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INVITRO ANALYTICAL EVALUATION OF NITROSAMINE- A CARCINOGENIC IMPURITIES IN SARTANS BY GC MS/MS METHOD

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ABSTRACT

N - Nitrosamine dimethyl amine (NDMA) and N Nitrosamine diethyl amine (NDEA) are the carcinogenic impurities detected in the sartan group of drugs in 2018 by FDA in the marketed formulations. A standard solution of NDMA and NDEA are solubilised in an aprotic solvent like DMS and the sample solutions are prepared from the marketed formulations of the reputed pharmaceutical company and were injected with a concentration of 0.004 and 4 ppm for NDMA and NDEA respectively to the GC with a programmed temperature controller using Elite Wax (30 m x 0.25 mm x 0.5 µm) column, helium as carrier gas and hyphenated to the Mass Spectrometer powered with Quadrupole mass analyser and photomultiplier tube detector. The retention and m/z can be found in 5.632,6.514 and 74.0 and 102.0 for NDMA and NDEA respectively. The method for these impurities can be validated as per the ICH Q2 R1 to its system suitability, specificity, Precision, accuracy, LOD and LOQ.

KEYWORDS: NDMA, NDEA, Carcinogenic Impurities, GC MS, Validation, Olmesartan Medoxomil.

INTRODUCTION

Food and Drug Administration, USA unexpectedly made a call back on valsartan drug in 2018 due to identification of N Nitrosamine Dimethyl amine and N Nitrosamine Diethyl amines in them. These are the carcinogenic solvents on long term usage. A clinical trial conducted on carcinogenic activity and proved that there are extra cases were recorded for

those patients taking the dose for six years.[1] The further studies made there is a need to conduct estimation of NDMA and NDEA in other sartan group of drugs. The Olmesartan is a class of sartans expecting a presence of NDMA and NDEA in their API and formulations.

The Olmesartan is a hypertensive drug, uses to reduce the blood pressure byacting on angiotensin system. The Olmesartan Medoxomilutilizes the NDMA and NDEA solvents for copolymerization into a product.[2] The fate of these chemicals on degradation produces dimethyl amine, formaldehyde and nitrate which can be eliminated from the body in urine by liver functions.

The present aim of work is to estimate the amount of NDMA and NDEA in the Olmesartan Medoxomil in the API and formulations by GC MS method and also validate the method as per ICH Q2R1 guidelines.

MATERIALS AND METHODS

Olmesartan Medoxomil was a gifted sample from the Apex Pharma, Bangalore, NDMA and NDEA were procured from Mumbai. All other materials procured for this purpose are Analytical grade having 98.9 – 100.2% range. A GC MS make Shimadzu model TQ 4080 which is a triple Quadruple mass analyzer having photo multiplier tube detector and column brand of Perkin Elmer, Elite WAX 30m x 0.25mm x 0.5 µm dimensions which is coated with carbowax and is generally used for the analysis of impurities in water and beverages.

Instruments

A GC MS make Shimadzu model TQ 4080 which is a triple Quadruple mass analyzer having photo multiplier tube detector and column brand of Perkin Elmer, Elite WAX 30m x 0.25mm x 0.5 µm dimensions which is coated with carbowax and is generally used for the analysis of impurities in water and beverages.[9] Sartorius weighing balance (0.1mg sensitive), Ultrasonic sonicator bath and Thermo fisher scientific refrigerator was used for the study.

The method can be carried out by preparing the standard solutions of NDMA, NDEA and sample solutions of Olmesartan by API and formulations. The solvent used for this method is Dimethyl Sulfoxides (DMS).

GC Conditions: the GC MS having Elite Wax 30m*0.25mm*0.5µm**,** the Helium carrier gas flowed through the column at 3 ml/min, the temperature programmed initially at 70^0C and slowly increased up to 240^0C at 20^0C raise per 5 Sec. Oven temperature, Sample line

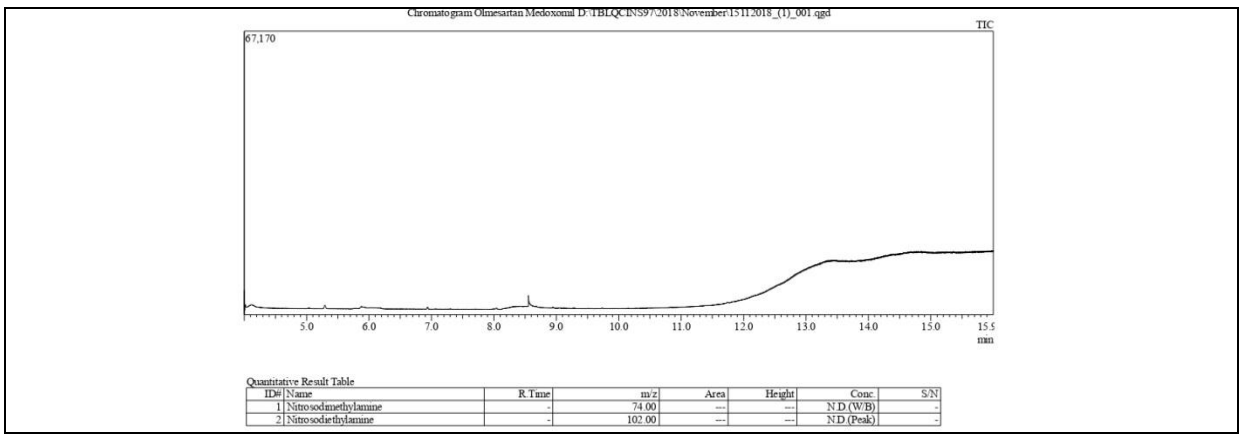
temperature and Transfer Line temperature are controlled at 120,125 and 130^{0}C temperature respectively. The pressurising time, pressure equilibrium time, load time, load Equilibrium time, injection time and GC cycle time are 0.50, 0.10, 0.50, 0.50, 1.0 and 23 min respectively. The sample injected about 1µl and runtime fixed to 16 min. The ion source temperature and interference temperature fixed at 230 and 250^{0}C respectively. The photomultiplier tube detector is used to identify the eluent. The mass spectrometer operated in positive electro spray ionization mode (ESI+) with the following conditions: capillary voltage 3.25 kV; source and desolation temperature 70°C and 280°C respectively. Acquisition was performed in Multiple Reaction Monitoring (MRM) mode, monitoring two specific transitions for analyte and IS with a dwell time of 100 ms. the analyte – dependent MS/MS parameters were optimized via direct infusion of tuning standard solution into mass spectrometer. Data analysis was performed using Mass Lynx 4.1 Software (Waters Corporation, Milford, USA)

The method can be carried out by preparing the standard solutions of NDMA, NDEA and sample solutions of Olmesartan by API and formulations. The solvent used for this method is dimethyl Sulfoxide (DMS).

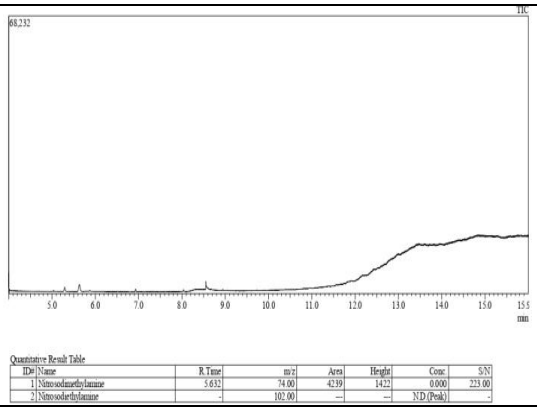
Preparation of Standard NDMA and NDEA solution: weigh accurately using Sartorius CPA225D balance about 25 mg and to be dissolve in 50 ml of Standard flask dilute with DMS, pipette out 0.1 ml and dilute with same diluent. Finally take 1 ml from above into 25 ml standard flask make up to volume with methanol. The resultant solution of NDMA and NDEA estimated as 0.16 PPM.

Preparation of sample Olmesartan Medoxomil: weigh about 1000 mg of tablet powder and dissolve in 20 ml of diluent. Pipette out 2 ml added to a head space vial.

Procedure: initially inject the blank solution with above stated GC MS conditions and then inject six replicates of the standard followed by sample solution and measure the peak area of each.



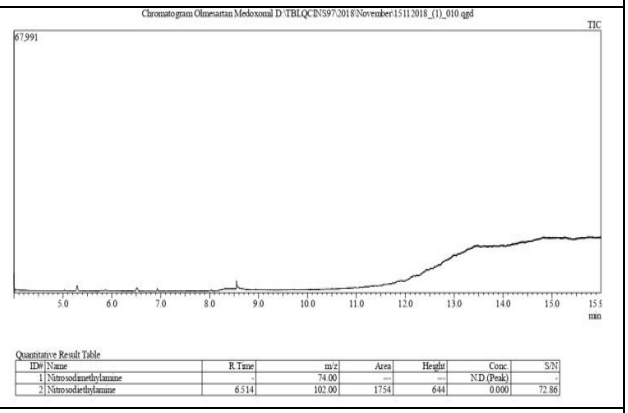
Placebo/ Blank Solution of NDMA and NDEA



Quantitative Result Table

ID#	Name	R.Time	m/z	Area	Height	Conc	S/N
1	Nitrosodimethylamine	5.632	74.00	4239	1422	0.000	223.00
2	Nitrosodiethylamine	-	102.00	---	---	N.D (Peak)	-

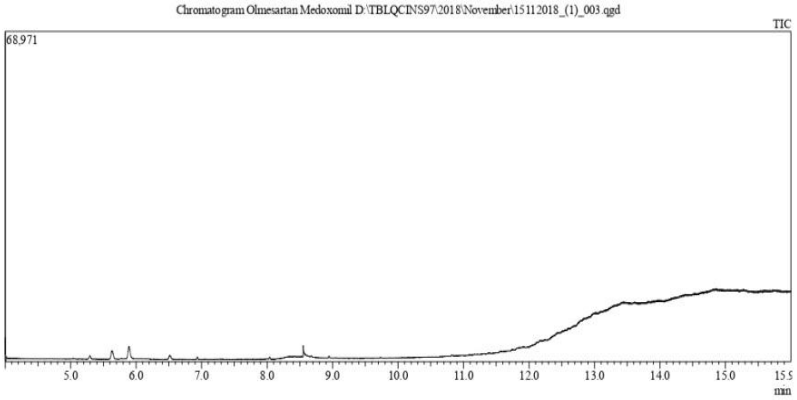
NDMA Std Chromatogram



Quantitative Result Table

ID#	Name	R.Time	m/z	Area	Height	Conc.	S/N
1	Nitrosodimethylamine	---	74.00	---	---	N.D (Peak)	-
2	Nitrosodiethylamine	6.514	102.00	1754	644	0.000	72.86

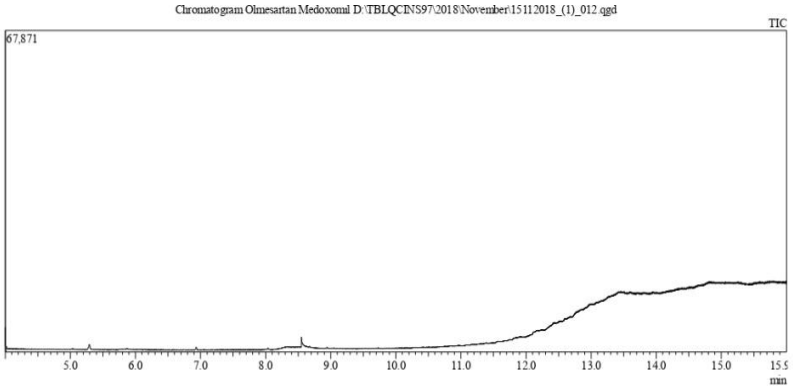
NDEA Std Chromatogram



Quantitative Result Table

ID#	Name	R.Time	m/z	Area	Height	Conc.	S/N
1	Nitrosodimethylamine	5.634	74.00	4371	1513	0.000	253.04
2	Nitrosodiethylamine	6.516	102.00	1802	671	0.000	55.38

NDEA and NDMA standard Chromatogram



Quantitative Result Table

ID#	Name	R.Time	m/z	Area	Height	Conc.	S/N
1	Nitrosodimethylamine	-	74.00	---	---	N.D (W/B)	-
2	Nitrosodiethylamine	-	102.00	---	---	N.D (Peak)	-

Sample Chromatogram of Olmesartan Medoxomil

Validation of method

System suitability: It is a procedure for verifying the method for the estimation of the selected impurities in the pharmaceuticals this is validated by measuring the standard deviation and relative standard deviation from the six replicate injections of the same of

concentration of NDMA and NDEA. The result of these replicate injections of NDMA and NDEA were found to be 2.7 and 2.1 which are within the specified limit of impurities stated by the regulatory agencies.

Specificity: this will access the unequivocally the analytes in the presence of components which may be expected to be present. This procedure can be validated by injecting Blank, six replicate injections of Standard NDMA and NDEA followed by sample as such and again a standard as bracketing. The retention of the components is found to be 5.632 and 6.514 min respectively for NDMA and NDEA. In the spiked sample the RT is found to be 5.630 and 6.513 min respectively for NDMA and NDEA.

LOD and LOQ: it is the procedure to find the lowest amount of analyte in a test sample which can be detected but not necessarily quantitated. LOQ is a process of determination of the analyte to its lowest level of quantification level. LOD and LOQ can be determined with suitable precision and accuracy.

The signal to noise ratio method is established for the determination of LOD and LOQ in NDMA and NDEA. The ratio limit of S/N is not exceeded than 3 and 10 and the concentration of about 0.032 and 0.016 PPM for both NDMA and NDEA.

Precision: for the method precision carried by repeatability, reproducibility and intermediate precision. The method precision or system precision can be carried by replicate injection of six similar concentrations of NDMA and NDEA initially with a blank. The method precision and intermediate precision for sample and standard were result in 2.7, 2.1,0.9, 2.2, 1.6 and 0 respectively for NDMA and NDEA.

Precision at LOQ level: under the similar conditions of GC MS inject the six replicate injections of the 0.032 and 0.016 PPM of NDMA and NDEA, calculate the relative standard deviation and compare with the acceptable criteria.

Linearity: the linearity follows beers law from 40% - 150% with respect to standard concentration. The correlation coefficient was found at 0.99 and 0.99 for both NDMA and NDEA respectively.

Accuracy: the accuracy for Olmesartan Medoxomil can be determined at LOQ, 50%, 100% and 150%. The recovery samples were prepared in triplicate for each concentration and chromatographed; calculate the percentage recovery for the amount added. The percentage recovery for 85.6 – 110.3 for NDMA and NDEA in pure and there were no level of detection found in the samples.

RESULTS

Table no. 1: Nitrosamine methyl amine solvent in olmesartan medoxomil.

Inj. No	System Suitability	Precision at LOQ	System Precision	Method Precision	Intermediate Precision
01	4433	1108	4433	4210	4040
02	4371	1145	4371	4221	3908
03	4119	1164	4119	4139	4018
04	4199	1095	4199	4196	3844
05	4239	1142	4239	4207	3896
06	4276	1135	4276	4262	3794
Avg.	4272.83	1131.50	4272.83	4205.83	3916.67
SD	114.51	25.48	114.51	39.92	96.24
RSD	2.7	2.3	2.7	0.9	2.5
Acceptance	LT 15.0	LT 15.0	LT 15.0	LT 15.0	
Result	Passes	Passes	Passes	Passes	

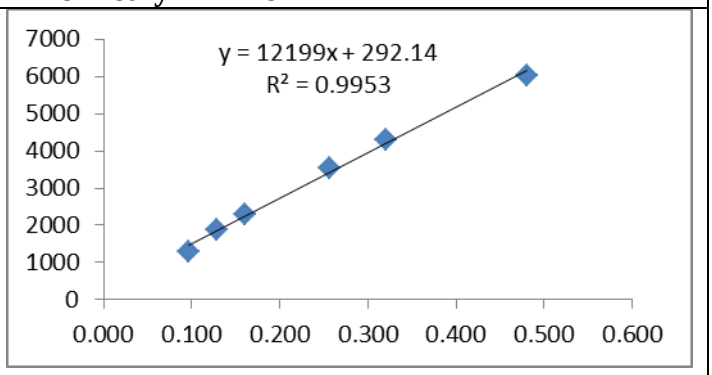
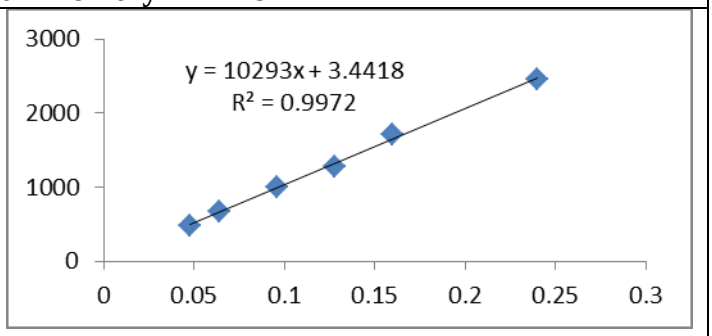
Table no. 2: Nitrosamine ethyl amine solvent in olmesartan medoxomil.

Inj. No	System Suitability	Precision at LOQ	System Precision	Method Precision	Intermediate Precision
01	1808	487	1808	1661	1649
02	1802	484	1802	1712	1587
03	1717	468	1717	1657	1647
04	1739	487	1739	1633	1599
05	1749	507	1749	1689	1600
06	1742	476	1742	1730	1526
Avg.	1759.50	484.83	1759.50	1680.33	1601.33
SD	36.88	13.14	36.88	36.62	45.27
RSD	2.1	2.7	2.1	2.2	2.8
Acceptance	LT 15.0	LT 15.0	LT 15.0	LT 15.0	LT 15.0
Result	Passes	Passes	Passes	Passes	Passes

Table no. 3: Accuracy/Recovery.

Inj. No	Nitrosamine Methyl Amine				Nitrosamine Ethyl Amine			
	50%	100%	150%	Controlled Samples	50%	100%	150%	Controlled Samples
1	89.3	89.2	91.4	Not Detected	114.6	90.2	84.6	Not Detected
2	88.5	88.6	93.0	Not Detected	109.2	93.1	86.6	Not Detected
3	83.9	88.1	94.2	Not Detected	107	93.9	86.1	Not Detected
Mean	87.23	88.63	92.87		110.27	92.40	85.77	
Acceptance	80 – 120	80 – 120	80 – 120		80 – 120	80 – 120	80 – 120	
Result	Passes	Passes	Passes		Passes	Passes	Passes	

Table no. 4: Linearity.

Nitrosamine Methyl Amine			
Linearity	**Concentration**	**Peak area**	
LOQ	0.096	1182	
40%	0.128	2092	
60%	0.160	2787	
80%	0.256	3535	
100%	0.320	4292	
150%	0.480	6031	
Correlation Coefficient		0.9953	
Slope		12199	
Nitrosamine Ethyl Amine			
Linearity	**Concentration**	**Peak area**	
LOQ	0.048	473	
40%	0.064	680	
60%	0.096	994	
80%	0.128	1284	
100%	0.160	1817	
150%	0.240	2448	
Slope		12199	

LOD and LOQ

S. No	Parameter	NDMA	NDEA
1	Retention time in GC	5.630	6.784
2	Concentration of Drug for LOD in PPM	0.032	0.016
3	Peak Area for LOD	365	203
4	S/N Ratio	18.82	6.17
5	Concentration of Drug for LOQ in PPM	0.10	0.05
6	Peak Area for LOQ	1139	480
7	S/N Ratio	69.47	16.87

CONCLUSION

A simple and rapid method is developed for the estimation of NDMA and NDEA carcinogenic impurities in the Olmesartan Medoxomil Standards and Sample marketed formulations. Retention is successfully achieved at 5.63 and 6.78 min for NDMA and NDEA respectively. The method is developed using GC MS make Shimadzu model TQ 4080 which is a triple Quadruple mass analyzer having photo multiplier tube detector and column brand of Perkin Elmer, Elite WAX 30m x 0.25mm x 0.5 µm dimensions. The method is validated for its specificity, system suitability, Precision, Intermediate and Method precision, Accuracy and Linearity, LOD and LOQ were all related and lie within the limits of Impurities

guidelines of ICH Q3A. Hence the method is precise and accurate for the estimation of NDMA and NDEA in the sartan group of drugs and formulations.

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