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Analytical Method Validation For The Content Estimation of N-Nitrosodimethylamine (NDMA) & N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA) & N-Nitrosodiisopropylamine (NDIPA) In Pantoprazole Sodium Sesquihydrate GCMS-HS

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ABSTRACT: The Objective of this paper is to validate an accurate, precise, and linear gas chromatographic-mass spectrometry selective ion monitoring (SIM) method for quantitative estimation of N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA) & N-Nitrosodiisopropylamine (NDIPA) as an impurity in Pantoprazole Sodium Sesquihydrate active pharmaceutical ingredient (API). Chromatographic separation of NDMA, NDEA, NEIPA, and NDIPA was achieved in, DB-WAX, 30.0 m X 0.25 mm, 0.5 µm Capillary column or Equivalent column, using helium carrier gas with 3.0 ml/min, Split ratio 1:2 and run time was 16.0 minutes. The method was fully validated, complying Food and Drug Administration, ICH, and European Medicines Agency guidelines. The retention times of NDMA, NDEA, NEIPA and NDIPA was 6.6, 7.4, 7.7 and 7.9 respectively. No blank interference was found in retention time of impurities. Linearity was achieved 0.99 for all known impurities. The LOD concentration for NDMA, NDEA, NEIPA and NDIPA is 0.033 ppm and LOQ concentration for all these impurities is 0.099 ppm. Method precision was found within predefined acceptance criteria. The methods were successfully validated to determination and quantification of above mentioned genotoxic impurities in pantoprazole API. Hence, the method holds good for the routine trace analysis of these impurities in Itraconazole and various pharmaceutical industries as well as academics.

KEYWORDS: NMDA, NMEA, Pantoprazole, Gas chromatography-mass spectrometry, Method validation.

I. INTRODUCTION:

Pantoprazole is a proton pump inhibitor (PPI) that binds irreversibly and specifically to the proton pump, thereby reducing gastric acid secretion. Pantoprazole has a relatively long duration of action compared with other PPIs, and a lower propensity to become activated in slightly acidic body compartments. To date, no drug-drug interactions have been identified with pantoprazole in numerous interaction studies [1-2]. Pantoprazole sodium (PPS), chemically described as 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-bezimadazole sodium salt is an antiulcervative agent. In the synthesis of PPS, 2-(chloromethyl)-3,4-dimethoxypyridine hydrochloride (CDP) is a key raw material and dimethyl sulphate (DMS) is an important reagent. Identification and determination of these two impurities in PPS is essential because CDP is toxic and DMS is genotoxic in nature [3-4]. NDMA and NDEA were classified as Class 2A carcinogens (probably carcinogenic to humans) according to the International Agency for Research on Cancer (IARC). In ICH M7 these two compounds are categorized as first class chemicals (substances with recognized genotoxicity/mutagenicity and carcinogenic), thus strict measures are in place to ensure that their levels are not higher than the acceptable limits (AL). From the datasheet released by the U.S. FDA and EMA, the acceptable limits for NDMA and NDEA in Valsartan Active Pharmaceutical Ingredient (API) were set to 0.3 ppm and 0.08 ppm, respectively, which is far below toxicological threshold (TTC) of most common genotoxic impurities (TTC of 1.5 ppm). Sensitive and reliable

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analytical instrumentations are therefore required for the detection and quantification of nitrosamines impurities in APIs and finished drug products [5-6]. Recently, the Chinese Food and Drug Administration (CFDA) and the U.S. FDA have released their recommended methods for the detection of nitrosamines (NDMA and NDEA) in various sartans drug product and drug substances on their websites [7-8]. Chromatography in different forms is the leading analytical method for separation of components in a mixture. Chromatographic methods can be classified according to the physical state of the mobile phase into the following basic categories: gas chromatography (GC), supercritical fluid chromatography (SFC) and liquid chromatography (LC) [9-13].

II. MATERIAL AND METHOD

Instruments/ chemicals & reagents /standards & samples:

S. No	Instrument/Materials	Make/Model/Lot No	Grade/Purity
1	GCMS	Shimadzu GCMS-TQ8040	NA
2	Analytical balance	RADWAG & XA 82/220.R2/LC&GC	NA
3	Column (DB-WAX)	30m X 0.250 mm, 0.5µm	NA
4	Methanol	SH8SA81209	HPLC
5	N-Methyl-2-Pyrollidinone	Spectrochem	GC
6	N-Nitrosodimethylamine	MNEA/001/08/2018	98.1
7	Nitraso Diethyl Amine	H5GMI	100
8	N-Nitrosoethylisopropylamine	L47-005	96.80%
9	N-Nitrosodiisopropylamine	L36-081(1)	96.70%
10	Pantoprazole Sodium Sesquihydrate	PTF(EU)PAA052/II/19-20	NA

III. METHODOLOGY:

Chromatographic Conditions:

Instrument	GCMS-HS-TQ8040
Column	DB-WAX, 30.0 m X 0.25 mm, 0.5 µm Capillary column or Equivalent
Detector	MS
Carrier gas	Helium
Column Oven Program	Initial: 70°C Hold time for 4.0 minutes
	Ramp rate: 20°C/minute at 240°C hold for 3.5 minutes
Injection Mode	Split
Split	1:2
Flow Control Mode	Linear velocity

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Run Time	16.00 minutes				
Column flow	3.00 mL/min				
Purge flow	3.00 mL/min				
Ion source temperature	230°C				
Interface temperature	240°C				
Event Time	0.200sec				
Start Time	4.00min				
End Time	12.00min				
Solvent cut time	4.00min				
Detector gain mode	Relative the Tuning result				
Acquisition mode	MRM				
Q1 Resolution	Unit				
Q3 Resolution	Unit				
Compound Name	N-Nitrosodimethylamine	Ch1-m/z	74.00>44.00	CE	5.00kV
Compound Name	N-Nitrosodiethylamine	Ch2-m/z	102.00>85.00	CE	5.00kV
Compound Name	N-Nitrosoethylisopropylamine	Ch3-m/z	116.00>99.00	CE	5.00kV
Compound Name	N-Nitrosodiisopropylamine	Ch4-m/z	130.00>88.00	CE	5.00kV

Head Space Parameters:

Oven Temperature	120°C
Sample Line Temperature	125°C
Transfer Line Temperature	130°C
Shaking Level	5
Pressurizing Gas Pressure	10 psi
Equilibrating Time	15.0 min
Pressurizing Time	0.2 min
Pressure Equilibration Time	0.2 min
Load Time	0.1 min

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Load Equilibration Time	0.05 min
Injection Time	1.00 min
Needle flush time	5.0 min
GC Cycle Time	23.0 min

Preparation of blank & standard solutions:

Preparation of Blank: Pipette 2mL of N-Methyl-2-Pyrrlodinone and transfer into 20mL HS vial crimp the vial immediately with cap and septa and place into GCMS-HS system.

Preparation of NDMA standard stock solution (30ppm w.r.t test conc.): Weigh about 10mg of NDMA standard into 10mL volumetric flask, mix with 3mL of diluent and make up to the volume with diluent and mix well. Transfer 0.7mL of above solution into 100mL volumetric flask and dilute to volume with diluent and mix well.

Preparation of NDEA standard stock solution (30ppm w.r.t test conc.): Weigh about 10mg of NDEA standard into 10mL volumetric flask, mix with 3mL of diluent and make up to the volume with diluent and mix well. Transfer 0.7mL of above solution into 100mL volumetric flask and dilute to volume with diluent and mix well.

Preparation of NEIPA standard stock solution (30ppm w.r.t test conc.): Weigh about 10mg of NEIPA standard into 10mL volumetric flask, mix with 3mL of diluent and make up to the volume with diluent and mix well. Transfer 0.7mL of above solution into 100mL volumetric flask and dilute to volume with diluent and mix well.

Preparation of NDIPA standard stock solution (30ppm w.r.t test conc.): Weigh about 10mg of NDIPA standard into 10mL volumetric flask, mix with 3mL of diluent and make up to the volume with diluent and mix well. Transfer 0.7mL of above solution into 100mL volumetric flask and dilute to volume with diluent and mix well.

Preparation of Standard solution: (0.3ppm of each NDMA, NDEA, NEIPA, NDIPA w.r.t test conc.): Pipette 1.0mL of NDMA, NDEA, NEIPA & NDIPA standard stock solutions into 100mL volumetric flask dilute to volume with diluent and mix well. Transfer accurately 2.0mL of Standard solution into a 20mL HS vial and immediately crimp the vial.

Preparation of NDMA, NDEA, NEIPA, NDIPA LOQ solution: (0.09ppm of each NDMA, NDEA, NEIPA, NDIPA w.r.t test conc.): Transfer 30mL of standard solution into 100mL volumetric flask and dilute to volume with diluent and mix well.

Preparation of NDMA, NDEA, NEIPA, NDIPA LOD solution: (0.03ppm of each NDMA, NDEA, NEIPA and NDIPA w.r.t test conc.): Transfer 10mL of standard solution into 100mL volumetric flask and dilute to volume with diluent and mix well.

Preparation of sample solution: (Prepare in duplicate): Weigh accurately 400 mg of Pantoprazole Sodium Sesquihydrate sample in to 20mL head space vial, add 2mL of diluent crimp the vial immediately with cap and septa and place into GCMS-HS system.

Procedure: Inject blank solution (two) and standard solution (six injections) into the GCMS system and check the system suitability parameters. Then inject sample solution record the chromatograms. Measure the area response of NDMA, NDEA, NEIPA and NDIPA peak. Disregard the peaks due to blank. The retention time of NDMA about 6.7 minutes and NDEA peak is about 7.5 minutes, NEIPA about 7.8 minutes and NDIPA about 8.0 minutes.

System Suitability Requirements: % RSD calculated for the peak areas of NDMA, NDEA, NEIPA & NDIPA peak areas obtained from six injections of standard solution should be not more than 15.0. The cumulative %RSD calculated for the peak areas of NDMA, NDEA, NEIPA & NDIPA from initial six injections and online standard solution should not be more than 15.0.

Calculation: Content of NDMA, NDEA, NEIPA & NDIPA in Pantoprazole Sodium Sesquihydrate calculated by µg/g

$$\frac{AT-AB}{AS-AB} \times \frac{CS}{CT} \times \frac{P}{100} \times 1000000$$

Calculate the content of NDMA, NDEA, NEIPA & NDIPA in preparation-1 and preparation-2 of Pantoprazole Sodium Sesquihydrate using above formula and report the average value.

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Specification Limits: 0.3ppm of each NDMA, NDEA, NEIPA and NDIPA w.r.t. test conc. As per the sponsor

Where,

AT = Peak area of NDMA, NDEA, NEIPA & NDIPA obtained in test solution.

AB = Area response of peak in the chromatogram of the respective blank

AS =Average area of NDMA, NDEA, NEIPA & NDIPA in standard solution

CS = Concentration of NDMA, NDEA, NEIPA & NDIPA in standard solution (mg/mL)

CT = Test concentration (mg/mL)

P = Purity/ Assay of NDMA, NDEA, NEIPA & NDIPA Standard (%)

IV. RESULT AND DISCUSSION:

System Suitability: The system suitability solutions were prepared by using NDMA, NDEA, NEIPA and NDIPA standard as per analytical test procedure describe above and injected into the GC-MS HS system. The system suitability parameters were evaluated and found within the limits. %RSD calculated for the peak areas of NDMA, NDEA, NEIPA and NDIPA in system suitability is 4.2, 3.6, 5.0 and 5.6 respectively. The results are summarized in Table No-1.

Table No-1: System Suitability Results for NDMA, NDEA, NEIPA and NDIPA

Preparation	Peak Area			
	NDMA	NDEA	NEIPA	NDIPA
Standard Solution-1	9967	5031	7497	3929
Standard Solution-2	11359	4895	7761	4245
Standard Solution-3	10574	5250	8080	3958
Standard Solution-4	10687	5206	8595	4277
Standard Solution-5	10803	5422	8239	4386
Standard Solution-6	10598	5237	8383	4529
Average	10665	5174	8093	4221
Standard Deviation	447	185	4.6	237
%RSD	4.2	3.6	5.0	5.6

Specificity (Blank Interference): Established the interference in blank. Specificity was conducted by preparing blank, individual standard solution at specification level, as such sample and spike preparation at specification level as per test procedure injected into the GC-MS HS system. No interference was observed in blank at the retention time of NDMA, NDEA, NEIPA and NDIPA. The results are summarized in Table No-2 and Figure No 1 to Figure no 4.

Table No-2: Blank interference results for NDMA, NDEA, NEIPA and NDIPA

Solution	Name	Retention Time (min)	Peak Area	Interference found at the retention time of NDMA, NDEA, NEIPA and NDIPA (Yes/No), in %
Standard	NDMA	6.626	13434	NA
	NDEA	7.412	7070	NA
	NEIPA	7.735	10768	NA
	NDIPA	7.969	5510	NA
Blank	NDMA	6.626	NA	NO
	NDEA	7.412	NA	NO
	NEIPA	7.735	NA	NO
	NDIPA	7.969	NA	NO

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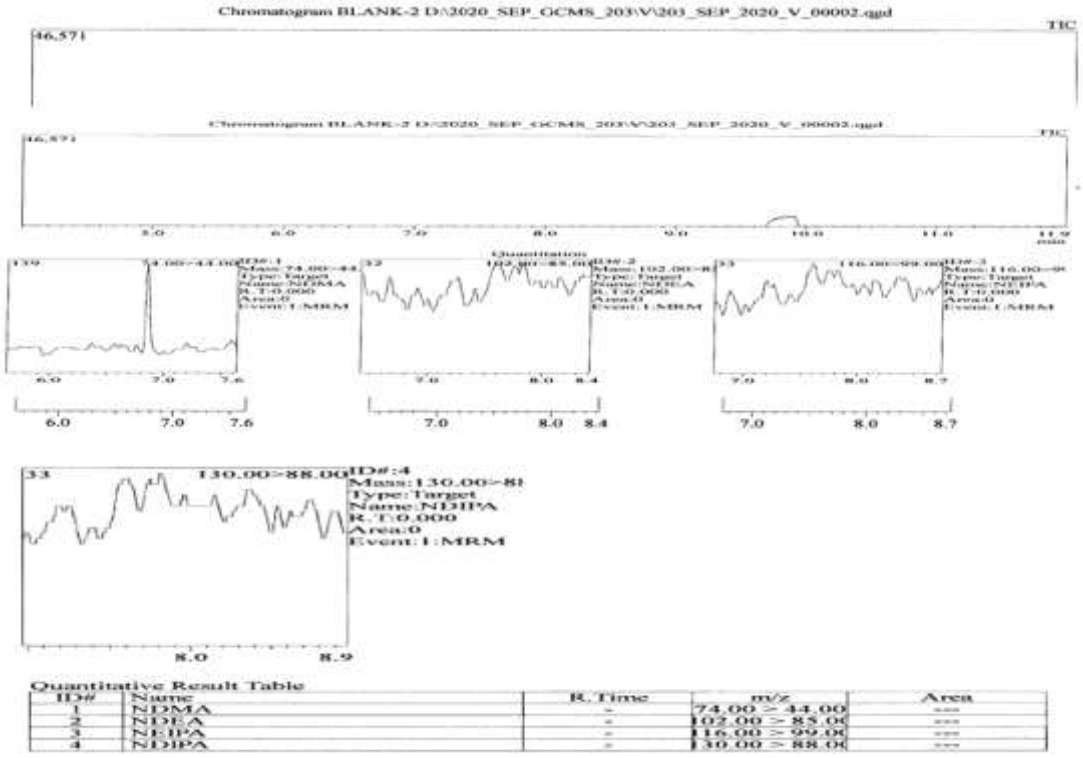

Figure 1: Reference Blank Chromatogram

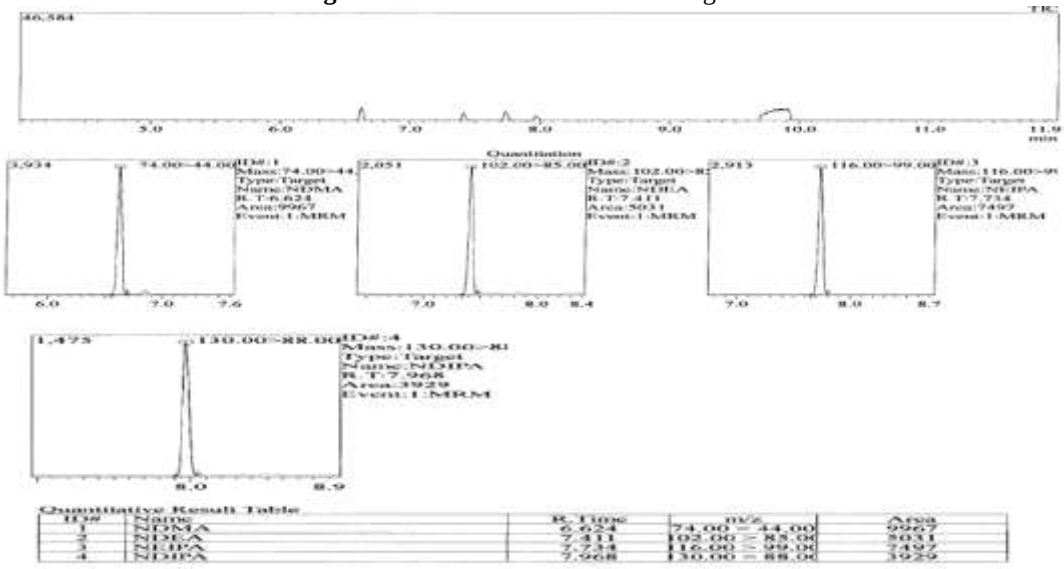

Figure 2: Reference Standard Chromatogram

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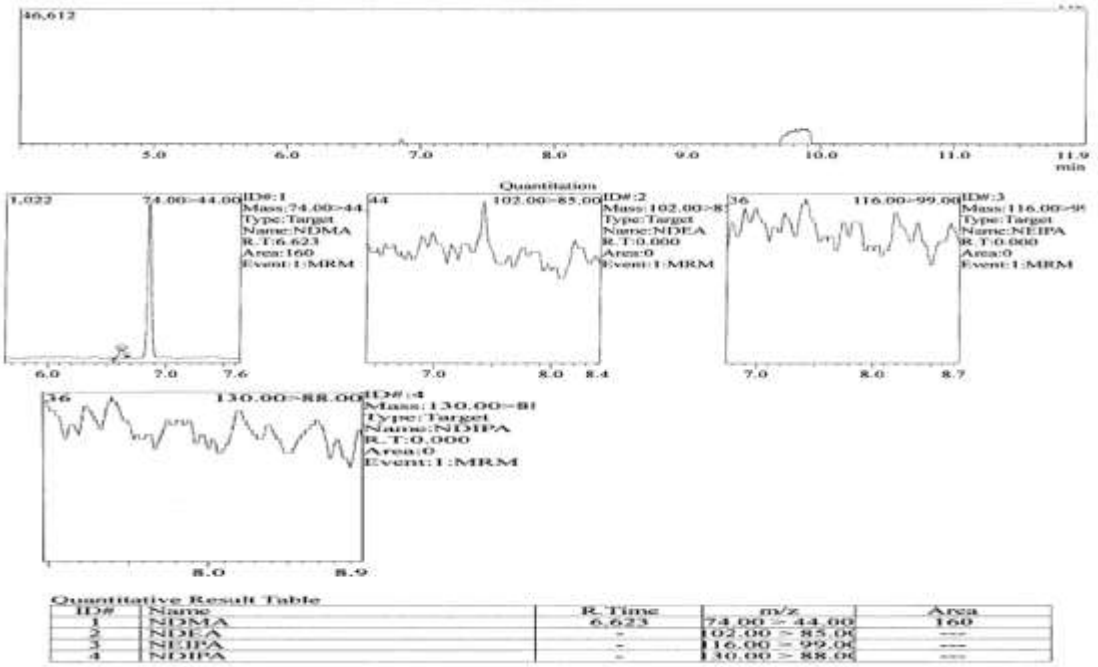

Figure 3: Reference Sample Chromatogram

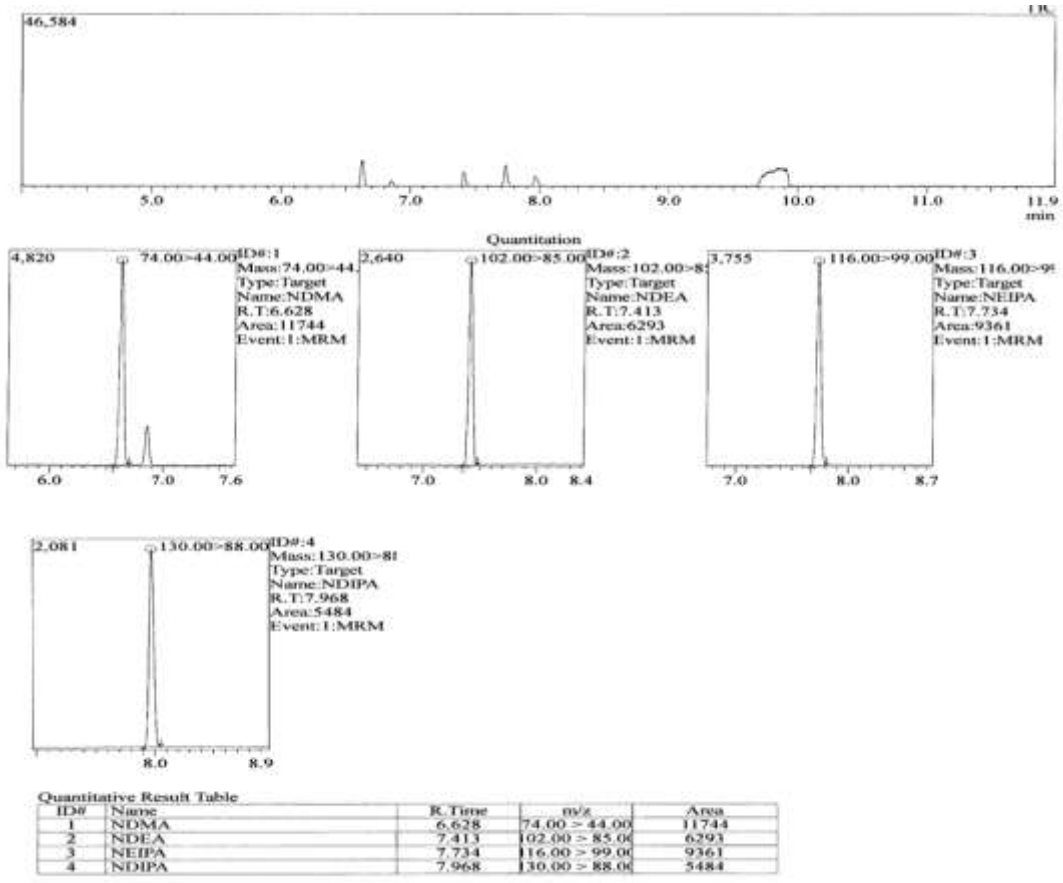

Figure 4: Reference Sample Spike Chromatogram

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LOD & LOQ Confirmation: Established and Confirmed Limit of Detection and Limit of Quantification for NDMA, NDEA, NEIPA and NDIPA. Based on the LOD and LOQ establishment by steyx method, LOD and LOQ solutions are prepared to obtain below define concentrations. The system suitability parameters were evaluated and found to be well within the limits. S/N Ratio for NDMA, NDEA, NEIPA and NDIPA from LOD solution 22.63, 17.32, 38.61 and 14.54. S/N Ratio for NDMA, NDEA, NEIPA and NDIPA from LOQ solution 76.57, 83.83, 106.37 and 53.20. The results of S/N ratio for NDMA, NDEA, NEIPA and NDIPA from LOD and LOQ concentrations are described in Table No.3 & 4.

Table No-3: LOD results for NDMA, NDEA, NEIPA and NDIPA

Name of analyte	LOD Concentration (ppm)	S/N Ratio
NDMA	0.033	22.63
NDEA	0.033	17.32
NEIPA	0.033	38.61
NDIPA	0.033	14.54

Table No-4: LOQ results for NDMA, NDEA, NEIPA and NDIPA

Name of analyte	LOQ Concentration (ppm)	S/N Ratio
NDMA	0.099	76.57
NDEA	0.099	83.83
NEIPA	0.099	106.37
NDIPA	0.099	53.20

LOQ Precision: Prepare six samples by spiking NDMA, NDEA, NEIPA and NDIPA at LOQ level. The system suitability parameters were evaluated and found to be well within the limits. The %RSD for content of NDMA, NDEA, NEIPA and NDIPA at LOQ level obtained from six preparations is found to be 4.8, 7.3, 3.2 and 8.0 respectively (% RSD calculated for the content of NDMA, NDEA, NEIPA and NDIPA obtained from six injections of LOQ standard solution should be not more than 20.0). The results are summarized in **Table No-5.**

Table No-5: LOQ Precision results for NDMA, NDEA, NEIPA and NDIPA

LOQ Preparation	content of Impurities (ppm)			
	NDMA	NDEA	NEIPA	NDIPA
Preparation-1	0.1318	0.1417	0.1219	0.1032
Preparation-2	0.1305	0.1210	0.1230	0.1197
Preparation-3	0.1248	0.1294	0.1269	0.1231
Preparation-4	0.1200	0.1233	0.1170	0.1173
Preparation-5	0.1160	0.01381	0.1194	0.1185
Preparation-6	0.1248	0.1192	0.1167	0.1017
Average	0.1247	0.1288	0.1208	0.1139
Standard Deviation	0.00603	0.00934	0.00391	0.00910
%RSD	4.8	7.3	3.2	8.0

Method Precision: The Method Precision was evaluated by preparing six spiked samples at the specification level. System suitability parameters were found to be well within the limits. The % Relative standard deviations for content of NDMA, NDEA, NEIPA and NDIPA in six spiked sample preparations were found 4.3, 2.9, 2.9 and 6.8 respectively (The % RSD for content of NDMA, NDEA, NEIPA and NDIPA from six replicate injections of spiked samples should be NMT 15.0%). The results are summarized in Table No-6.

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Table No-6: Method Precision results for NDMA, NDEA, NEIPA and NDIPA

Preparation	Contents of Impurities (ppm)			
	NDMA	NDEA	NEIPA	NDIPA
Spiked at 100% Level Preparation-1	0.4346	0.4676	0.4239	0.4910
Spiked at 100% Level Preparation-2	0.4265	0.4480	0.4318	0.4766
Spiked at 100% Level Preparation-3	0.4077	0.4400	0.4339	0.4250
Spiked at 100% Level Preparation-4	0.4182	0.4528	0.4392	0.4389
Spiked at 100% Level Preparation-5	0.3932	0.4468	0.4219	0.4419
Spiked at 100% Level Preparation-6	0.3904	0.4283	0.4042	0.4122
Average	0.4118	0.4473	0.4258	0.4476
Standard Deviation	0.01788	0.01309	0.01238	0.03032
%RSD	4.3	2.9	2.9	6.8

V. CONCLUSIONS:

The results presented in this work clearly demonstrate that the GCMS-HS platforms can be used to produce results that are compliant with the CFDA and U.S. FDA standard methods for nitrosamines detection and quantification in Pantoprazole, providing excellent flexibility and analytical performance for routine laboratory use. The proved analytical performance of the GCMS-HS configurations met the regulation requirements in terms of sensitivity and repeatability, exceeding the expected requirements of the control limits. The static headspace injection technique, offers a simplified workflow for sample handling, not requiring additional steps of sample preparation, but still providing high recovery and suitable sensitivity in compliance with U.S. FDA limits of detection. The method are consistent for the determination of NDMA, NDEA, NEIPA and NDIPA in Pantoprazole Sodium Sesquihydrate by the use of GC-MS/MS offers higher sensitivity and more accurate quantitative results, resulting in the method of choice for the quantification of trace level of nitrosamines in drugs. The method is found to be, Specific, precise and can be used for routine analysis.

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ABBREVIATIONS:

GC	:	Gas Chromatography
MS	:	Mass Spectrometer
HS	:	Head Space
LOD	:	Limit Of Detection
LOQ	:	Limit of Quantification
RSD	:	Relative Standard Deviation
S No.	:	Serial Number
%	:	Percentage
NDMA	:	N-Nitrosodimethylamine
NDEA	:	N-Nitrosodiethylamine
NEIPA	:	N-Nitrosoethylisopropylamine

NDIPA	:	N-Nitrosodiisopropylamine
MP	:	Method Precision

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