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METHOD DEVELOPMENT AND VALIDATION OF FIVE NITROSAMINES IMPURITIES CONTENT IN TELMISARTAN DRUG SUBSTANCE BY GC-MS/MS

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Abstract
A proficient GC-MS/MSS method was developed for determination of N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine, N-Nitrosodibutylamine impurities in Telmisartan Drug Substance using column Rtx-5 Amine (30m length X 0.32mm diameter) 1.5µm film thickness, Part No.: 12369, Make: Restek. Helium is used as Carrier gas at with Linear velocity of 44.3 cm/sec. The proposed method was validated for System suitability, Specificity, Linearity, LOD and LOQ, Method precision, Intermediate precision and Recovery. All the parameters were found within the acceptable limits. Linearity in the range of LOQ to 150% for each impurity. The established methodology was commercially useful, specific, accurate, precise and suitable for the analysis of Nitrosamines impurities in Telmisartan drug substance

***Keywords**: Gas chromatography with mass spectrometry (GC-MS/MS), Telmisartan drug substance, Nitrosamine impurities, ICH guideline and Method Validation.*

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INTRODUCTION:

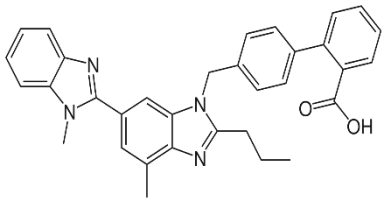
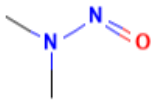
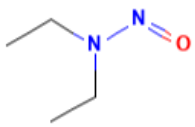
Telmisartan is used in the treatment of Hypertension (high blood pressure), prevention of heart attack and stroke and Heart failure. Telmisartan is an angiotensin receptor blocker (ARB). It relaxes blood vessels by blocking the action of a chemical that usually makes blood vessels tighter. This lowers the blood pressure, allowing the blood to flow more smoothly to different organs and the heart to pump more efficiently.

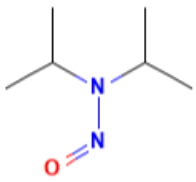
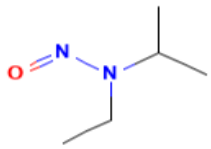
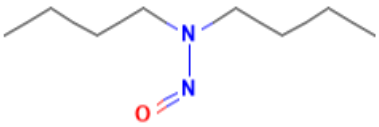
Nitrosamine impurities are unwanted chemicals, have no therapeutic value and are potentially harmful to the body. Nitrosamine substances are those which impact genetic material by means of mutations. Mutations can be chromosomal breaks, rearrangements, covalent binding or insertion into DNA during replication. The focus of this study is on reactive substances they have a potential to directly cause DNA damage when present low levels leading to mutations and there for potentially causing cancer. Because of this, it is important to identify Nitrosamine substances followed by monitoring and control at very low levels to ensure safety to the public health.

There are many sources of Nitrosamine impurities in pharmaceuticals active ingredient and drug products i.e. starting materials, byproducts, reagents, intermediates, degradation products, solvents or unreacted products that get carried over into the final product. In addition, some of cases the Active pharmaceutical ingredient itself can decompose to form Nitrosamine impurities or they can form in the drug product by reaction between excipients and Active pharmaceutical ingredient or oxidation of drug products. The employment of these compounds within the synthetic process is logical as these substances are reactive building blocks that come together to form complex drug substances.

Impurity guidelines have been developed by international Conference on Harmonization (ICH). ICH Q3A regulates impurities in new drug substances with thresholds for reporting, identifying, and qualifying impurities. ICH Q3B is the equivalent guideline for impurities in new drugs. ICH Q3C controls residual solvent, and is the first time the ICH applied substance specific limits. Depending on their potential risk to human health. ICH Q3D is currently published and will include elements and limits for heavy metal impurities. Currently released ICH guidelines for impurity limits are not suitable for most Nitrosamine impurities. The Nitrosamine compounds considered unsafe at any level. The limit for Nitrosamine impurities with an understood toxicity can be calculated based upon the know PDE.

Chemical Structure of N-Nitrosodimethylamine and Telmisartan:

Telmisartan: ***Chemical Name:*** *2-(4-{[4-Methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid* ***Molecular weight:*** *514.6*	
N-Nitrosodimethylamine(NDMA)***:*** ***Chemical Name:*** *N,N*-dimethylnitrous amide ***Molecular weight:*** 74.04	
N-nitrosodiethylamine(NDEA): **Chemical Name:** *N,N-diethylnitrous amide* **Molecular weight:** 102.07	

N-Nitrosodiisopropylamine (NDIPA): ***Chemical Name:*** *N,N*-di(propan-2-yl)nitrous amide ***Molecular weight:*** 130.11	
N-Nitrosoisopropylethylamine (NIPEA): ***Chemical Name:*** *N*-ethyl-*N*-propan-2-ylnitrous amide ***Molecular weight:*** 116.09	
N-Nitrosodibutylamine (NDBA): ***Chemical Name:*** *N,N*-dibutylnitrous amide ***Molecular weight:*** 158.14	

METHOD DEVELOPMENT:

Instrument, Chemicals and Reagents

Instrumentation

A Shimadzu Gas chromatograph with Auto sampler with Triple Quadrupole Mass spectrometer Detector (Shimadzu GC-2010 plus with TQ8050 MS), and Rtx-5 Amine (30m length X 0.32mm diameter) 1.5µm film thickness, Part No.: 12369, Make: Restek column was employed in the method. All the weighing in the experiments was done with Mettler toledo electronic balance (Mettler Toledo / XSE 205) capable of measuring with an accuracy of 0.01 mg.

Chemicals and Reagents: Methanol (GCMS Grade, Make Merck), Dichloromethane (GCMS Grade, Make Merck), Acetone (GCMS Grade, Make Merck), Methane sulfonyl chloride (GCMS Grade, Make Merck), Anhydrous Sodium Sulphate (GCMS Grade, Make Merck), Sodium Hydroxide (AR Grade, Make Merck), Water (Milli-Q)

Chromatographic Conditions for GC:

Column	RTX-5 Amine, (30.0m x 0.32mm), 1.5µm, P. No. 12369, Make: Restek with Base deactivated guard column (5.0m x 0.32mm) Make: Restek, P. No: 10001.
Flow control Mode	Constant Linear velocity
Pressure	12.8 kPa
Column Flow	1.49 mL/min
Linear Velocity	44.3 cm/sec.
Total Flow	11.9mL/min
Injector Temperature	200°C
Injection mode	Split less

Sampling time	2.0 minutes	
Injection Volume	1.0 µL	
Carrier Gas	Helium	
Split Ratio	1:5	
Purge Flow	3.0 mL/min	
GC Equilibration time	0.10 minutes	
Split Ratio Program	**Time (min)**	**Split ratio**
	2.10	30
Oven Programing	Initial 50.0°C,hold for 5.0min, Increase @ 20.0°C per min to 250.0°C, hold for 0 minute, Increase @ 30.0°C per min to 280.0°C, hold for 4.0 minutes	
Run time	20.0 Minutes	
Retention Time	**Name**	**Typical RT (minutes)**
	N-Nitrosodimethylamine	6.8
	N-Nitrosodiethylamine	9.7
	N-Nitrosoisopropylethylamine	10.5
	N-Nitrosodiisopropylamine	11.1
	N-Nitrosodibutylamine	13.3

GC-MS Programming

Ion source temperature	210.0°C
Interface temperature	230.0°C
Solvent cut time	5.50 min
Detector gain mode	Relative to tuning result
Detector gain	+0.4 kV

MS Conditions:

MS/MS Table	
N-Nitrosodimethylamine	
Ch1-m/z (Precursor>Product) [Collision Energy]	74.00>44.10 [6 V] {Quantifier Ion}
Ch2-m/z (Precursor>Product) [Collision Energy]	74.00>42.10 [15 V] {Qualifier Ion}
N-Nitrosodiethylamine	
Ch1-m/z (Precursor>Product) [Collision Energy]	102.00>85.10 [6 V] {Quantifier Ion}
Ch2-m/z (Precursor>Product) [Collision Energy]	102.00>56.10 [15 V] {Qualifier Ion}
N-Nitrosoisopropylethylamine	
Ch1-m/z (Precursor>Product) [Collision Energy]	116.00>99.10 [6 V] {Quantifier Ion}
Ch2-m/z (Precursor>Product) [Collision Energy]	116.00>70.10 [15 V] {Qualifier Ion}
N-Nitrosodiisopropylamine	
Ch1-m/z (Precursor>Product) [Collision Energy]	130.00>88.10 [6 V] {Quantifier Ion}

Ch2-m/z (Precursor>Product) [Collision Energy]	130.00>42.00 [10 V] {Qualifier Ion}
N-Nitrosodibutylamine	
Ch1-m/z (Precursor>Product) [Collision Energy]	116.00>99.10 [6 V] {Quantifier Ion}
Ch2-m/z (Precursor>Product) [Collision Energy]	158.00>99.10 [10V] {Qualifier Ion}

Preparation of blank, standard and sample solution: The diluent used was homogeneous mixture of 4 gm sodium hydroxide/1 litre water. Nitrosamines impurities standard solution was prepared by using N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine reference standards to attain a concentration of about 0.0084 ppm of each impurity. For sample solution attain a concentration of about 2/mg/ml of sample.

Acceptance criteria for System Suitability:
% RSD: The %RSD for the area response of each impurity peak from initial six replicates of Standard Solution analyzed in the sequence should be not more than 25.

Calculations and results:
Calculate the impurity in the sample using the following formula (Result in ppm each nitrosamine impurity):

$$\text{Impurity(ppm)} = \frac{(AT-AB) \times WS \times DT \times P \times 106}{(AS-AB) \times DS \times WT \times 100}$$

AB = Average Peak area of respective impurity analysed in the chromatogram obtained from Blank, AT = Peak area counts of respective impurity analysed in the chromatogram of the sample solution, AS = Average peak area counts of respective impurity analysed in standard in the chromatogram of the standard Solution, WS = Weight of respective impurity analyzed Standard, WT = Weight of sample solution, P = Purity/Potency of Impurity Standard used, DT = Dilution factor of sample solution, DS = Dilution factor of standard Solution.

VALIDATIONOF GC-MS/MS METHOD
Specificity:

The specificity is defined as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as residual, degradation product and matrix components. In HPLC method, it is assured/proved by complete separation of peak of analyte from other peaks that are of other impurities that might be present in sample or blank.

Inject the Blank and Standard solution, Check the interference at the retention time of all five analytes. There should not be any interference in blank and standard at the retention time of each impurity and each impurity shall be well separated from each other. If any peak is present at the retention time of analyte its response should not be more than 20% of the response at the quantification limit (LOQ).

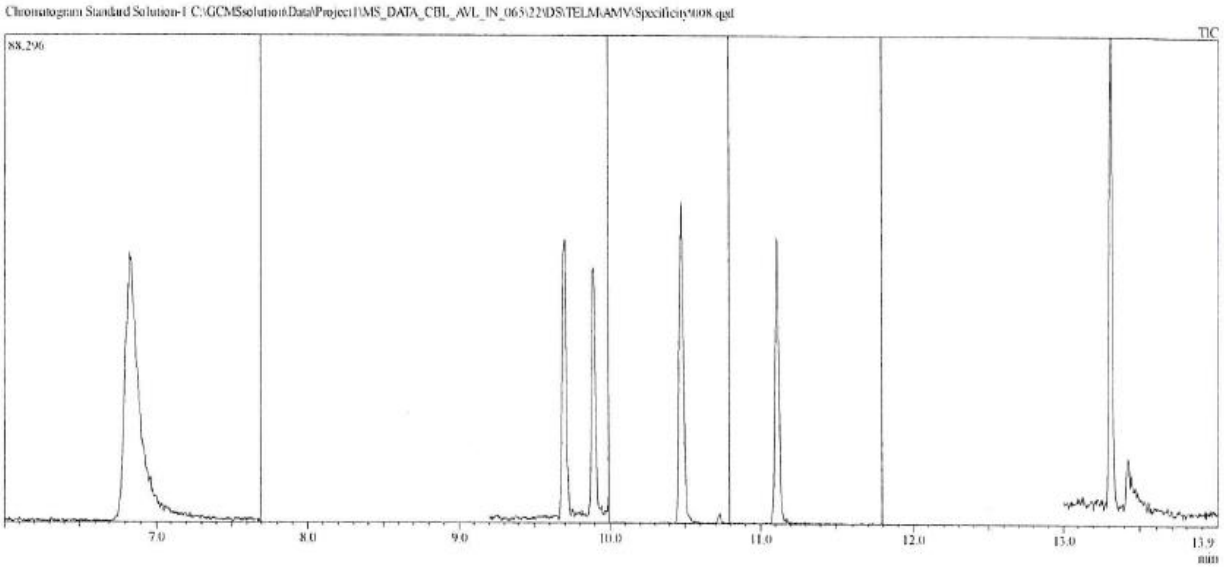
There is not any interference at retention time of all five analyte (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine). Refer Table-I

Table-I

Name of Solvent	Retention Time (Minutes)	
	Blank	standard
N-Nitrosodimethylamine	ND	6.8
N-Nitrosodiethylamine	ND	9.7
N-Nitrosoisopropylethylamine	ND	10.5
N-Nitrosodiisopropylamine	ND	11.1
N-Nitrosodibutylamine	ND	13.3

No peak was observed in blank at the retention time of any impurity peak and all peaks are well separated from *each other.*

Chromatograms of study



Quantitative Result Table

ID#	Name	Conc.	R.Time	m/z	Area	Height	S/N
1	N-Nitrosodimethylamine	0.000	6.838	74.00 > 44.10	235165	26091	590
2	N-Nitrosodiethylamine	0.000	9.702	102.00 > 85.10	71146	13026	1183
3	N-Nitrosoisopropylethylamine	0.000	10.480	116.00 > 99.10	103856	19146	2584
4	N-Nitrosodiisopropylamine	0.000	11.114	130.00 > 88.10	59157	11070	700
5	N-Nitrosodibutylamine	0.000	13.315	116.00 > 99.10	94751	18277	708

Linearity:

A linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration of analyte. The linearity of method was determined using different concentration of N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine. Calibration curve found to be linear from LOQ to 150% of specification level.

The test method linearity was established from, six levels of concentration over the range LOQ to 150% of, ICH limit for each residual solvent impurity. A linear correlation, and regression were determined among the concentrations, and peak area responses of each residual solvent. The correlation coefficient (r) and regression coefficient (R2) values, for all five nitrosamine impurities found to be higher than, 0.990. The statistical characteristics like slope, y-intercept and, % y-intercept were interpreted and found within the acceptable limit for all five analyte (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine). The data tabulated in Table-II demonstrate the linearity of procedure.

Table-II

Linearity Conc. level	N-Nitrosodimethylamine		N-Nitrosodiethylamine		N-Nitrosoisopropylethylamine		N-Nitrosodiisopropylamine		N-Nitrosodibutylamine	
	Conc. (ppm)	Mean area	Conc. (ppm)	Mean area	Conc. (ppm)	Mean area	Conc. (ppm)	Mean area	Conc. (ppm)	Mean area
LOQ level	0.33	68688	0.09	21410	0.09	25996	0.08	17886	0.08	29825
50% level	0.55	112796	0.15	34416	0.14	43371	0.14	30929	0.14	44530
75% level	0.82	168478	0.23	52078	0.22	65093	0.2	45812	0.2	67532
100% level	1.09	218388	0.3	68885	0.29	87293	0.27	61320	0.27	86587
125% level	1.37	274823	0.38	84299	0.36	105499	0.34	77277	0.34	110513
150% level	1.64	331561	0.45	105303	0.43	127351	0.41	91751	0.41	134100
Correlation coefficient		1.00	1.00		1.00		1.00		1.00	
Squared Correlation coefficient		0.99975	0.99725		0.9991		0.99949		0.9979	
Slope		199386.6	228824.0754		294072.3241		225686.1039		318436.7532	
Y-Intercept		3048.66	45.41322		778.72402		-2.16493		2423.01255	
Residual sum of square		11958731	13505980.5		6577226.572		1981326.631		16427990.82	

Limit of detection (LOD):

It is the smallest amount or concentration of an analyte that can be estimated. The detection limit is determined by the analysis of standard with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The limit of detection is determined by establishing the signal to noise ratio. Inject the blank and standard solutions at lower concentration and calculate the signal to noise ratio.

A signal-to-noise ratio between 3:1 estimating the detection limit.

The detection limit for N-Nitrosodimethylamine is found 0.12ppm and for remaining four nitrosamine impurities i.e. N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine is found 0.03 ppm w.r.t. Telmisartan sample concentration. For details, refer Table III.

Table-III

Residual Solvent	LOD Level (ppm) w.r.t SPL	Mean S/N ratio
N-Nitrosodimethylamine (10.0%)	0.12	7
N-Nitrosodiethylamine (10.0%)	0.03	11
N-Nitrosoisopropylethylamine (10.0%)		9
N-Nitrosodiisopropylamine (10.0%)		7
N-Nitrosodibutylamine (10.0%)		10

Limit of quantitation (LOQ):

The Quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

The limit of quantification is determined by establishing the signal to noise ratio. Inject the blank sample and the spiked sample at LOQ level in six replicates and calculate signal to noise ratio and the % RSD at LOQ level.

A signal-to-noise ratio between 10:1 estimating the quantification limit.

The quantification limit for N-Nitrosodimethylamine is found 0.36ppm and for remaining four nitrosamine impurities i.e. N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine is found 0.09 ppm w.r.t. Telmisartan sample concentration. For details, refer Table IV.

Table-IV

Residual Solvent	LOQ Level (ppm) w.r.t SPL	Mean S/N ratio	%RSD (six replicates)
N-Nitrosodimethylamine (30.0%)	0.36	22	7.7
N-Nitrosodiethylamine (30.0%)	0.09	19	6.3
N-Nitrosoisopropylethylamine (30.0%)		17	8
N-Nitrosodiisopropylamine (30.0%)		17	6.3
N-Nitrosodibutylamine (30.0%)		18	2.8

Recovery:

Recovery means the percentage of the true concentration of a substance recovered during the analytical procedure.

Recovery assessed using a minimum of 6 determinations over a minimum of 3 concentration levels.

Acceptable limits for a recovery result during validation should be within the range of 80% - 120%.

The percentage of average recovery for all five analyte (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine) in Telmisartan found >90% at LOQ, 100% and 150%. For details, refer Table V.

Table V

Accuracy of N-Nitrosodimethylamine					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.361	0.3517	97.42	94.3
	Sample-2		0.3191	88.39	
	Sample-3		0.3505	97.09	
100	Sample-1	1.2035	1.2126	100.75	100.2
	Sample-2		1.2004	99.74	
	Sample-3		1.2032	99.97	
150	Sample-1	1.8053	1.6531	91.56	96.6
	Sample-2		1.7522	97.05	
	Sample-3		1.825	101.09	

N-Nitrosodiethylamine					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.0891	0.0863	96.85	97.3
	Sample-2		0.0847	95.06	
	Sample-3		0.0891	100	
100	Sample-1	0.2971	0.3078	103.6	102.3
	Sample-2		0.294	98.95	
	Sample-3		0.3104	104.47	
150	Sample-1	0.4457	0.4235	95.01	98
	Sample-2		0.4378	98.22	
	Sample-3		0.4494	100.83	

N-Nitrosoisopropylethylamine					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.0883	0.086	97.39	97.5
	Sample-2		0.0862	97.62	
	Sample-3		0.086	97.39	
100	Sample-1	0.2946	0.3088	104.82	101.8
	Sample-2		0.2943	99.89	
	Sample-3		0.2967	100.71	
150	Sample-1	0.4419	0.4146	93.82	96.8
	Sample-2		0.4317	97.69	
	Sample-3		0.4373	98.95	

N-Nitrosodiisopropylamine					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.0899	0.0841	93.54	96.1
	Sample-2		0.0832	92.54	
	Sample-3		0.0919	102.22	
100	Sample-1	0.2999	0.3021	100.73	100.1
	Sample-2		0.2963	98.79	
	Sample-3		0.3026	100.9	
150	Sample-1	0.4498	0.4237	94.19	97
	Sample-2		0.4403	97.88	
	Sample-3		0.4444	98.79	

N-Nitrosodibutylamine					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.0857	0.083	96.84	97.2
	Sample-2		0.0819	95.56	
	Sample-3		0.0851	99.29	
100	Sample-1	0.2856	0.2905	101.71	100.7
	Sample-2		0.2794	97.82	
	Sample-3		0.2932	102.66	
150	Sample-1	0.4285	0.4019	93.79	96.6
	Sample-2		0.4187	97.71	
	Sample-3		0.4216	98.38	

Precision: (Method Precision)

The precision determined under equal conditions with same homogeneous spiked sample (six different sample preparation) as per recommended test method and % RSD of the results obtained shall be calculated.

The repeatability is established by estimating the six replicates of spiked sample and calculates the % RSD of the results obtained for each analyte.

The %RSD of results for the analysis of spiked sample should not be more than 25%.

The % RSD of six different sample preparation found <5% refer Table-VI.

Table-VI

Preparation	Results (ppm)				
	N-Nitroso dimethylamine	N-Nitroso diethylamine	N-Nitroso Isopropylethylamine	N-Nitroso Diisopropylamine	N-Nitroso Dibutylamine
1	1.2	0.28	0.29	0.29	0.27
2	1.2	0.29	0.29	0.28	0.27
3	1.21	0.28	0.29	0.3	0.28
4	1.18	0.29	0.3	0.3	0.28
5	1.27	0.29	0.29	0.31	0.27
6	1.26	0.31	0.3	0.31	0.28
Mean	1.22	0.29	0.29	0.29	0.27
SD	0.036	0.01	0.005	0.011	0.005
%RSD	3	3.4	1.7	3.8	1.9

Precision: (Intermediate Precision)

Intermediate Precision means the susceptibility of an analytical method to changes in experimental conditions which can be expressed as different columns, different analyst and different days.

Intermediate Precision of the method is established by estimating the six replicates of spiked sample by different analysts, on different days and on different columns. Calculate the % RSD of the results obtained.

The %RSD of results for the analysis of spiked sample should not be more than 210%.

The % RSD of six different sample preparation found 5%, refer Table-VII.

Table-VII

Preparation	Results (ppm)				
	N-Nitroso dimethylamine	N-Nitroso diethylamine	N-Nitroso Isopropylethylamine	N-Nitroso Diisopropylamine	N-Nitroso Dibutylamine
1	1.06	0.27	0.27	0.26	0.25
2	1.16	0.27	0.28	0.27	0.26
3	1.11	0.26	0.27	0.25	0.25
4	1.15	0.29	0.28	0.27	0.27
5	1.11	0.27	0.28	0.26	0.27
6	1.16	0.28	0.3	0.28	0.27
Mean	1.12	0.27	0.28	0.26	0.26
SD	0.039	0.01	0.01	0.01	0.009
%RSD	3.5	3.7	3.6	3.8	3.5

DISCUSSION:

A chromatographic method involves demonstrating specificity, which is the ability of the method to accurately measure the all five Nitrosamines impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine) response in the presence of all potential sample components. The chromatographic and mass spectroscopy parameters were fixed and GC-MS/MS system was studied for suitability of Nitrosamine impurity analysis. The developed method was performed for linearity, precision, Accuracy, specificity, LOD and LOQ.

CONCLUSION:

A simple and sensitive method for the determination all five Nitrosamines impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine) in Telmisartan drug substance by using GC-MS/MS was developed, validated and applied for the analysis of Telmisartan drug substance samples. The method was validated to ensure the feasibility of the method for its application in routine analysis. The LOQs achieved through this method were lower than the genotoxic impurities limit.

REFERENCES:

1. Beard, Jessica C.; Swager, Timothy M. (21 January 2021). "An Organic Chemist's Guide to N-Nitrosamines: Their Structure, Reactivity, and Role as Contaminants". The Journal of Organic Chemistry. 86 (3): 2037–2057. doi:10.1021/acs.joc.0c02774. PMID 33474939.
2. Yang, Chung S.; Yoo, Jeong-Sook H.; Ishizaki, Hiroyuki; Hong, Junyan (1990). "Cytochrome P450IIe1: Roles in Nitrosamine Metabolism and Mechanisms of Regulation". Drug Metabolism Reviews. 22 (2–3): 147–159. doi:10.3109/03602539009041082. PMID 2272285.
3. Anselme, Jean-Pierre (1979). "The Organic Chemistry of N-Nitrosamines: A Brief Review". N-Nitrosamines. ACS Symposium Series. 101. pp. 1–12. doi:10.1021/bk-1979-0101.ch001. ISBN 0-8412-0503-5.
4. Vogel, A. I. (1962). Practical Organic Chemistry (3rd ed.). Impression. p. 1074.
5. G. Subba Rao (1980), "NNitrosarnines from drugs and nitrite: potential source of chemical carcinogens in humans", Pharmacy International, Volume 1, pp. 187-190.
6. Hecht, Steven S.; Borukhova, Anna; Carmella, Steven G. "Tobacco specific nitrosamines" Chapter 7; of "Nicotine safety and toxicity" Society for Research on Nicotine and Tobacco; 1998 - 203 pages.
7. Hecht, Stephen S. (1998). "Biochemistry, Biology, and Carcinogenicity of Tobacco-Specific N-Nitrosamines". Chemical Research in Toxicology. 11 (6): 559–603. doi:10.1021/tx980005y. PMID 9625726
8. Nowak M, Seubert A. Application of experimental design for the characterization of a novel elution system for high-capacity anion chromatography with suppressed conductivity detection. Journal of Chromatography A. 1999;855: 91-109.
9. ICH Q2 (R1) Validation of Analytical Procedures: Definitions and Methodology, Geneva, 2005, in 2005 incorporated in Q2 (R1).
10. Wang Y, Harrison M, Clark B. Optimizing reversed-phase liquid chromatographic separation of an acidic mixture on a monolithic stationary phase with the aid of response surface methodology and experimental design. Journal of Chromatograhy A, 2006;1105: 199-207.

11. Jakszyn, P; Gonzalez, CA (2006). "Nitrosamine and related food intake and gastric and oesophageal cancer risk: A systematic review of the epidemiological evidence". World Journal of Gastroenterology. 12 (27): 4296–4303. doi:10.3748/wjg.v12.i27.4296. PMC 4087738. PMID 16865769.
12. Combet, E.; Paterson, S; Iijima, K; Winter, J; Mullen, W; Crozier, A; Preston, T; McColl, K. E. (2007). "Fat transforms ascorbic acid from inhibiting to promoting acid-catalysed N-nitrosation". Gut. 56(12): 1678–1684. doi:10.1136/gut.2007.128587. PMC 2095705. PMID 17785370
13. Honikel, Karl-Otto (2008). "The use and control of nitrate and nitrite for the processing of meat products". Meat Science. 78 (1–2): 68–76. doi:10.1016/j.meatsci.2007.05.030. PMID 22062097.
14. Raj T, Bharathi C, Saravana M, Prabahar J, Kumar P, Sharma H, Parikh K. Identification, isolation and characterization of process – related impurities in Rizatriptan benzoate. Journal of Pharmaceutical and Biomedical Analysis, 2009;49: 156-162.
15. Combet, E; El Mesmari, A; Preston, T; Crozier, A; McColl, K. E. (2010). "Dietary phenolic acids and ascorbic acid: Influence on acid-catalyzed nitrosative chemistry in the presence and absence of lipids". Free Radical Biology and Medicine. 48 (6): 763–771. doi:10.1016/j.freeradbiomed.2009.12.011. PMID 20026204.
16. Wisler J, Black K, Assessment of genotoxic impurities in small molecule drug candidates. Comparative Biology and Safety Sciences, Amgen Inc., Northern California SOT Meeting, 06-May-2010.
17. ICH M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to limit potential Carcinogenic Risk, Business plan 2010. Position paper 2010.
18. Elder, D.P., Snodin. D., and Teasdale, A Control and Analysis of Hydrazine, Hydrazides, Hydrazones Genotoxic Impurities in Active Pharmaceutical ingredients (APIs) and Drug products. J Pharm Biomed Anal., 2011, 54:900-910.
19. R. Nilsson (July, 2011), "The molecular basis for induction of human cancers by tobacco specific nitrosamines", Regulatory Toxicology and Pharmacology, Volume 60, Issue 2, pp. 268-80. Available at https://doi.org/10.1016/j.yrtph.2011.02 .014.
20. Joyce I. Boye; Yves Arcand (2012-01-10). Green Technologies in Food Production and Processing. Springer Science & Business Media. p. 573. ISBN 978-1-4614-1586-2
21. Indian Pharmacopoeia, Volume-II, 2014.
22. "Information on nitrosamines for marketing authorization holders", European Medicines Agency, pp. 1-10, EMA/CHMP/428592/2019 Rev. 2.
23. U. S. Food and Drug Administration (February, 2019), "FDA update table of interim limits for nitrosamine impurities in ARBs", from https://www.fda.gov.
24. European Medicines Agency (2019), "Temporary interim limits for NMBA, DIPNA and EIPNA impurities in sartan blood pressure medicines", EMA/351053/2019 rev. 1.
25. Aloka Srinivasan (2019), "Proactive Evaluation of Possible Genotoxic Impurities during the Early Stages of Drug Development", http://www.pharmtech.com.
26. "EMA to provide guidance on avoiding nitrosamines in human medicines". European Medicines Agency *(EMA) (Press release). 13 September 2019*. Retrieved 19 September 2019.
27. Method Development and Validation of 2-[(2-Methoxyphenoxy) Methyl] Oxirane Content In Ranolazine Drug Substance By LC-MS/MS. Journal of Chemistry and Chemical Sciences, Vol.10(12), 377-388, December, 2020.
28. Method Development and Validation of Epichlorohydrin content in Ranolazine drug substance by GC-MS/MS. Asian Journal of Research in Chemistry and Pharmaceutical Sciences. 8(3), 306-316, 2020.
29. Analyzing three Genotoxic impurities of Atorvastatin calcium employing GC-MS single quad detector with electron impact technology. Rasayan J. Chem. Vol. 14, No. 2, 1081-1086, April – June, 2021.
30. Method Development and Validation 0f N, N dimethylaminopropyl Chloride (DMPC) Content in Clomipramine Hydrochloride Drug Substance By GC-MS/MS. Asian Journal of Research in Chemistry and Pharmaceutical Sciences. 9(3), 121-131, 2021.