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Research Article

IN VITRO ANALYTICAL EVALUATION OF NITROSAMINE – A CARCINOGENIC IMPURITIES IN OLMESARTAN MEDOXIMIL BY GC MS/MS METHOD

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ABSTRACT

Objective: A simple and sensitive method development and validation for the simultaneous determination of the N-nitrosamine dimethylamine (NDMA) and N Nitrosamine diethylamine (NDEA) in Olmesartan medoxomil (OLM) API and formulations by a tandem mass spectrometer (GC-MS/MS).

Methods: Gas chromatography with a programmed oven temperature controller, Elite Wax (30 m × 0.25 mm × 0.5 µm) column, Helium as carrier gas and hyphenated to the tandem mass spectrometer powered with triple quadrupole mass analyzer, and photomultiplier tube detector. The method was validated as per the United States Food and Drug Administration (USFDA) guidelines.

Results: With the selected GC-MS/MS conditions, the NDMA and NDEA 0.08 µg/ml (80 ng/ml) and 0.16 µg/ml (160 ng/ml) injected and Rt. for NDMA 5.634 and NDEA 6.516 min, respectively. A linear/range lies in between 0.024 and 0.120 µg/ml and 0.048 and 0.240 µg/ml for NDMA and NDEA with r2 >0.99. The precision, accuracy, and system suitability are established as per USFDA and ICH guidelines, the sensitivity of NDMA limit of detection and limit of quantification 0.08, 0.024 and NDEA 0.16, 0.048.

Conclusion: Other nitrosamine impurities are not involved in the determination of NDMA and NDEA in the OLM using GC-MS/MS and the method is simple, sensitive, rapid, accurate, and precise.

Keywords: Nitrosamine Dimethylamine, Nitrosamine diethylamine, Carcinogenic impurities, Gas chromatography-mass spectrometry, Validation, Olmesartan Medoxomil.

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INTRODUCTION

N nitrosamine dimethylamine (NDMA) and N nitrosamine diethylamine (NDEA) are the carcinogenic solvents on long-term usage which were found first in valsartan. A clinical trial conducted on carcinogenic activity and proved that there are extra cases which were recorded for those patients taking the dose for 6 years (WHO, 2018). Further study is a needed to conduct estimation of NDMA and NDEA in other sartan group of drugs. The Olmesartan Medoxomil (OLM) is of sartan expecting a presence of NDMA and NDEA in their API and formulations.

The OLM is an ARB class of drug, uses to reduce blood pressure by acting on angiotensin system. The OLM structurally (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazole-4-carboxylate [1], the structure consists tetrazole ring, sodium nitrile, and utilizing the solvents employed either were amines or contain traces of amines and this likely to afforded the observation of NDMA and NDEA [2]. Olmesartan is organic in nature and when it is dissolved in a solvent of dimethyl sulfoxide (DMS), the sample will be evaporated, leaving the Olmesartan in the vial and the solvent carries the NDMA and NDEA escaped from the headspace when exposed to gas headspace 70–240°C program temperature [3]. Impurity assay performed by a tandem mass spectrometer to determine the NDMA and NDEA in Olmesartan is limited to 2.400 and 0.663 µg/ml in active pharmaceuticals. [4]. The present aim of work is to estimate the amount of NDMA and NDEA in the OLM in the API and formulations by gas chromatography-mass spectrometry (GC-MS) method and also validate the method as per the United States Food and Drug Administration (USFDA) and ICH Q2R1 guidelines [5,6]. With extensive literature review, there were few GC MS methods for the estimation of NDMA and NDEA individually in the formulations, no method is published for the simultaneous estimation of NDMA and NDEA in the API and marketed formulations [7-35] (Fig.1-3).

METHODS

OLM, NDMA, and NDEA gift sample having 99.98–99.99% procured from Apotex labs, Bangalore Purity, and all chemicals/reagents are analytical grade having 99.8–100.2%. A GC MS makes Shimadzu model TQ 4080 NX with triple quadrupole mass analyzer make Agilent and GC MS empowered with SIM mode with a dwell time 60 GC Column Perkin Elmer, Elite WAX 30 m × 0.25 mm × 0.5 µm dimensions internal coating with Carbowax, and the Semi microbalance of make Sartorius Secura 225D-10N is used.

GC Conditions

The GC having Elite Wax 30 m × 0.25 mm × 0.5 µm column**,** Helium as carrier gas flowed through the column at 3 ml/min, the temperature-programmed initially at 70°C and slowly increased up to 240°C at 20°C raise per minute. Oven temperature, sample line temperature, and transfer line temperature are controlled at 120,125 and 130°C temperature, respectively. The pressurizing 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. Sample 1 µl injected and runtime fixed to 16 min. The ion source temperature and interference temperature fixed at 230 and 250°C, respectively.

Mass Spectrometer

The effluent vapors are directly introduced into ion source of MS equipped with EI as ionizing source and TQM mass analyzer (triple quadrupole mass analyzers) consists of two quadrupoles are arranged in sequence and a radio frequency quadrupole analyzer in between them. Selected

ion monitoring fixed at m/z 74, 102 with a dwell time of 60 ms, SIM is more advantageous in scanning analyte, the interference temperature is 250°C and total runtime 16 min, solvent cutoff time is 4 min.

Table 1: NDMA and NDEA in sample and standards

S. No.		Concentration	Rt.	m/z	Area	S/N
1.	Placebo	0.00	--	--	--	--
2.	NDMA Std.	0.16 µg/ml	5.632	74.0	4239	223.0
3.	NDEA Std.	0.08 µg/ml	6.514	102.0	1754	72.86
4.	Olmesartan medoxomil	50.0 µg/ml	--	--	--	--

NDMA: Nitrosamine dimethyl amine, NDEA: Nitrosamine diethylamine

Table 2: Sensitivity of method by LOD and LOQ

S. No.	Parameter	NDMA	NDEA
1.	Rt.	5.630	6.784
2.	Concentration for LOD in µg/ml	0.0016	0.008
3.	Peak Area for LOD	365	203
4.	S/N ratio	18.82	6.17
5.	Concentration of drug for LOQ in µg/ml	0.0048	0.024
6.	Peak area for LOQ	1139	480
7.	S/N ratio	69.47	16.87

NDMA: Nitrosamine dimethyl amine, NDEA: Nitrosamine diethylamine, LOD: Limit of detection, LOQ: Limit of quantification

Table 3: Validation studies

Parameter	NDMA	NDEA	Acceptability
System suitability	2.7	2.1	LT 15%
LOQ precision	2.3	2.7	
System precision	2.7	2.1	
Method precision	0.9	2.2	
Intermediate precision	2.5	2.8	
Recovery study 50%	87.23	110.27	80–120%
Recovery study 100%	88.63	92.40	
Recovery study 150%	92.87	85.77	
Controlled samples	Not detected	Not detected	
Linearity r^2	0.994	0.997	0.99–1.00
Slope	11798	10293	No limits
Range	0.096–0.480 µg/ml	0.048–0.240 µg/ml	

*Validation procedures for above parameters are conducted at 6 replicate injections of each for selected concentration, the mean±SD, %CV are calculated. NDMA: Nitrosamine dimethyl amine, NDEA: Nitrosamine diethylamine, CV: Correlation variance, LOQ: Limit of quantification

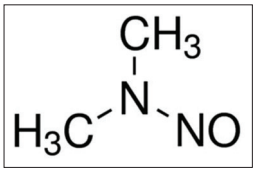


Fig. 1: Structure of NDMA

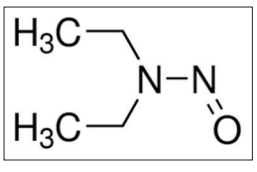


Fig. 2: Structure of NDEA

RESULTS AND DISCUSSION

Preparation of standard NDMA and NDEA solution (0.16 and 0.08 µg/ml)

Weigh 50 mg and 25 mg of NDMA and NDEA in a 50 ml standard flask dilute with DMS sonicated and degasses and Pipette out 0.1 in another 25 ml VF and diluted with DMS. Further, take 1 ml in a 25 ml VF and diluted. The final concentration of NDMA and NDEA was 0.16 and 0.08 µg/ml, respectively.

Preparation of sample OLM (50 µg/ml)

Weigh 100 mg of tablet powder and dissolve in 20 ml of diluent, filtered, sonicated, and degassed; pipette out 2 ml added to a headspace vial.

Procedure

Stabilize the GC for 60 min, initially inject the blank solution, then inject six replicates of the standard followed by sample and measure the peak area (Fig.4-8).

Validation of Method

a. 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 correlation variance (%CV) from the six replicate injections of the same concentration of NDMA and NDEA. The 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
b. Specificity: This will access the unequivocally the analyte 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 Rt. is 5.632 and 6.514 min for NDMA and NDEA, respectively. In the spiked sample, the Rt. is found to be 5.630 and 6.513 min, respectively, for NDMA and NDEA Table 1.
c. Limit of detection (LOD) and limit of quantification (LOQ): It is the procedure to find the lowest amount of analyte in a test sample which can be detected but not necessarily quantities. 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
d. 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 LOD conc. of 0.0016 and 0.008 µg/ml and for LOQ conc. of 0.0048 and 0.024 µg/ml for NDMA and NDEA. The results are detailed in Table 2
e. 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
f. Precision at LOQ level: Under the similar conditions of GC MS inject, the six replicate injections of the 0.032 and 0.016 µg/ml of NDMA and NDEA, calculate the relative standard deviation, and compare with the acceptance criteria

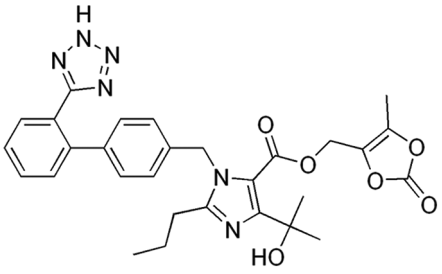


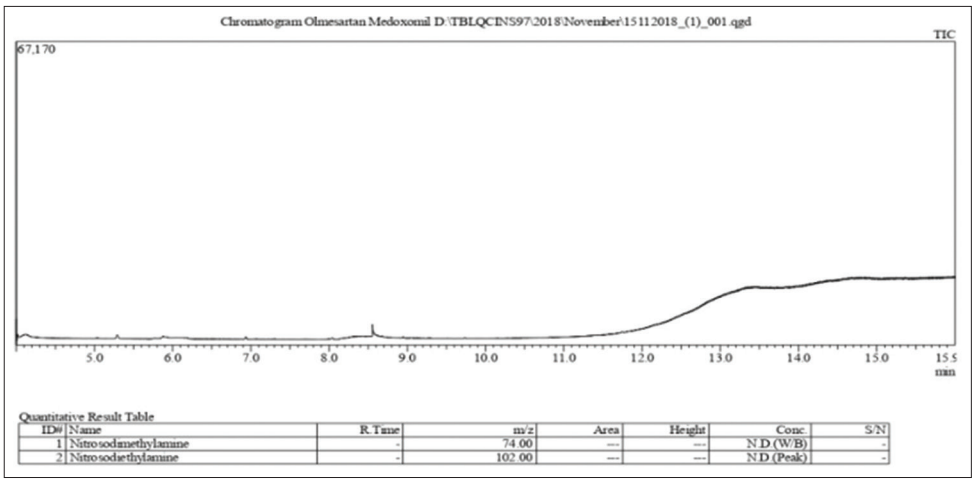
Fig. 3: Olmesartan medoxomil

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

h. Accuracy: The accuracy for OLM 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 was no level of detection found in the samples.

All the validation parameters are explained in the following Table 3.

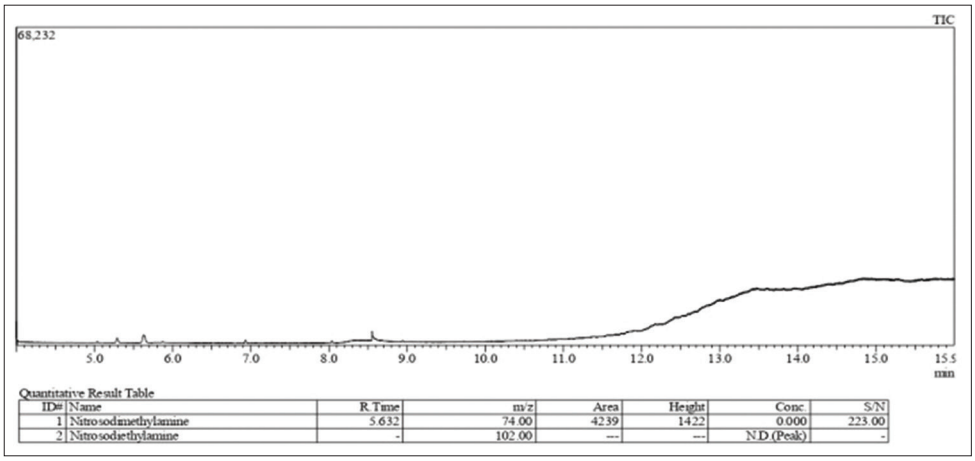
The % CV for NDMA and NDEA for six replicate injections are calculated using excel, results for system suitability, precision at LOQ



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)	-

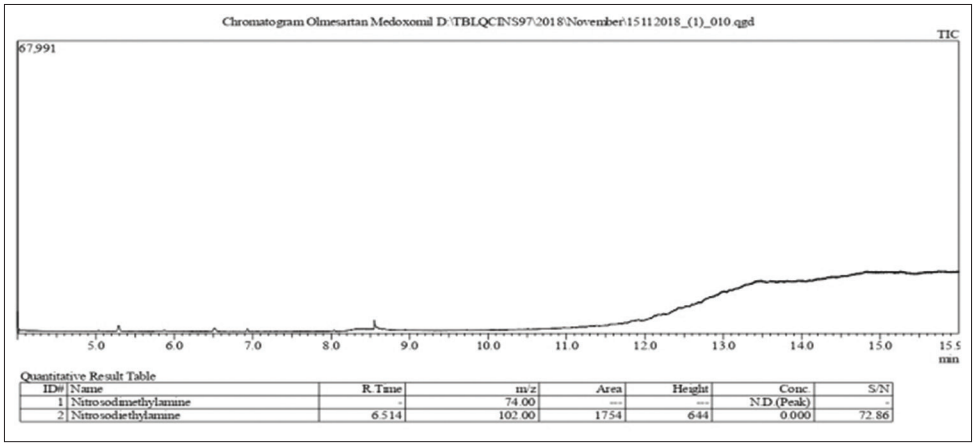
Fig. 4: Placebo/blank solution of NDMA and NDEA



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)	-

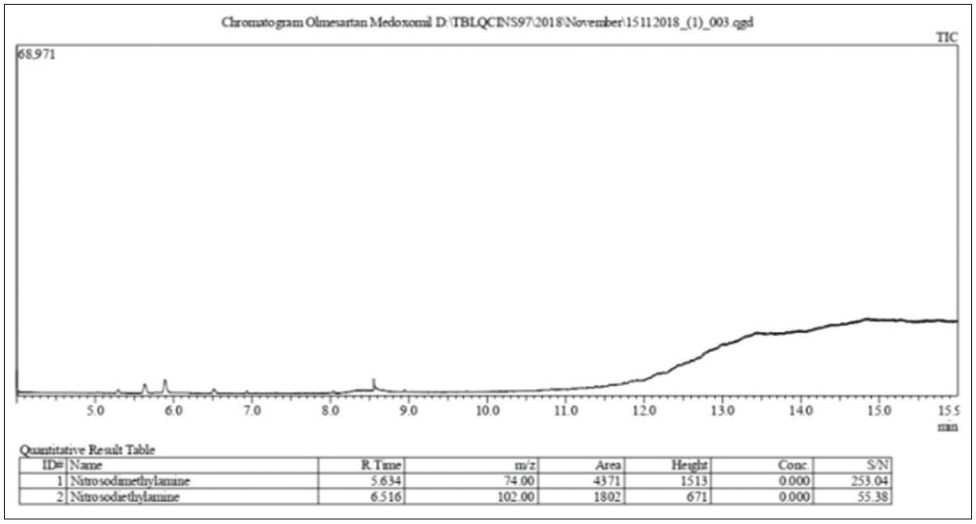
Fig. 5: NDMA Std chromatogram



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

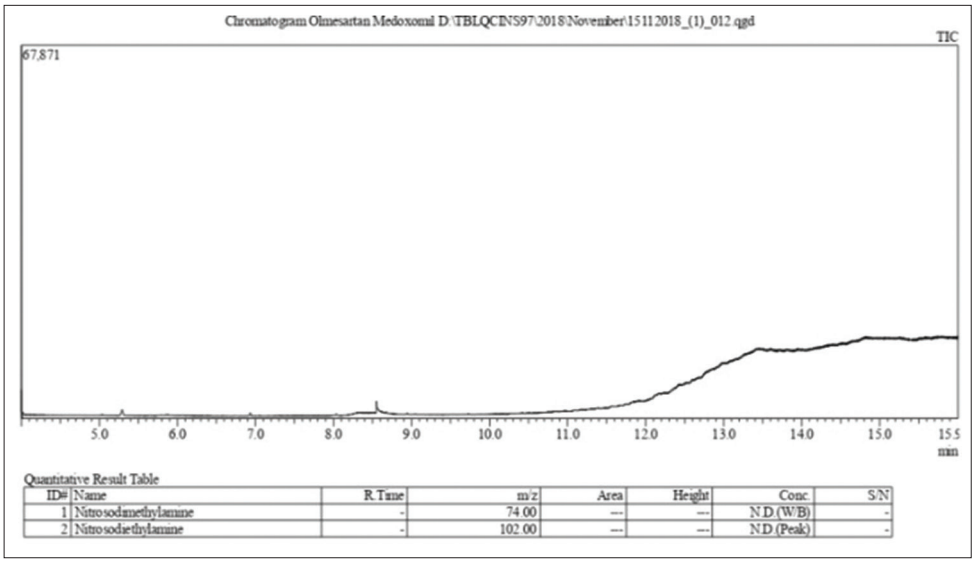
Fig. 6: NDEA Std chromatogram



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

Fig. 7: 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)	-

Fig. 8: Sample chromatogram of olmesartan medoxomil

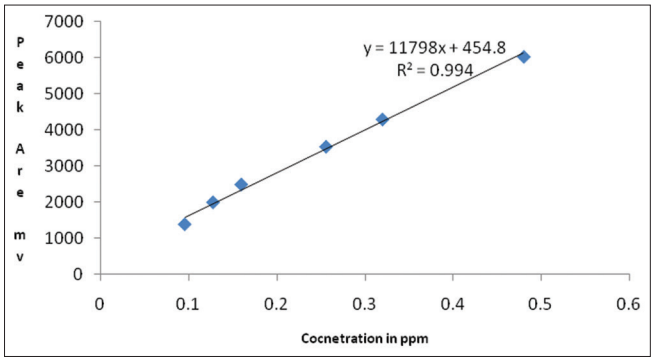


Fig. 9: Linearity graph for NDMA

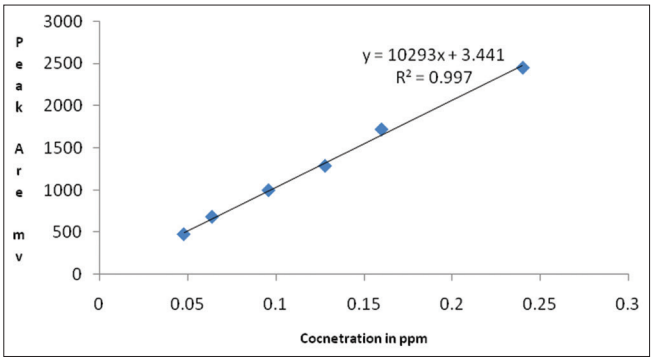


Fig. 10: Linearity graph for NDEA.

level, system precision, method precision, and intermediate precision are carried as per the procedure, the recovery studies for 50%, 100%, and 150% are by spiking method and recovery yield using the assay formulae, linearity constructed from LOQ level to 150% W.R.T the working standard, slope, and correlation calculated using excel sheets [36-38] (Fig.9 and 10).

CONCLUSION

A simple and rapid method is developed for the simultaneous estimation of NDMA and NDEA carcinogenic impurities in the OLM standard and sample marketed formulations. Rt. 5.63 and 6.78 min for NDMA and NDEA achieved using GC MS make Shimadzu model TQ 4080 which is a triple quadrupole mass analyzer having a photomultiplier tube detector and column brand of Perkin Elmer, Elite WAX 30 m × 0.25 mm × 0.5 µm dimensions. The method is validated for its specificity, system suitability, precision, intermediate and method precision, accuracy and linearity, and LOD and LOQ were all related and lie within the limits of impurities guidelines of ICH Q2R1 and USFDA. There was no limit of identification of NDMA and NDEA in the sample Olmesartan marketed formulation. Hence, the method is precise and accurate for the estimation of NDMA and NDEA in the sartan group of drugs and formulations.

AUTHORS' CONTRIBUTIONS

Dr. NDVR Saradhi concepted the research work, collected materials, and experimented and authored the manuscript, Mr. KK Kalyan Kumar collected the data and analyzed. Dr. M Venkata Reddy provided the research support and analyzed the data.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest in publishing this research article.

AUTHORS' FUNDING

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