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ORIGINAL ARTICLE

Cost Effective Accurate & Precise Analytical Method Validation For The Content Estimation Of N-Nitrosodimethylamine & N-Nitrosodiethylamine in Olmesartan Medoxomil by GCMS

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ABSTRACT
A simple, precise and accurate GCMS method was validated for estimation the content estimation of N-Nitrosodimethylamine (NDMA) & N-Nitrosodiethylamine (NDEA) in olmesartan medoxomil (OLM) in drug substances. The content was determined by GCMS on DB-CAM 30.0 m X 0.32 mm, 0.5µm Capillary column and helium was used as carrier gas, using methanol as diluent at column flow rate of 2.0 mL/min and Ion source temperature & Interface temperature at 200ºC and Detector gain mode relative to tuning file with acquisition mode Q3 SIM. The method was validated as per ICH guidelines for Specificity, linearity, LOQ accuracy and LOQ precision. The method shows good linearity over the concentration range of 0.24-3.61 for NDMA and 0.07-0.99 for NDEA Concentration in ppm (µg/g) (r2=0.998) for olmesartan. The average percentage recoveries were in the range of 108.0-113.4% and 97.6-106.7% for N-Nitrosodimethylamine (NDMA) & N-Nitrosodiethylamine (NDEA) in olmesartan medoxomil, respectively. The limits of detection (LODs) were 0.23 concentrations in ppm (µg/g) and 0.10 concentrations in ppm (µg/g) for NDMA and NDEA, and limits of quantification (LOQs) were 0.71 µg/g and 0.31µg/g, respectively. Therefore, the proposed method can be applied for routine analysis of the bulk drugs as well as combined pharmaceutical dosage forms of olmesartan medoxomil.
KEYWORDS: *Olmesartan medoxomil (OLM), N-Nitrosodimethylamine (NDMA) &, N-Nitrosodiethylamine (NDEA) GCMS, Validation, Analytical validation.*

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INTRODUCTION

Olmesartan medoxomil (OLM), (5-methyl-2oxo-1,3-dioxol-4-yl)methylester of 4-(1hydroxy-1-methylethyl)-2-propyl-1-{[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl}-1H-imidazole-5-carboxylic acid (Figure 1), is a novel selective angiotensin II type 1 (AT_1) receptor antagonist having antihypertensive efficacy. It is an ester prodrug which is completely and rapidly hydrolysed to the active form, olmesartan. It works by blocking the binding of angiotensin II to the AT_1 receptors in vascular smooth muscle and as a result of this blockade olmesartan reduces vasoconstriction. This lowers blood pressure by decreasing total peripheral resistance in hypertensive individuals. Olmesartan medoxomil is obtained as colourless crystalline powder, practically insoluble in water and sparingly soluble in methanol [1-3].

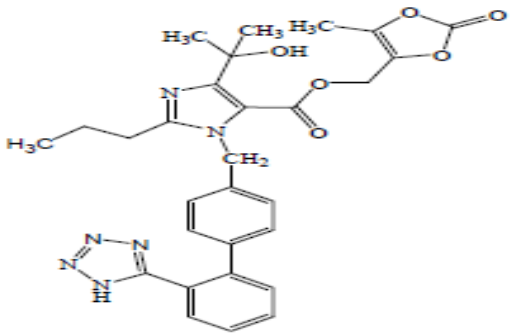

Figure 1: Chemical structures of olmesartan

Nitrosamines in Drug Substances

European Medicine Agencyin March 2020 describes the currently identified sources of nitrosamine impurities. The use of sodium nitrite (NaNO2), or other nitrosating agents, in the presence of secondary, tertiary amines or quaternary ammonium salts within the same or different process steps (if carry over can occur).Use of sodium nitrite (NaNO2), or other nitrosating agents, in combination with reagents, solvents and catalysts, which are susceptible to degradation to secondary or tertiary amines, within the same or different process steps (if carry over can occur). Contaminated raw materials in the API manufacturing process (e.g. solvents, reagents and catalysts) are also one of the identified root causes for presence nitrosamine impurities. Use of recovered materials (e.g. solvents, reagents and catalysts), including recovery outsourced to third parties who are not aware of the content of the materials they are processing and routine recovery processes carried out in non-dedicated equipment. Cross-contaminations due to different processes run on the same line and due to operator-related errors such as inadequate phase separations and degradation processes of starting materials, intermediates and drug substances, including those induced by inherent reactivity in combination with carry-over of sodium nitrite (NaNO2), or other nitrosating agents. This could potentially occur also during finished product formulation or storage. Methods for determination of NDMA and NDEA in some drug substances (Like Sartans) have already been developed by the Official Medicines Control Laboratories and are available for reference on the European Directorate for the Quality of Medicines & HealthCare (EDQM) website. These may serve as a starting point for the development and validation of analytical methods appropriate for other APIs. Depending on the manufacturing process used, other nitrosamines could potentially be present in medicinal products. Uses of accurate mass techniques are required (MS/MS or high- resolution accurate mass systems) in order to overcome interferences in the identification of the specific peak of a certain nitrosamine (e.g. DMF co-eluting with NDMA) [4-6].

Recently, many countries have banned the use of sartans due to reports that carcinogenic N-nitrosodimethylamine (NDMA) or/and. N-nitrosodiethylamine (NDEA) are present as impurities in drug substances[7]. NDMA impurities were also recently observed in ranitidine tablets that belong to the class of drugs known ashistamine-2 blockers. It has been found that the NDMA impurities of ranitidine products increase over time and during storage at temperatures above room temperature, and that a large amount of NDMA is produced, especially when heated to high temperatures [8-9]. The U.S. FDA has reported that low concentrations of NDMA have been detected in some metformin products, and no sample of metformin exceeds the acceptable daily intake for NDMA [10].

GC–MS/MS is recognized as efficient analytical equipment for NA analysis due to its advantages, including low interference, high sensitivity and reasonable price. Because ranitidine thermally degrades to NDMA in a GC instrument, GC analysis is inappropriate for analysis of NDMA in ranitidine. In order to prevent interference and thermal degradation, a clean-up method is required to completely remove the drug substances such as sartans, ranitidine and metformin. If a clean-up method for completely removing drug substances is developed, it can be applied to analyze NAs in different types of pharmaceutical products with an analysis method using GC–MS/MS. The aim of this study was to develop a method for the simultaneous analysis of NDMA and NDEA in drug substances such olmesartan and metformin or their medicinal products by GC–MS/MS. This study focused on optimizing the extraction and clean-up methods for NDMA and NDEA from olmesartans and metformin. Also, a highly sensitive GC–MS/MS method was established by selecting the optimal multiple reactions monitoring (MRM) transition for low interference and high sensitivity of the analytes in the medium [11-12].

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MATERIAL AND METHODS

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-CAM)	(Dimension) 30m X 0.32mm, 0.5µm	NA
4	Methanol	SH8SA81209	HPLC
5	N-Nitrosodimethylamine	MNEA/001/08/2018	98.1
6	Nitraso Diethyl Amine	H5GMI	100
7	Olmesartan Medoxomil	OMS/1602003	NA

Methodology:

Chromatographic Conditions:

Instrument	GCMS-TQ8040 (Gas Chromatograph equipped with MS detector (GC-MS)
Column	DB-CAM 30.0 m X 0.32 mm, 0.5µm Capillary column or Equivalent
Detector	MS
Carrier gas	Helium
Column Oven Program	Initial: 40°C Hold time for 2.0 minutes
	Ramp rate: 15°C/minute at 150°C Hold time for 2.0 minutes
	Ramp rate: 30°C/minute at 200°C Hold time for 2.0 minutes
Injector temperature	200°C
Injection Mode	Split
Flow Control Mode	Linear velocity
Column flow	2.0mL/min
Purge flow	3mL/min
Split ratio	1:5
Injection volume	2µL
Run time	15.00 minutes
Ion source temperature	200°C
Interface temperature	200°C
Solvent cut time	2.0min
Detector gain mode	Relative to Tuning file
Detector gain	+0.2kV
Start time	6.00min
End time	10.00min
Acquisition mode	Q3 SIM
Event time	0.03sec
Q3 Resolution	High
NDMA Ch-1 m/z	74.00
NDEA Ch-2 m/z	102.00
Name of Diluent	Methanol

Preparation of Blank: Diluent (methanol) use as blank.

Preparation of standard stock solution: Weigh about 66mg of N-Nitrosodiethylamine and 245mg of N-Nitrosodimethylamine into a 10mL volumetric flask, dissolve in 3 mL of diluent and make upto the volume with diluent and mix well.

Preparation of Intermediate stock solution-1: Pipette 0.5mL of Standard stock solution into 10 mL volumetric flask Containing 3 mL of diluent and make up to the volume with diluent and mix well.

Preparation of Intermediate stock solution-2: Pipette 0.5mL of Intermediate stock solution-1 into 10 mL volumetric flask Containing 3 mL of diluent and make up to the volume with diluent and mix well.
Preparation of Intermediate stock solution-3: Pipette 1.0 mL of Intermediate stock solution-2 into 10 mL volumetric flask Containing 3 mL of diluent and make up to the volume with diluent and mix well.
Preparation of Standard solution: Pipette 1.0 mL of Intermediate stock solution-3 into 10 mL volumetric flask Containing 3 mL of diluent and make up to the volume with diluent and mix well.
Preparation of sample solution: Weigh and transfer about 250mg of sample (Olmesartan Medoxomil) into a 5mL vial and add 1mL of diluent and mix well collect the supernatant solution, filter through PVDF filter and transfer into 2mL vial.
Injection sequence:

S.No	Description	Number of injections
1.	Blank	2
2.	Standard solution	6
3.	Blank	1
4.	Sample solution-1	1
5.	Sample solution-2	1
6.	Blank-1	1
7.	B. Standard solution	1

Procedure: Inject blank, standard and sample solution into the GCMS system and record the chromatograms. Disregard the peaks due to blank. The retention time of NDMA is 7.6 & NDEA is 8.4 minutes.
System Suitability Requirements: The %RSD for NDMA & NDEA peak areas from six replicate standard injections should be NMT 15.0. **(**The %RSD for bracketing standard of NDMA & NDEA peak areas by replacing sixth standard injection with bracketing standard should be NMT 15.0).
Calculation: Content of NDMA & NDEA

$$\frac{A_T}{A_S} \times \frac{C_S}{C_T} \times \frac{P}{100} \times 1000000$$

Where, AT = Peak area of NDMA & NDEA obtained in sample solution.
A_S = Average peak area of NDMA & NDEA obtained with standard solution
C_S = Standard concentration
C_T = Sample concentration
P = Purity

RESULT AND DISCUSSION:
System Suitability: The Standard solution was prepared by using NDMA & NDEA standard as per test method and injected into the GCMS system. The system suitability parameters were evaluated and % RSD for NDMA & NDEA peak areas obtained from six replicate injections of standard solution was found within limits (NMT 15.0). The results are summarized in Table No-1**.**

Table No-1: System Suitability Results for NDMA & NDEA

Injection No.	Peak Area	Peak Area
	NDMA	NDEA
1	74214	13866
2	76631	15099
3	76676	15197
4	75950	15106
5	76318	15219
6	77055	15178
Average	76141	14944
% RSD	1.3	3.5

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Specificity (Blank Interference): Established the interference of blank peaks. Specificity was conducted by preparing blank, standard as per test procedure injected into the GCMS system. No interference was observed from blank. The results are summarized in Table No-2 and Figure 2 to 5.

Table No-2: Blank interference results for NDMA & NDEA

Name	Retention Time (min)	Peak Area	Interference (Yes/No)
NDMA	7.639	76141	No
NDEA	8.407	14944	No
Blank	NA	NA	No

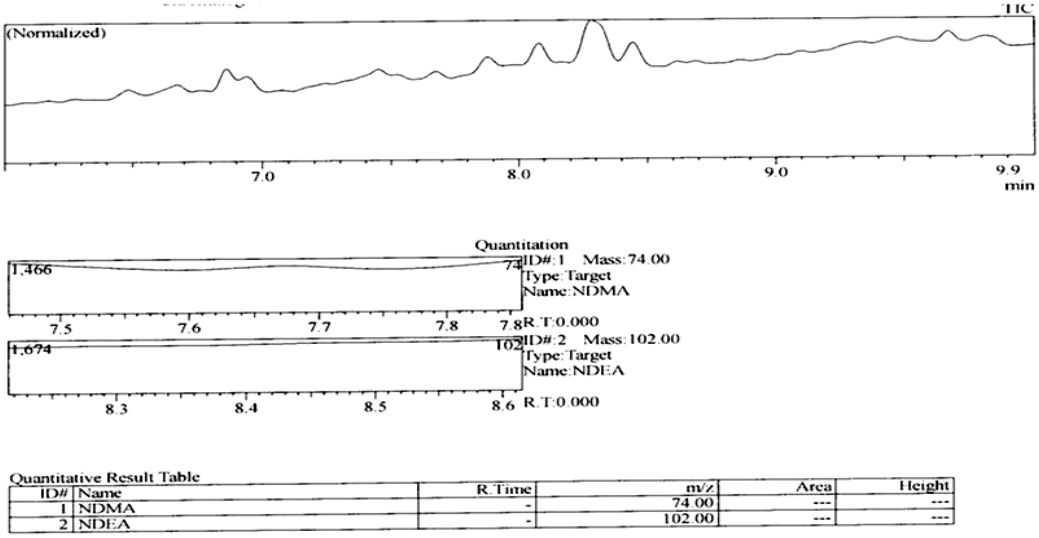

Figure 2: Typical Chromatogram of Blank

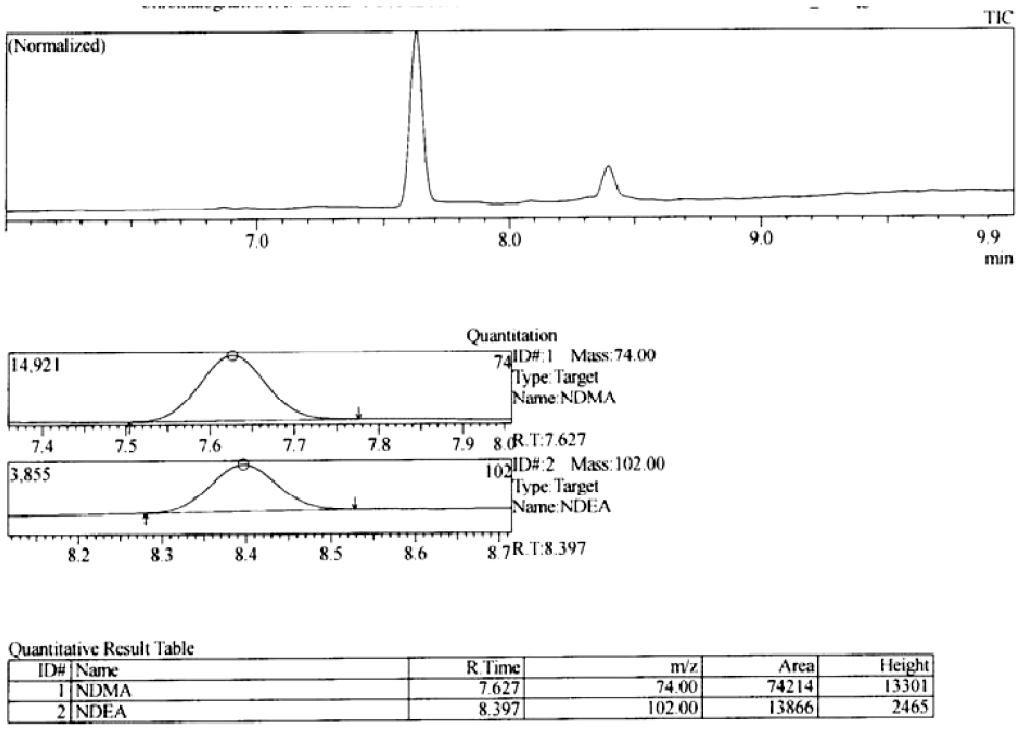

Figure 3: Typical Chromatogram of Standard

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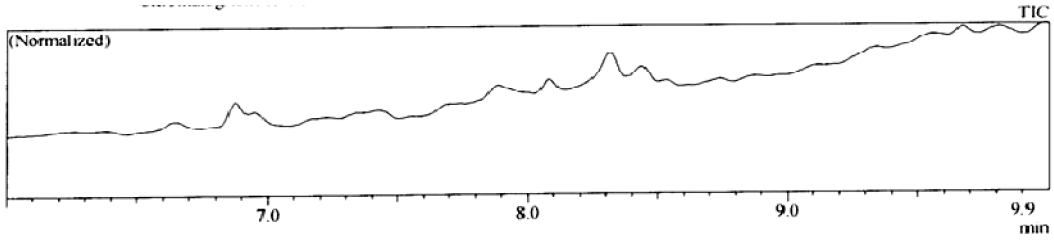
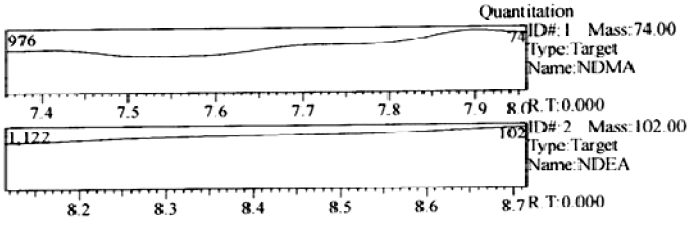
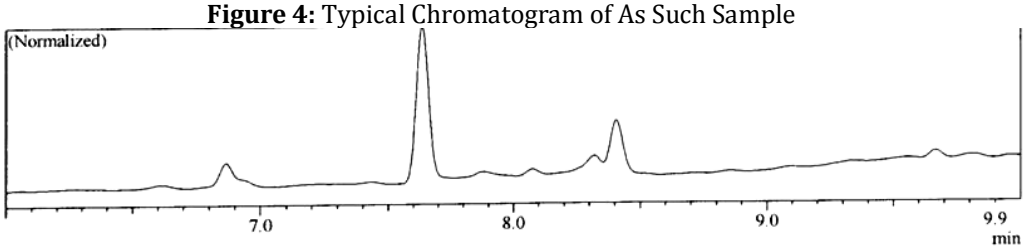


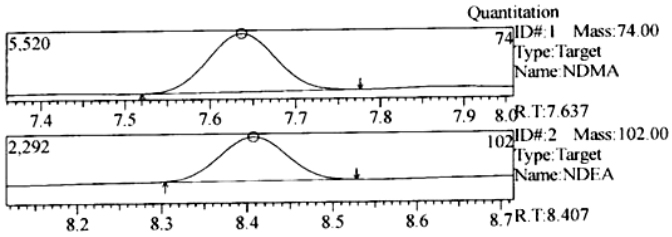


Quantitative Result Table

ID#	Name	R.Time	m/z	Area	Height
1	NDMA	-	74.00	---	---
2	NDEA	-	102.00	---	---

Figure 4: Typical Chromatogram of As Such Sample





Quantitative Result Table

ID#	Name	R.Time	m/z	Area	Height
1	NDMA	7.637	74.00	25912	4670
2	NDEA	8.407	102.00	7978	1450

Figure 5: Typical Chromatogram of LOQ Spiked Sample

LOQ & LOD Establishment and Confirmation: A series of NDMA & NDEA in the concentration ranging from 10% Level to 150% level of the target concentration and injected into the GCMS system. The detector response was found to be linear with a correlation coefficient 0.99 for NDMA & NDEA. LOD and LOQ established from STEYX method. The results are summarized in Table No 3 and 4.

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Table No-3: Linearity Results for NDMA & NDEA

LOQ & LOD Establishment Levels	NDMA		NDEA	
	Conc. in ppm(µg/g)	Area	Conc. in ppm(µg/g)	Area
10% Level	0.24	8851	0.07	1781
25% Level	0.60	18578	0.17	3820
50% Level	1.20	39140	0.33	7552
75% Level	1.80	55972	0.50	10274
100% Level	2.41	81075	0.66	16302
125% Level	3.01	94702	0.83	19014
150% Level	3.61	113951	0.99	23169
Slope	**31619**		**23388**	
Intercept	**755**		**-97**	
Correlation coefficient	**0.99864**		**0.99669**	

NDMA

$$\text{LOQ Concentration} = \frac{10 \times 2252}{31619}$$

$$\text{LOD Concentration} = \frac{3.3 \times 2252}{31619}$$

NDEA

$$\text{LOQ Concentration} = \frac{10 \times 714}{23388}$$

$$\text{LOD Concentration} = \frac{3.3 \times 714}{23388}$$

Table No-4: LOD and LOQ Establishment results for NDMA & NDEA

Peak Name	Limit of Detection	Limit of Quantification
	Concentration in PPM (µg/g)	Concentration in PPM (µg/g)
NDMA	0.23	0.71
NDEA	0.10	0.31

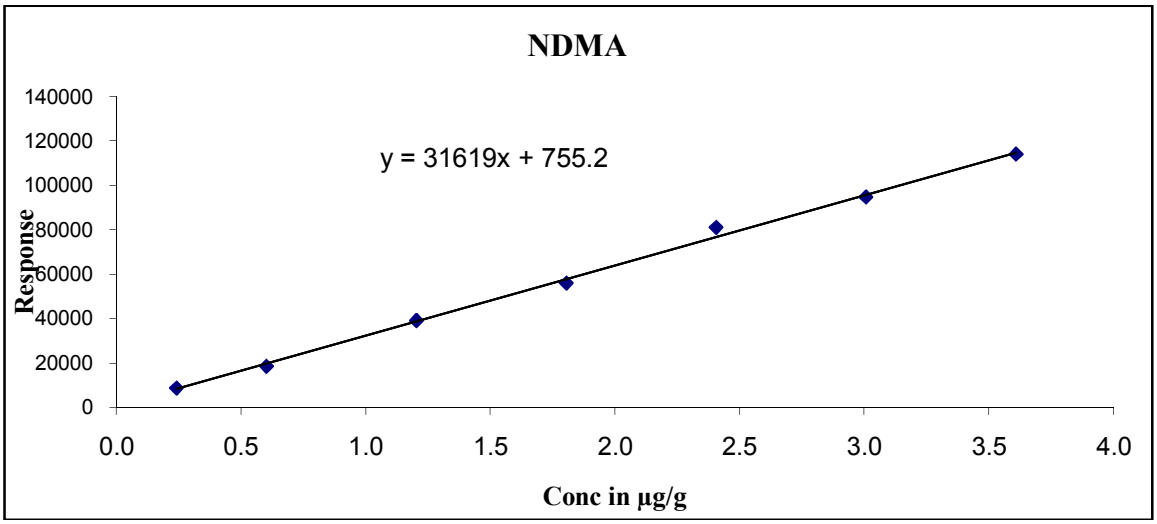

Figure 6: Calibration curve for NDMA

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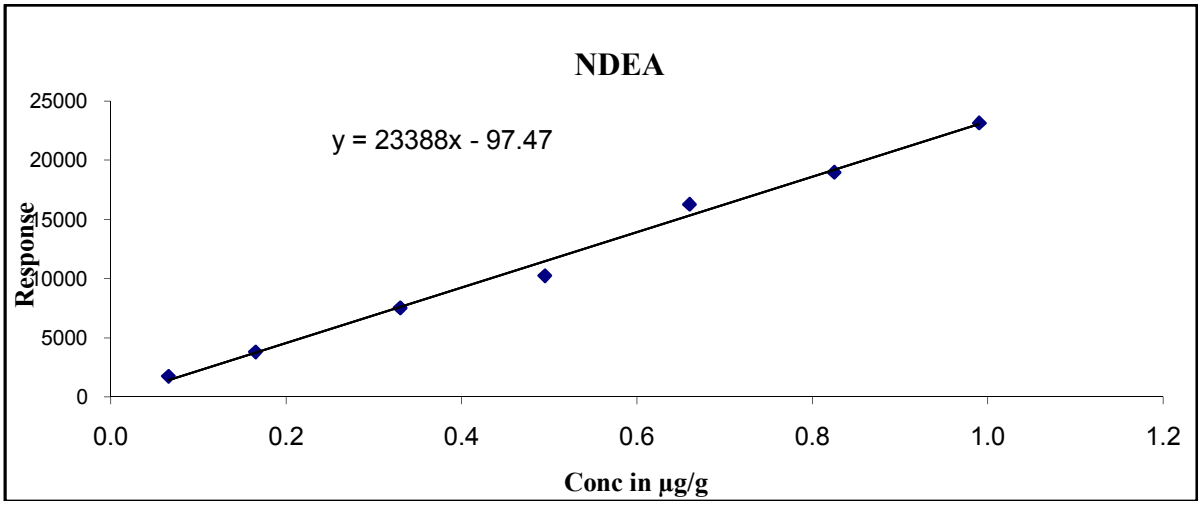

Figure 7: Calibration curve for NDEA

LOQ PRECISION: Prepared six samples having NDMA & NDEA at about limit of quantification level on sample. The % Relative standard deviation for content of NDMA was found to be 1.9% & NDEA was found to be 2.9% and is well within the limits (The% RSD for content of NDMA & NDEA should be NMT 20.0). The results are summarized in Table No-5.

Table No-5: LOQ Precision results for NDMA & NDEA

LOQ Preparation	Content of NDMA(µg/g)	Content of NDEA(µg/g)
Preparation-1	0.82	0.35
Preparation-2	0.79	0.34
Preparation-3	0.78	0.34
Preparation-4	0.78	0.34
Preparation-5	0.80	0.34
Preparation-6	0.79	0.32
Average	**0.79**	**0.34**
%RSD	**1.9**	**2.9**

LOQ ACCURACY: Prepared six samples having NDMA & NDEA at about limit of quantification level on sample (The individual % recovery of NDMA & NDEA at LOQ level should be NLT 70.0 and NMT 130.0). The results are summarized in Table No-6.

Table No-6: LOQ Accuracy results for NDMA & NDEA

LOQ Preparation	% Recovery for NDMA	% Recovery for NDEA
Preparation-1	113.4	106.7
Preparation-2	109.2	101.9
Preparation-3	108.0	102.8
Preparation-4	108.5	102.3
Preparation-5	111.1	103.7
Preparation-6	108.8	97.6

CONCLUSION

The test method for the estimation of NDMA & NDEA in Olmesartan Medoxomil by GCMS has been validated. The proposed method is found to be Precise, Specific, Linear, Accurate at LOQ Level and can be used for routine analysis. The method was free from Interferences. Therefore, this method may be useful for routine analysis of olmesertan in bulk drugs.

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ABBREVIATIONS:

GC	:	Gas Chromatograph
MS	:	Mass Spectrometer
MVP	:	Method Validation Protocol
MVR	:	Method Validation Summary Report
RSD	:	Relative Standard Deviation
S No.	:	Serial Number
%	:	Percentage
QA	:	Quality Assurance
ATP	:	Analytical Test Procedure
NDMA	:	N-Nitrosodimethylamine
NDEA	:	N-Nitrosodimethylamine

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