 3, Masayuki Yokota 3 Selective formation of a phenanthridine derivative by photodegradation of azilsartan; *Bioorganic & Medical chemistry letters*, volume 41, June 2021, 128011 ; <https://doi.org/10.1016/j.bmcl.2021.128011>.
14. Bhaskara P.V. Mantena, Sumathi V. Rao , K.M. Ch. Appa Rao, K. Ramakrishna, R. Srikanth Reddy; Method Development and Validation for the Determination of Potential Impurities Present in Azilsartan medoxomil Tablets by Reverse Phase-Ultra Performance Liquid Chromatography; *Journal of Analytical chemistry letters*, volume 4, 2014-issue 5-6 ; <https://doi.org/10.1080/22297928.2014.1000966>.
15. Dhiraj Kaushik, Jasmeen Kaur, Vaneet Paul Kaur, Balraj Saini, Yogita Bansal, Gulshan Bansal; Forced degradation, LC–UV, MSⁿ and LC–MS–TOF studies on azilsartan: Identification of a known and three new degradation impurities; *Journal of pharmaceutical and biomedical analysis*, volume 120, 20 February 2016, pages 202-211; <https://doi.org/10.1016/j.jpba.2015.12.027>.