CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.6983632

Available online at: http://www.iajps.com *Research Article*

QUANTITATIVE EVALUATION OF N-NITROSAMINE IMPURITIES IN TELMISARTAN TABLETS USP 20MG, 40MG AND 80MG BY GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETER

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Article Received: July 2022 **Accepted:** July 2022 **Published: August** 2022

Abstract:

Determination of Nitrosamine Impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine, N-Nitrosodibutylamine) in Telmisartan Tablets USP 20 mg, 40 mg and 80 mg by GC-MS/MS. Using column Rtx-5 Amine fused silica capillary column with Base deactivated guard column. The validation of optimized method was carried out in accordance with relevant validation principles. The authenticated procedure was noticed to be specific, precise, linear, accurate and rugged with concentration ranging from limit of quantification (LOQ) to 200% specification level for Nitrosamine Impurities. As per daily dose of Telmisartan Tablets, the specification limits of N-Nitrosodimethylamine is 1.2mcg/gm and other Nitrosamine Impurities is 0.33mcg/gm. In this method the limit of detection for N-Nitrosodimethylamine is 0.12mcg/gm and other Nitrosamine Impurities is 0.033mcg/gm. The established method was productively useful to determine the N-Nitrosamine Impurities in Telmisartan Tablets.

Keywords: *Gas chromatography coupled with mass spectrometry (GC-MS/MS), Telmisartan tablets USP, Nitrosamine impurities, ICH guideline.*

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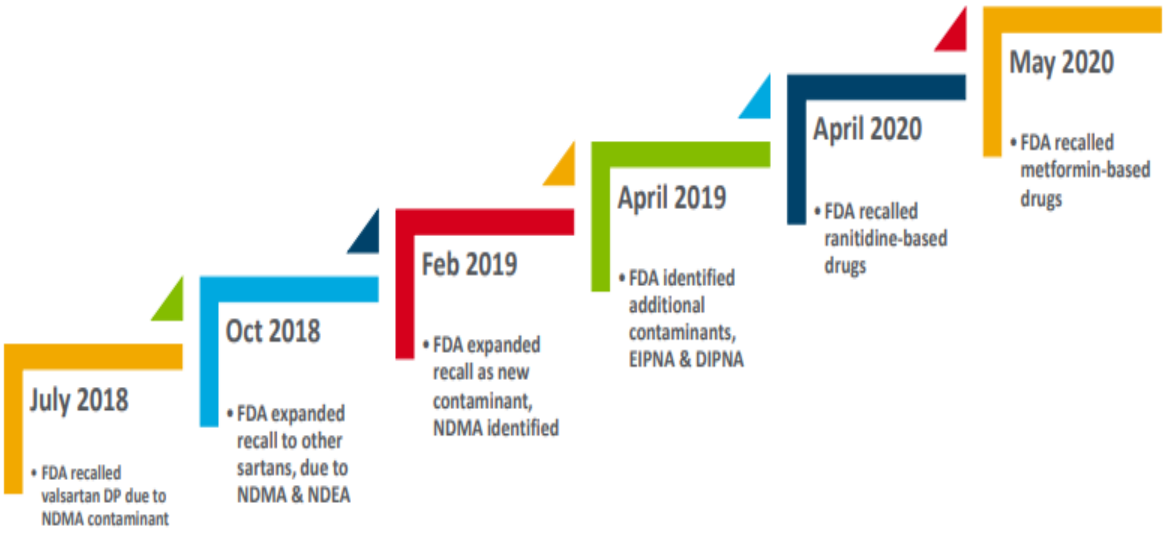
Please cite this article in press Rahul Kumar et al, ***Quantitative Evaluation Of N-Nitrosamine Impurities In Telmisartan Tablets USP 20mg, 40mg And 80mg By Gas Chromatography Coupled With Mass Spectrometer****., Indo Am. J. P. Sci, 2022; 09(8).*

INTRODUCTION:

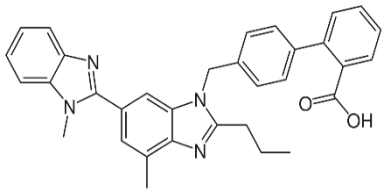
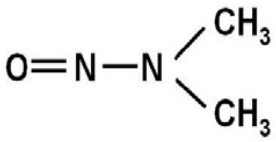
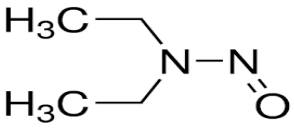
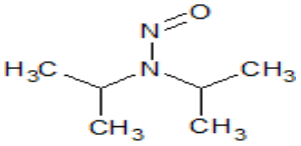
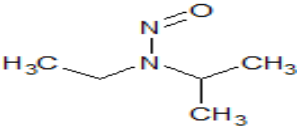
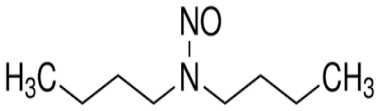
Telmisartan is used in the treatment of Hypertension (high blood pressure), prevention of heart attack and stroke and Heart failure. Telmisartan is an angiotensin receptor blocker (ARB). It relaxes blood vessels by blocking the action of a chemical that usually makes blood vessels tighter. Nitrosamine impurities in drug products carriage a significant risk to human health and safety. Even the presence of small quantities of Nitrosamine impurities in drug products is a major concern for regulatory agencies and Manufacturers. Nitrosamine impurities can damage DNA, leading to mutations and potentially cancer. Nitrosamines are formed by chemical reactions that occur during API manufacturing whether from starting materials, intermediates, reactants, reuse of solvents and by-products; they can form through degradation products generated during formulation or storage or from environmental contaminants. Recently, nitrosamines have been found in sartan drugs, a class of medications used to treat high blood pressure and heart failure, prompting recalls of angiotensin receptor blockers (ARBs) Telmisartan, valsartan, losartan, and irbesartan which were contaminated with N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA), two carcinogenic impurities. Since then, several other N-nitrosamines have also been identified and are being investigated by regulators: N-Nitrosodiispropylamine (NDIPA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodibutylamine (NDBA), and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA). Nitrosamines have now also been identified in ranitidine medications (which are used to treat heartburn and acid reflux) and metformin, an oral diabetes medication.

The presence of trace levels of Nitrosamines impurities is of special concern to global regulators. As a result, US FDA and other regulatory agencies have taken steps to address the issue of Nitrosamines impurities in pharmaceuticals. Detection and quantification of these trace nitrosamines in APIs and drug products can be challenging and necessitates the use of advanced and sensitive tools to meet regulatory requirements.



Impurity guidelines have been developed by international Conference on Harmonization (ICH). ICH M7 (R1) assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. 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 (R7) 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-Nitroso dimethylamine 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	

EXPERIMENTAL METHODOLOGY:

Instrumentation

Gas chromatograph coupled with Triple Quadrupole Mass spectrometer and Auto sampler (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 and during solution preparations Eppendorf Micropipettes (research plus) were used.

Chemicals and Reagents: Methanol, Dichloromethane, Acetone, Methane sulfonyl chloride and Anhydrous Sodium Sulphate using GC-MS Grade, Sodium Hydroxide (AR Grade), Water (Milli-Q)

Chromatographic Conditions for GC: Column oven temperature; Initial temperature (50.0ºC) for 5 min; increased to 250.0ºC @ 20.0ºC/min, hold for 0 min; then increased to 280ºC @ 30.0ºC/min, hold for 4 min; Helium gas used a carrier gas and linear velocity is 44.3 cm/sec. Injection mode is used splitless for 1 min; and injector temperature is 200ºC. During injection high pressure injection mode is used (150kPa).

Conditions for MS: Ion source temperature is 230.0ºC and interface temperature is 250.0ºC. Detector voltage 0.6kV and CID gas is on. Solvent cut time is 5.50 min. Qualifier and Qualifier ions for N-Nitrosamine impurities are given below:

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 diluent, standard and sample solution: The diluent used was homogeneous mixture of 4 gm sodium hydroxide/1 litre water. Reference standards of Nitrosamines impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine) was used for standard stock solution. All Nitrosamines impurities stock solution was prepared in Methanol and stable of these solutions are at least 14 days when stock solutions stored under dark conditions at room temperature. The final standard solution of N-Nitrosamines impurities was prepared in diluent.was used the final concentration is about 0.0026 mcg/ml for all N-Nitrosamine impurity apart from N-Nitrosodimethylamine. The final concentration of N-Nitrosodimethylamine is about 0.0095 mcg/ml. The concentration of sample solution is 8.0 mg/ml of Telmisartan

Procedure for derivatization of blank, standard and sample solution: Accurately pipette and transfer 3.0 mL of acetone into a 50ml centrifuge tube placed in an ice bath. Add 150µL of diluent to it and mix. Further, add 100 µL of Methane sulfonyl chloride to it and mix gently. Allow this solution mixture to stand in the ice bath for 10-15 minutes. Transfer 7.0 mL of diluent to this mixture and placed in the ice bath, cover with lid and shake well to mix. Bring this solution mixture to room temperature. Add accurately 5.0 mL of Dichloromethane to this mixture in the centrifuge tube (Appropriate precautions for the pipetting of Dichloromethane to be taken). Cover with lid and mix the solutions well using a vortex mixture for 10-15 seconds and centrifuge at 3000 rpm for 30 minutes. Carefully extract about 3.0 mL of the lower organic layer using a 5 mL syringe fitted with a needle and filter the solvent using a 0.22 µm PTFE syringe filter in a 20 mL Headspace vial containing about 1000 mg anhydrous sodium sulphate. Label this solution as blank Solution.

For sample solution, weight the crushed tablet powder (active ingredient of Telmisartan approx. 80 mg) in into a 50ml centrifuge tube and follow the above procedure. For standard solution, transfer 7.0 mL of standard solution instead of diluent and follow the blank solution procedure.

System suitability criteria:

a) **%RSD:** The %RSD for the area response of each impurity peak from initial six replicates injection of Standard Solution NMT 25.

b) **S/N Ratio:** The signal to noise ratio (From Quantifier ion) for the peaks observed of the analytes from Sensitivity Standard Solution analysed in the sequence should not be less than 10.

Calculations and results:

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

$$\text{Impurity(ppm)} = \frac{(AT-AB) \times WS \times 7 \times 10 \times P \times \text{Avg. wt.} \times 10^6}{(AS-AB) \times SD \times 10 \times WT \times 100 \times LC}$$

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 standard in mg, SD = dilution of standard, WT = Weight of sample in mg, P = Purity/Potency of Impurity Standard used, LC = Label claim, Avg. wt.: Average weight of tablet in mg.

VALIDATION OF GC-MS/MS METHOD

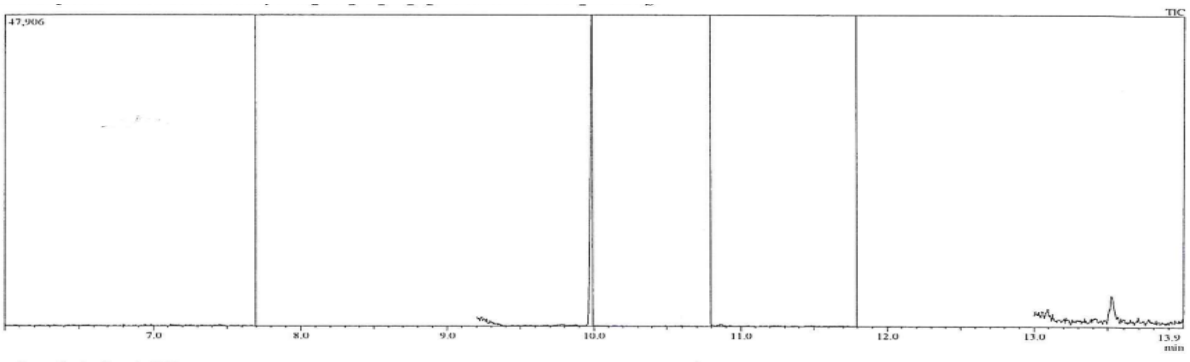
Specificity:

Specificity was determined by injecting blank solution, sensitivity solution, standard solution, placebo solution, sample solution, individual standard solution and sample spiked solution with N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosoisopropylethylamine, N-Nitrosodiisopropylamine and N-Nitrosodibutylamine impurities at specification level.

There is no interference observed on the retention time of N-Nitrosamines impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine) from blank solution and placebo solution and all Nitrosamine impurities are separated to each other. Hence based on this study it is concluded that that method is specific. The results of specificity study are given in Table-I from blow Chromatograms.

Table-I

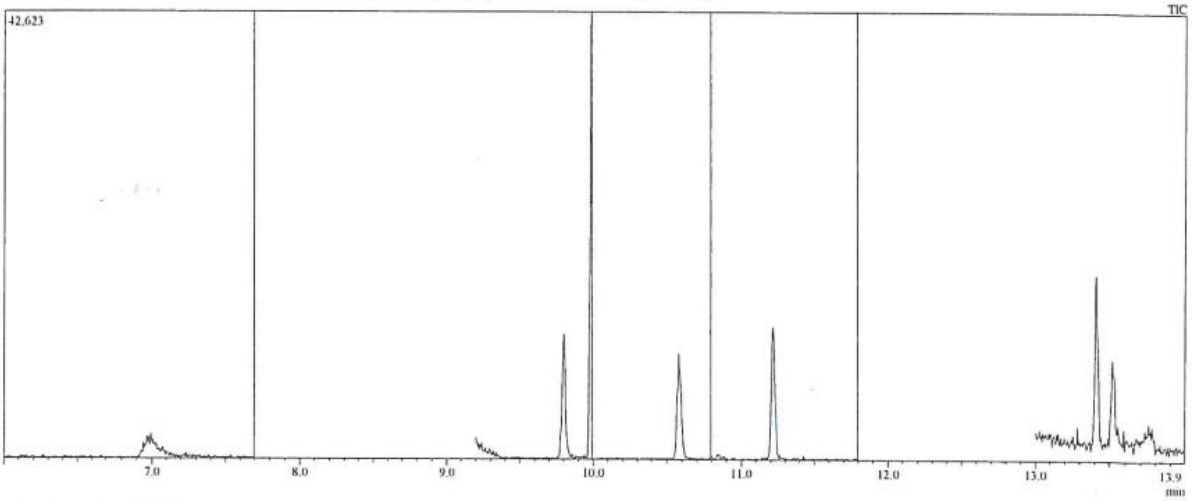
Name of Impurity	Retention Time (Minutes)				
	Blank	Placebo Solution	Un spiked Sample	Spiked Sample	Individual Solution
N-Nitrosodimethylamine (NDMA)	ND	ND	ND	7.0	7.0
N-Nitrosodiethylamine (NDEA)	ND	ND	ND	9.8	9.8
N-Nitrosoisopropylethylamine (NIPEA)	ND	ND	ND	10.6	10.6
N-Nitrosodiisopropylamine (NDIPA)	ND	ND	ND	11.2	11.2
N-Nitrosodibutylamine (NDBA)	ND	ND	ND	13.4	13.4



Quantitative Result Table

ID#	Name	Conc.	R.Time	m/z	Area	Height	S/N
1	N-Nitrosodimethylamine	N.D.(Peak)	-	74.00 > 44.10	---	---	-
2	N-Nitrosodiethylamine	N.D.(Peak)	-	102.00 > 85.10	---	---	-
3	N-Nitrosoisopropylethylamine	N.D.(Peak)	-	116.00 > 99.10	---	---	-
4	N-Nitrosodiisopropylamine	N.D.(Peak)	-	130.00 > 88.10	---	---	-
5	N-Nitrosodibutylamine	N.D.(Peak)	-	116.00 > 99.10	---	---	-

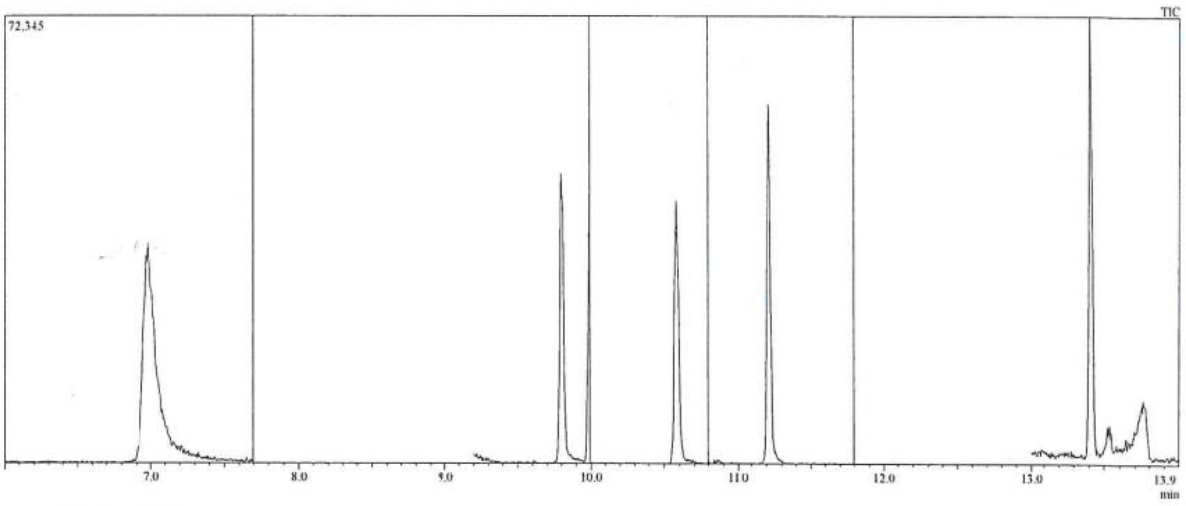
. Chromatograms of Blank solution



Quantitative Result Table

ID#	Name	Conc.	R.Time	m/z	Area	Height	S/N
1	N-Nitrosodimethylamine	0.000	6.982	74.00 > 44.10	9730	1339	12
2	N-Nitrosodiethylamine	0.000	9.802	102.00 > 85.10	14788	6209	242
3	N-Nitrosoisopropylethylamine	0.000	10.583	116.00 > 99.10	17973	6905	306
4	N-Nitrosodiisopropylamine	0.000	11.218	130.00 > 88.10	15813	6435	34
5	N-Nitrosodibutylamine	0.000	13.415	116.00 > 99.10	18695	8494	27

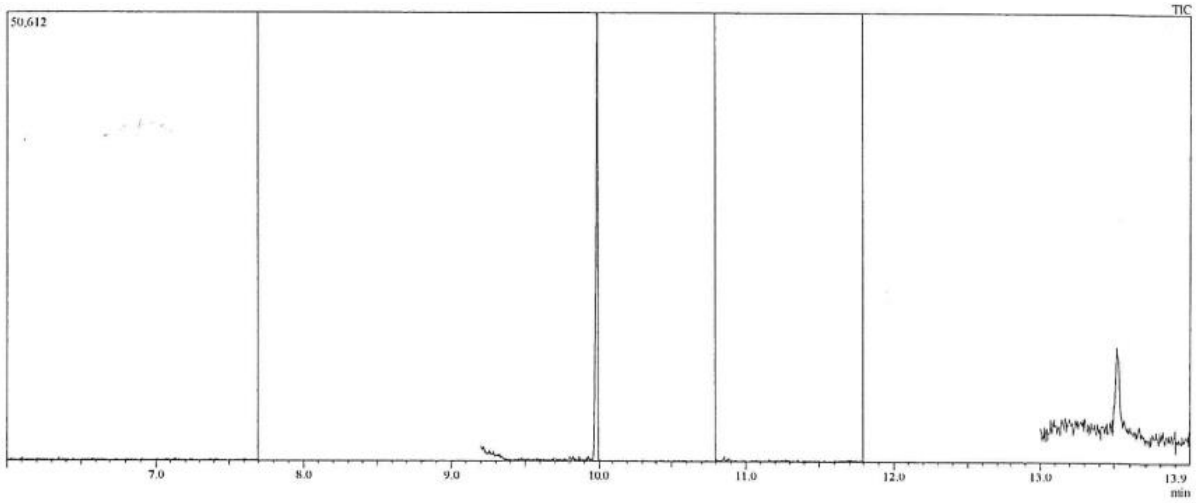
Chromatograms of sensitivity solution



Quantitative Result Table

ID#	Name	Conc.	R.Time	m/z	Area	Height	S/N
1	N-Nitrosodimethylamine	0.000	6.980	74.00 > 44.10	155147	24068	53
2	N-Nitrosodiethylamine	0.000	9.803	102.00 > 85.10	70188	27749	817
3	N-Nitrosoisopropylethylamine	0.000	10.583	116.00 > 99.10	81321	30641	1043
4	N-Nitrosodiisopropylamine	0.000	11.218	130.00 > 88.10	67339	27443	91
5	N-Nitrosodibutylamine	0.000	13.415	116.00 > 99.10	83687	38486	135

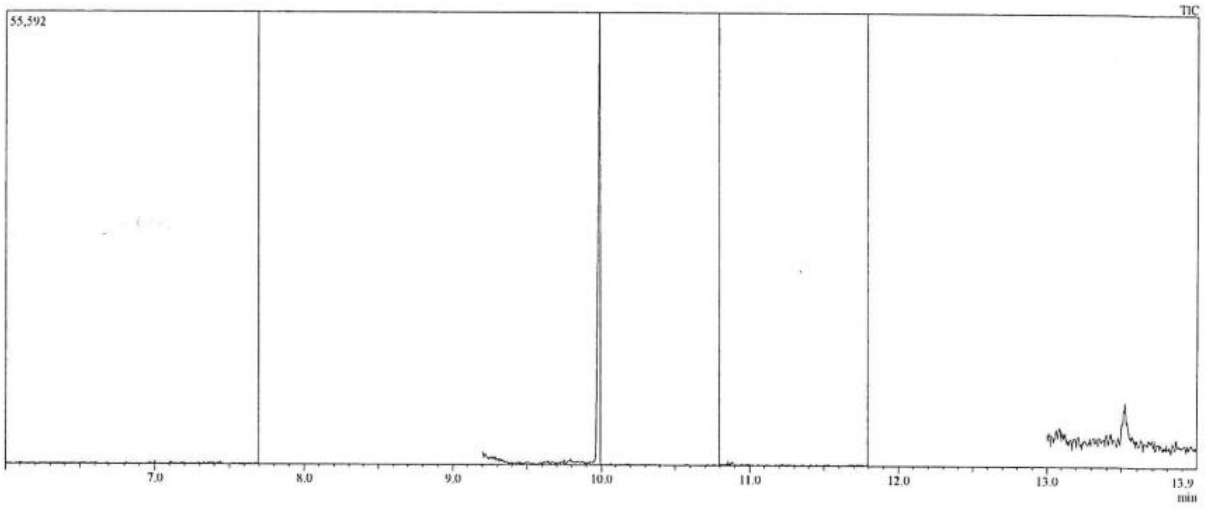
Chromatograms of standard solution



Quantitative Result Table

ID#	Name	Conc.	R.Time	m/z	Area	Height	S/N
1	N-Nitrosodimethylamine	N.D.(Peak)	-	74.00 > 44.10	---	---	-
2	N-Nitrosodiethylamine	N.D.(Peak)	-	102.00 > 85.10	---	---	-
3	N-Nitrosoisopropylethylamine	N.D.(Peak)	-	116.00 > 99.10	---	---	-
4	N-Nitrosodiisopropylamine	N.D.(Peak)	-	130.00 > 88.10	---	---	-
5	N-Nitrosodibutylamine	N.D.(Peak)	-	116.00 > 99.10	---	---	-

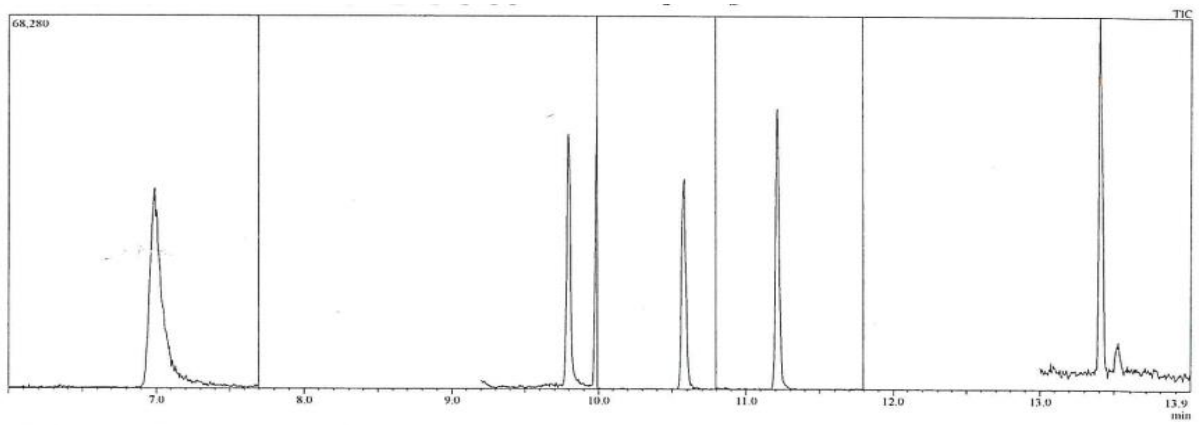
Chromatograms of placebo solution



Quantitative Result Table

ID#	Name	Conc.	R.Time	m/z	Area	Height	S/N
1	N-Nitrosodimethylamine	N.D.(Peak)	-	74.00 > 44.10	---	---	-
2	N-Nitrosodiethylamine	N.D.(Peak)	-	102.00 > 85.10	---	---	-
3	N-Nitrosoisopropylethylamine	N.D.(Peak)	-	116.00 > 99.10	---	---	-
4	N-Nitrosodiisopropylamine	N.D.(Peak)	-	130.00 > 88.10	---	---	-
5	N-Nitrosodibutylamine	N.D.(Peak)	-	116.00 > 99.10	---	---	-

Chromatograms of sample solution (Telmisartan Tablets USP 80 mg)



Quantitative Result Table

ID#	Name	Conc.	R.Time	m/z	Area	Height	S/N
1	N-Nitrosodimethylamine	0.000	6.987	74.00 > 44.10	152790	25167	66
2	N-Nitrosodiethylamine	0.000	9.800	102.00 > 85.10	66617	27517	3931
3	N-Nitrosoisopropylethylamine	0.000	10.580	116.00 > 99.10	72117	28421	1194
4	N-Nitrosodiisopropylamine	0.000	11.215	130.00 > 88.10	59702	24993	171
5	N-Nitrosodibutylamine	0.000	13.412	116.00 > 99.10	82387	37171	70

Chromatograms of spiked sample solution (Telmisartan Tablets USP 80 mg)

Limit of detection and limit of quantitation (LOD & LOQ):

Limit of detection (LOD) was determined through prediction linearity from 0.5% to 40% concentration of N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosoisopropylethylamine, N-Nitrosodiisopropylamine and N-Nitrosodibutylamine impurities with respect to specification limit.
Limit of quantitation (LOQ) = 3× Limit of detection (LOD).

Limit of detection (LOD) and Limit of quantitation (LOQ) for N-Nitrosamines impurities in Telmisartan Tablets USP 20 mg, 40mg and 80 mg are given below table-2.

Table-2

Impurity	LOD Level (ppm)	LOQ Level (ppm)
	w.r.t test	
NDMA	0.12	0.36
NDEA	0.033	0.099
NIPEA		
NDIPA		
NDBA		

Confirmation of LOD was performed by injecting six replicate injections of concentration equivalent to LOD as determined in determination of limit of detection and calculate the %RSD. The %RSD of six replicate injections for LOD concentration is not more than 33. Details of LOD precision is given below in table-3.

Table-3

Impurity	Area						
	1	2	3	4	5	6	%RSD
NDMA	13776	16786	16499	14108	15327	16751	8.7
NDEA	7547	6592	7967	6573	7205	7710	8.0
NIPEA	7036	7194	8129	8012	7127	8357	7.7
NDIPA	6965	5938	6265	6710	6686	6480	5.6
NDBA	8617	8096	9834	9332	9608	9773	7.6

Precision of LOQ was established by injecting six replicate injections of concentration equivalent to LOQ as determined in limit of quantitation and % relative standard deviation was calculated. The % RSD of six replicate injections for LOQ concentration is not more than 20. Details of LOQ precision is given below in table-4.

Table-4

Impurity	Area						
	1	2	3	4	5	6	%RSD
NDMA	48675	43464	42067	42367	47994	46854	6.5
NDEA	21149	20460	20519	20176	20992	21817	2.8
NIPEA	22296	24029	23853	23641	23934	24266	3.0
NDIPA	19342	18377	19441	18362	19932	19259	3.3
NDBA	26411	26113	26588	26975	26417	27776	2.2

Linearity:

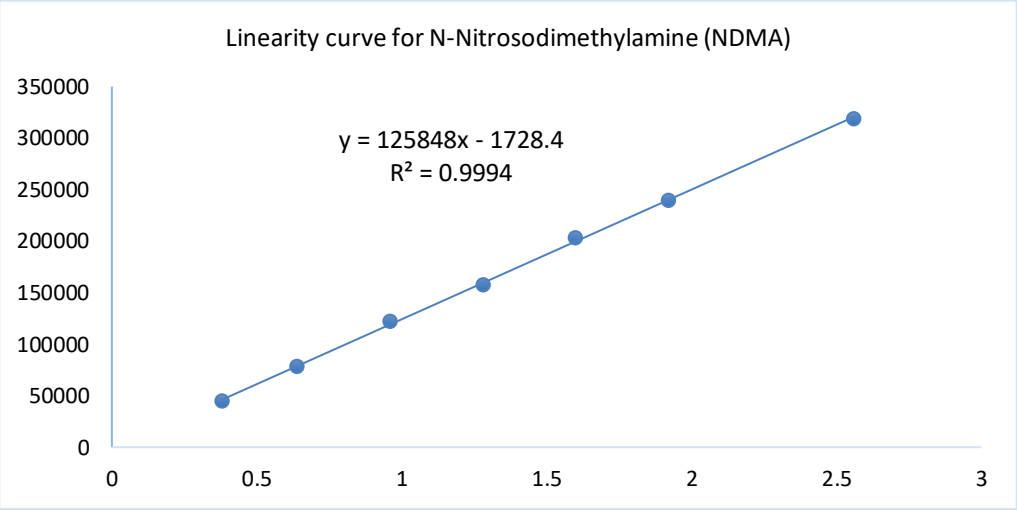
A linearity study was performed at seven concentration levels from LOQ to 200% of specification limit of each Nitrosamine impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosoisopropylethylamine, N-Nitrosodiisopropylamine and N-Nitrosodibutylamine) and injected in duplicate.

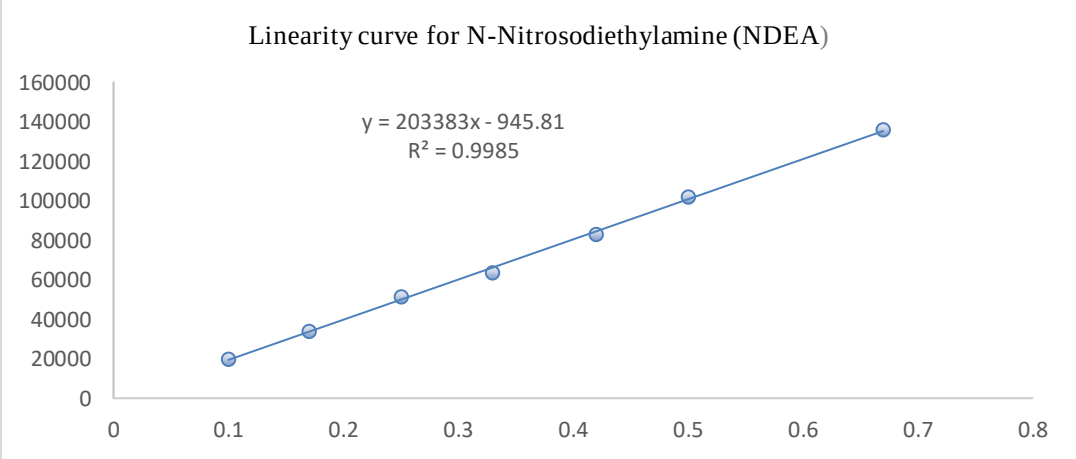
Linearity graph was plotted between area response and concertation of analyte. Squared correlation coefficient, Correlation coefficient, Residual sum of square, slope and Y- intercept were calculated. Data obtained from linearity study, the correlation coefficient of each Nitrosamine impurities is within acceptance limit of NLT 0.98. The method is found linear from LOQ to 200 % of specification limits. Details of Squared correlation coefficient, Correlation coefficient, Residual sum of square, slope and Y- intercept is given below in table-5.

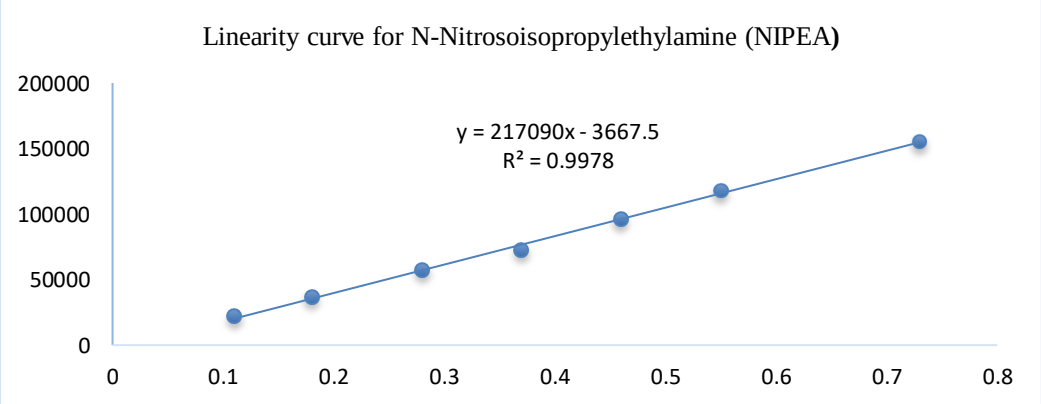
Table-5

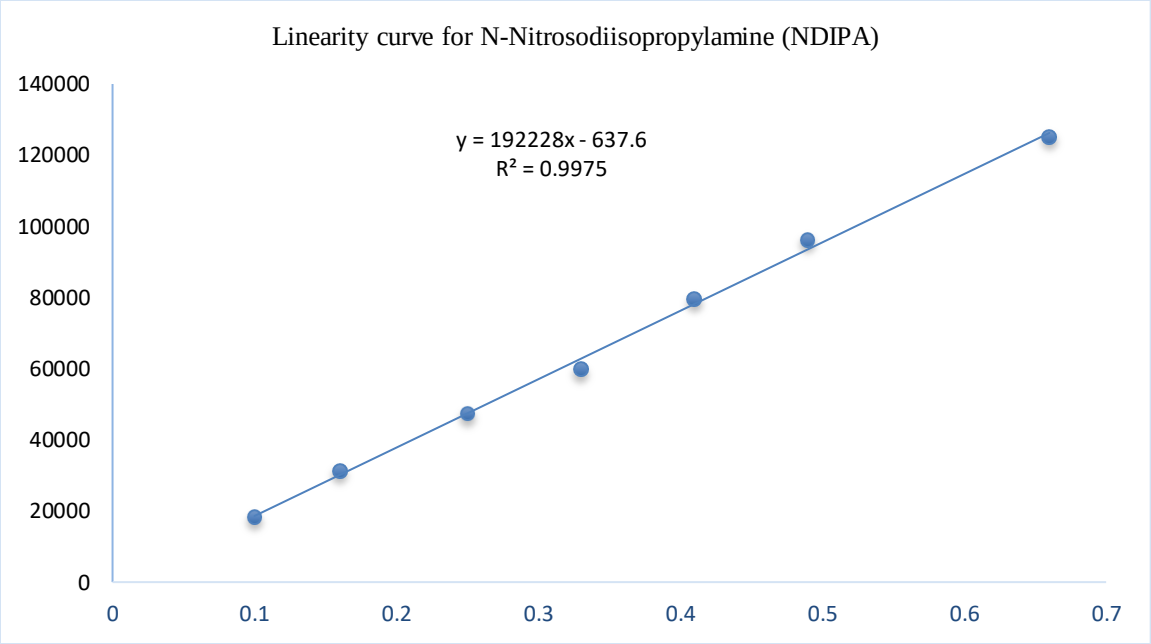
Linearity level	N-Nitroso dimethyl amine		N-Nitroso diethyl amine		N-Nitroso isopropylethyl amine		N-Nitroso-diisopropyl amine		N-Nitroso dibutyl amine	
	Conc. (ppm)	Mean area	Conc. (ppm)	Mean area	Conc. (ppm)	Mean area	Conc. (ppm)	Mean area	Conc. (ppm)	Mean area
LOQ level	0.38	44364	0.10	19970	0.11	21554	0.10	18292	0.09	24694
50% level	0.64	77720	0.17	33900	0.18	36524	0.16	31183	0.16	41724
75% level	0.96	121900	0.25	51396	0.28	56821	0.25	47220	0.24	62279
100% level	1.28	157775	0.33	63300	0.37	72216	0.33	59693	0.32	74804
125% level	1.60	203428	0.42	83162	0.46	95693	0.41	79319	0.39	102787
150% level	1.92	239510	0.50	101988	0.55	118075	0.49	96218	0.47	123932
2000% level	2.56	318629	0.67	135917	0.73	155245	0.66	124960	0.63	163143
Correlation coefficient		1.00	1.00		1.00		1.00		1.00	
Squared Correlation coefficient		0.9994	0.9985		0.9978		0.9975		0.9947	
Slope		125848	203383		217090		192228		259448	
Y-Intercept		-1728.4	-945.81		-3667.50		-637.60		-480.96	
Residual sum of square		32490709.46	14508110.10		28774983.52		20920070.80		74417418.33	

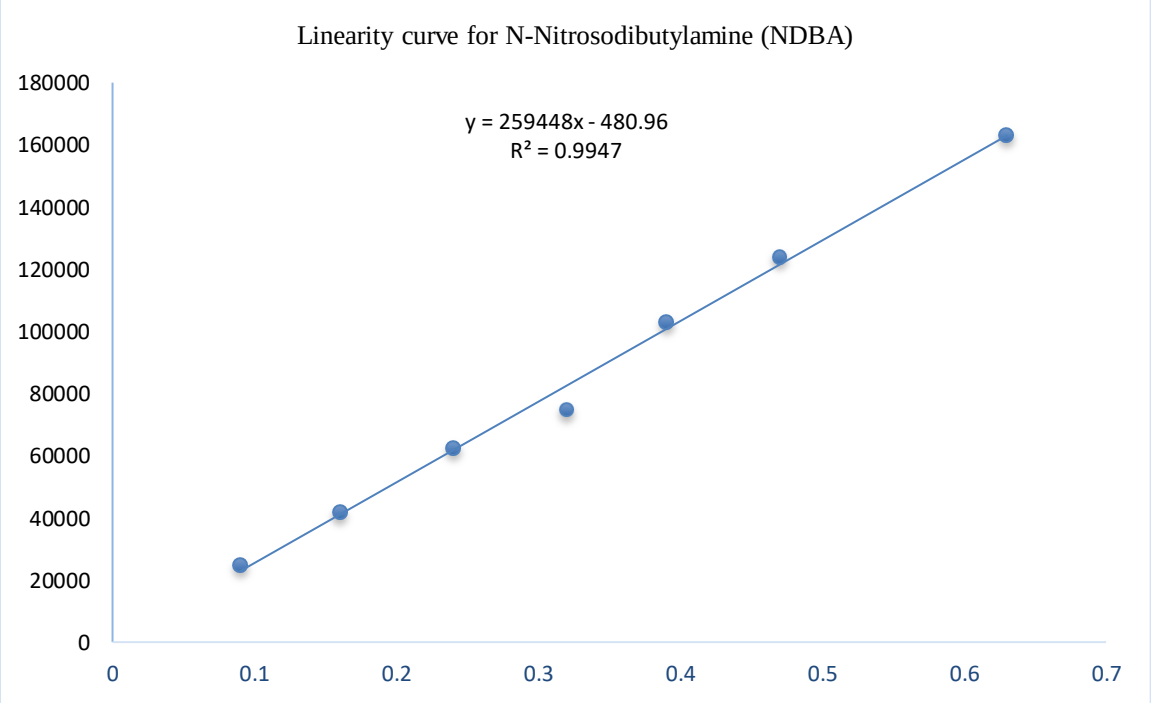
Linearity curve of each Nitrosamine impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosoisopropylethylamine, N-Nitrosodiisopropylamine and N-Nitrosodibutylamine) are given below.











Precision: (System Precision)

System precision was determined by injecting blank, sensitivity standard solution and six replicates of standard preparation. %RSD was calculated for area response.

The %RSD for the area response (from Quantifier ion) of each Nitrosamine impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosoisopropylethylamine, N-Nitrosodiisopropylamine and N-Nitrosodibutylamine) from the initial six replicates of Standard Solution were found below 25% and the signal to noise ratio (From Quantifier ion) for the peaks observed of the analytes from Sensitivity Standard Solution was found more than 10. Details are given below in table-6.

Table-6

Injection	AREA				
	NDMA	NDEA	NIPEA	NDIPA	NDBA
1	172692	56398	108512	51374	64520
2	171643	56797	106690	50364	67715
3	162273	52561	102815	48339	64024
4	159441	52307	102978	47020	63279
5	168179	53824	105176	49008	62922
6	171598	54124	103425	49386	62572
Mean	167638	54335	104933	49249	64172
SD	5541	1891	2304	1525	1877
% RSD	3.3	3.5	2.2	3.1	2.9
S/N ratio	15	186	281	15	53

Precision: (Method Precision)

Method precision was determined by analyzing six sample preparations as per the method representing a single batch. Determined the results of these samples and evaluate the precision of the method by computing the %RSD results for (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosoisopropylethylamine, N-Nitrosodiisopropylamine, N-Nitrosodibutylamine) N-Nitrosamine impurities.

%RSD for result for each N-Nitrosamine impurity of six sample for tablet strength 80mg is not applicable as it was found not detected except NDBA is below detection limit in sample preparation-2 and sample preparation-5. Details of results are given below in table-7.

Table-7

Sample	Results (ppm)				
	NDMA	NDEA	NIPEA	NDIPA	NDBA
1	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	BDL
3	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	BDL
6	ND	ND	ND	ND	ND
Mean	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA
%RSD	NA	NA	NA	NA	NA

Since the results of each N-Nitrosamine impurity was concluded as above Hence performed the spiked test repeatability by spiking the N-Nitrosamine impurities at specification level in the sample and injected in six replicate sample preparation. Calculated the results of N-Nitrosamine impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosoisopropylethylamine, N-Nitrosodiisopropylamine, N-Nitrosodibutylamine). The % RSD for result of each N-Nitrosamine impurity from six spiked sample was found below 25%. Details of spiked samples results are given below in table-8.

Table-8

Sample	Results (ppm)				
	NDMA	NDEA	NIPEA	NDIPA	NDBA
1	1.338	0.303	0.311	0.285	0.310
2	1.229	0.330	0.330	0.312	0.330
3	1.299	0.314	0.317	0.297	0.330
4	1.370	0.323	0.338	0.325	0.340
5	1.309	0.321	0.327	0.309	0.331
6	1.340	0.309	0.338	0.320	0.347
Mean	1.31	0.32	0.33	0.31	0.33
SD	0.049	0.010	0.011	0.015	0.012
%RSD	3.7	3.1	3.3	4.8	3.6

Precision: (Intermediate Precision)

Intermediate precision was determined by analyzing six spiked sample preparations as per the method representing a single batch by different analyst on different day. % RSD for each impurity results were calculated. On the basis of method precision study, intermediate spiked study was performed as follow. Since results of each impurity were concluded in method precision study hence six individually test sample spiked with each impurity for the all tablet Strength was analyzed in intermediate precision. The % RSD for result of each N-Nitrosamine impurity from six spiked sample was found below 25%. Details of spiked samples results are given below in table-9.

Table-9

Sample	Results (ppm)				
	NDMA	NDEA	NIPEA	NDIPA	NDBA
1	1.123	0.335	0.327	0.293	0.328
2	1.208	0.332	0.358	0.312	0.334
3	1.200	0.340	0.361	0.307	0.332
4	1.249	0.343	0.352	0.318	0.326
5	1.181	0.334	0.361	0.304	0.329
6	1.229	0.348	0.363	0.321	0.345
Mean	1.20	0.34	0.35	0.31	0.33
SD	0.044	0.006	0.014	0.010	0.007
%RSD	3.7	1.8	4.0	3.2	2.1

Recovery:

Accuracy was done by adding known amount of N-Nitrosamine impurities in the sample. Accuracy was done at LOQ, 100% and 150% concentration of specification limit with three preparations at each level and Injected all levels in single. Individual and mean % Accuracy was calculated for each level. The Individual and Mean % Accuracy results for N-Nitrosamine impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosoisopropylethylamine, N-Nitrosodiisopropylamine and N-Nitrosodibutylamine) during validation study were found between 80.0% to 120.0% for LOQ, 100% and 150% level of specification concentration. Details of spiked samples results are given below in table-10.

Table-10

Accuracy of N-Nitrosodimethylamine (NDMA)					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.38	0.377	99.21	98.2
	Sample-2		0.365	96.05	
	Sample-3		0.377	99.21	
100	Sample-1	1.27	1.216	95.75	94.7
	Sample-2		1.191	93.78	
	Sample-3		1.200	94.49	
150	Sample-1	1.90	1.747	91.95	92.8
	Sample-2		1.836	96.63	
	Sample-3		1.709	89.95	
Accuracy of N-Nitrosodiethylamine (NDEA)					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.10	0.092	92.00	94.3
	Sample-2		0.098	98.00	
	Sample-3		0.093	93.00	
100	Sample-1	0.33	0.320	96.97	96.2
	Sample-2		0.323	97.88	
	Sample-3		0.309	93.64	
150	Sample-1	0.49	0.455	92.86	94.8
	Sample-2		0.469	95.71	
	Sample-3		0.469	95.71	
Accuracy of N-Nitrosoisopropylethylamine (NIPEA)					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.11	0.097	88.18	94.8
	Sample-2		0.107	97.27	
	Sample-3		0.109	99.09	
100	Sample-1	0.36	0.363	100.83	99.4
	Sample-2		0.365	101.39	
	Sample-3		0.346	96.11	
150	Sample-1	0.54	0.501	92.78	95.8
	Sample-2		0.519	96.11	
	Sample-3		0.532	98.52	
Accuracy of N-Nitrosodiisopropylamine (NDIPA)					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.10	0.098	98.00	97.3
	Sample-2		0.101	101.00	
	Sample-3		0.093	93.00	
100	Sample-1	0.32	0.324	101.25	97.4
	Sample-2		0.313	97.81	
	Sample-3		0.298	93.13	

150	Sample-1	0.49	0.446	91.02	93.3
	Sample-2		0.462	94.29	
	Sample-3		0.464	94.69	
Accuracy of N-Nitrosodibutylamine (NDBA)					
Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
LOQ	Sample-1	0.09	0.099	110.00	112.6
	Sample-2		0.104	115.56	
	Sample-3		0.101	112.22	
100	Sample-1	0.31	0.311	100.32	101.9
	Sample-2		0.331	106.77	
	Sample-3		0.306	98.71	
150	Sample-1	0.47	0.448	95.32	98.6
	Sample-2		0.476	101.28	
	Sample-3		0.466	99.15	

DISCUSSION:

A gas chromatography coupled with mass spectrometry (GC-MS/MS) 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 gas chromatographic coupled with mass spectrometry (GC-MS/MS) system was suitable for the determination of N-Nitrosamine impurities analysis in Telmisartan Tablets USP 20 mg, 40 mg and 80 mg. The developed method was performed for specificity, LOD and LOQ determination & precision, precision, linearity and Accuracy.

CONCLUSION:

Only few methods were reported on the determination of N-Nitrosamine impurities in drug products. Based on the above results it can be concluded that method for the determination of N-Nitrosamine Impurities {N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosoisopropylethylamine (NIPEA), N-Nitrosodibutylamine (NDBA)} in Telmisartan Tablets USP 20 mg, 40 mg and 80 mg by GC-MS/MS is successfully validated and the results of all validation parameters are found well within the acceptance criteria.

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