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Cost Effective Accurate and Precise Analytical Method Development of Content Estimation of N-Nitrosodimethyl Amine and N-Nitrosodiethyl Amine in Olmesartan Medoxomil by GC-MS

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Abstract

A simple, precise and accurate GCMS method was developed 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 developed and evaluated for validation parameter as per ICH guidelines for Specificity, linearity, accuracy and precision. The method shows good linearity over the range of 10%-150% for NDMA and NDEA for olmesartan. The average percentage recoveries were found within predefined acceptance criteria (10% and 100%) for N-Nitrosodimethylamine (NDMA) & N-Nitrosodiethylamine (NDEA) in olmesartan medoxomil, 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].

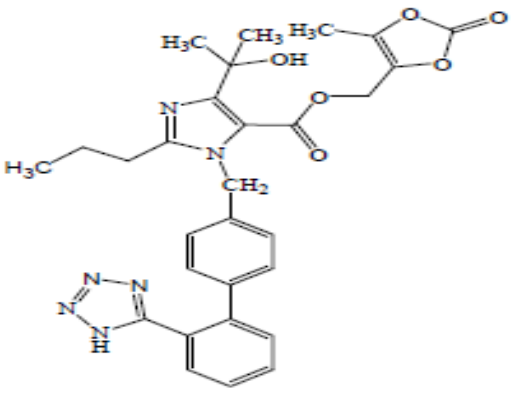


Figure 1: Chemical structures of olmesartan

Nitrosamines in Drug Substances

European Medicine Agency in March 2020 describes the currently identified sources of nitrosamine impurities. 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[4-7]. 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 by GC–MS/MS. This study focused on optimizing the extraction and clean-up methods for NDMA and NDEA from olmesartans[11-12].

MATERIALS 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:

Identification of Compound: Initially 100ppm of Impurity solution was injected in full scan mode & optimizes the SIM method (74 & 102) of N-Nitrosodimethyl amine and N-Nitrosodiethyl amine impurity was determined in GCMS.

Experiment No 1: Optimize the method for N-Nitrosodimethyl amine and N-Nitrosodiethyl amine impurity in Olmesartan Medoxomil by GCMS.

Name of diluent: Methanol

Chromatographic Conditions:

Instrument	GCMS-TQ8040
Column	Stabilwax-MS (Restek PN 10673), 30 m x 0.25 mm x 0.25 um df
Detector	MS
Carrier gas	Helium
Column Oven Program	Initial: 50°C Hold time for 2.0 minutes
	Ramp rate: 15°C/minute at 130°C Hold time for 1minutes
	Ramp rate: 20°C/minute at 220°C Hold time for 4minutes
Injector temperature	200°C
Injection Mode	Splitless
Flow Control Mode	Linear Velocity
Column flow	2.43mL/min
Injection Volume	2µL
Ion source temperature	200°C
Interface temperature	220°C
Solvent cut time	6.50 min
Detector gain mode	Relative to the tuning result
Detector gain	+0.2kv
Compound-1 Name	Nitraso Dimethyl Amine
Acquisition mode	MRM
Ch1 m/z	74.00
Diluents	Methanol

Preparation of Blank: Diluent is used as blank.

Preparation of Standard stock solution-1: Pipetted 50µL of NDMA & NDEA into a 50mL volumetric flask, and made upto the volume with MDC and mixed well.

Preparation of Standard stock solution-2: Pipetted 50µL of NDMA & NDEA into a 50mL volumetric flask, and made upto the volume with DMSO and mixed well.

Preparation of Standard stock solution-3: Pipetted 50µL of NDMA & NDEA into a 50mL volumetric flask, and made upto the volume with MTBE and mixed well.

Preparation of Standard stock solution-4: Pipetted 50µL of NDMA & NDEA into a 50mL volumetric flask, and made upto the volume with Methanol and mixed well.

Preparation of Standard solution: Pipetted 1mL of above stock solution into a 10mL volumetric flask, and made upto the volume with Methanol and mixed well.

Experiment No 2:

Chomatographic conditions:

Instrument	GCMS-TQ8040
Column	DB-CAM 30.0 m X 0.32 mm, 0.5 µm Capillary column or Equivalent
Column id	ASC143-18
Detector	MS
Carrier gas	Helium
Column Oven Program	Initial: 80°C Hold time for 2.0 minutes
	Ramp rate: 15°C/minute at 150°C Hold time for 2minutes
	Ramp rate: 30°C/minute at 200°C Hold time for 2minutes
Injector temperature	200°C
Injection Mode	Split
Flow Control Mode	Linear Velocity
Column flow	2.00mL/min
Purge flow	3.00mL/min
Split ratio	5:1
Injection Volume	2µL
Ion source temperature	200°C
Interface temperature	200°C
Solvent cut time	2.00 min
Detector gain mode	Relative to the tuning result
Detector gain	+0.2kV
Compound-1 Name	Nitraso Dimethyl Amine
Compound-2 Name	Nitraso Diethyl Amine
Acquisition mode	Q3 SIM
Ch1 m/z	74.00
Ch2 m/z	102.00
Diluents	Methanol

Preparation of Blank: Diluent is used as blank.

Preparation of Standard stock solution: Weighed and transferred 244.15mg of NDMA & 66.09mg of NDEA into a 10mL volumetric flask, and made upto the volume with Methanol and mixed well.

Preparation of Intermediate standard stock solution-1: Pipetted 0.5mL of Standard stock solution into a 10mL volumetric flask, and made upto the volume with diluent and mixed well.

Preparation of Intermediate standard stock solution-2: Pipetted 0.5mL of intermediate standard stock solution-1 into a 10mL volumetric flask, and made upto the volume with diluent and mixed well.

Preparation of Intermediate standard stock solution-3: Pipetted 1mL of intermediate standard stock solution-2 into a 10mL volumetric flask, and made upto the volume with diluent and mixed well.

Preparation of Standard solution (2.4ppm & 0.6ppm, 100%): Pipetted 1mL of intermediate standard stock solution-3 into a 10mL volumetric flask, and made upto the volume with Methanol and mixed well.

Preparation of Standard solution (10%): Pipetted 1mL of standard solution (2.4 ppm, 0.6ppm) into a 10mL volumetric flask, and made upto the volume with Methanol and mixed well.

Preparation of as such sample solutions: Below samples mixed well and filtered through 0.45µ filters, transferred to AOC vial and placed in GC MS system.

Table 1: Preparation of as Such Sample Solution

S. No	Weight of sample taken(mg)			Transferred to vial(mL)	Diluent added(mL)
	Gross weight	Tare weight	Net weight		
1	251.88	0.66	251.22	5mL vial	1
2	250.39	0.16	250.23	5mL vial	1

Preparation of 10% Spiked sample solutions**:** Below samples mixed well and filtered through 0.45µ filters, transferred to AOC vial and placed in GC MS system.

Table 2: Preparation of as 10% Spike Sample Solution

S. No	Weight of sample taken(mg)			Transferred to vial(mL)	10% std added(mL)
	Gross weight	Tare weight	Net weight		
1	251.41	0.20	251.21	5mL vial	1
2	250.60	0.00	250.60	5mL vial	1
3	250.67	0.34	250.33	5mL vial	1

Preparation of 100% Spiked sample solutions**:** Below samples mixed well and filtered through 0.45µ filters, transferred to AOC vial and placed in GC MS system.

Table 3: Preparation of as 100% Spike Sample Solution

S. No	Weight of sample taken(mg)			Transferred to vial(mL)	100% std added(mL)
	Gross weight	Tare weight	Net weight		
1	251.18	0.42	250.76	5mL vial	1
2	250.52	0.00	250.52	5mL vial	1
3	250.63	0.00	250.63	5mL vial	1
4	250.60	0.00	250.60	5mL vial	1
5	250.47	0.27	250.20	5mL vial	1
6	250.33	0.00	250.33	5mL vial	1

Preparation of Linearity level solutions**:** Below linearity solutions transferred to AOC vial and placed in GC MS system.

Table 4: Preparation of Linearity level Solution

S. No	Linearity level	Volume of intermediate stock solution-3 taken	Transferred to flask	Made up with
01	10% level	0.10mL	10mL flask	diluent
02	25% level	0.25mL	10mL flask	diluent
03	50% level	0.50mL	10mL flask	diluent
04	75% level	0.75mL	10mL flask	diluent
05	100% level	1.00mL	10mL flask	diluent
06	125% level	1.25mL	10mL flask	diluent
07	150% level	1.50mL	10mL flask	diluent

Specification Limit: Content of NDMA is 2.39µg/g and NDEA is 0.66µg/g.

Chomatographic conditions:

Instrument	GCMS-TQ8040
Column	DB-CAM 30.0 m X 0.32 mm, 0.5 µm Capillary column or Equivalent
Column id	ASC143-18
Detector	MS
Carrier gas	Helium
Column Oven Program	Initial: 80°C Hold time for 2.0 minutes
	Ramp rate: 15°C/minute at 150°C Hold time for 2minutes
	Ramp rate: 30°C/minute at 200°C Hold time for 2minutes
Injector temperature	200°C
Injection Mode	Split
Flow Control Mode	Linear Velocity
Column flow	2.00mL/min
Purge flow	3.00mL/min
Split ratio	5:1
Injection Volume	2µL
Ion source temperature	200°C
Interface temperature	200°C
Solvent cut time	2.00 min
Detector gain mode	Relative to the tuning result
Detector gain	+0.2kV
Compound-1 Name	Nitroso Dimethyl Amine
Compound-2 Name	Nitroso Diethyl Amine
Acquisition mode	Q3 SIM
Ch1 m/z	74.00
Ch2 m/z	102.00
Diluents	Methanol

Preparation of Blank: Diluent is used as blank.

Preparation of Standard stock solution: Weighed and transferred 244mg of NDMA & 66mg of NDEA into a 10mL volumetric flask, and made upto the volume with Methanol and mixed well.

Preparation of Intermediate standard stock solution-1: Pipetted 0.5mL of Standard stock solution into a 10mL volumetric flask, and made upto the volume with diluent and mixed well.

Preparation of Intermediate standard stock solution-2: Pipetted 0.5mL of intermediate standard stock solution-1 into a 10mL volumetric flask, and made upto the volume with diluent and mixed well.

Preparation of Intermediate standard stock solution-3: Pipetted 1mL of intermediate standard stock solution-2 into a 10mL volumetric flask, and made upto the volume with diluent and mixed well.

Preparation of Standard solution (2.4ppm & 0.6ppm): Pipetted 1mL of intermediate standard stock solution-3 into a 10 mL volumetric flask, and made upto the volume with Methanol and mixed well.

Preparation of as such sample solutions: Weighed and transferred 250 mg of sample in 5 mL vial and add 1mL diluent and mixed well filter the solution through PVDF filter.

Injection sequence:

S.N.	Description	Number
1.	Blank	2
2.	Standard	6
3.	Blank	1
4.	Sample	1
5.	Sample	1
6.	Blank	1
7.	Bracketing	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 N-Nitrosodimethylamine about 7.6 and N-Nitrosodiethylamine peak is about 8.4 minutes.

System Suitability Requirements: The %RSD for N-Nitrosodimethylamine and N-Nitrosodiethylamine peak areas from six replicate standard injections are NMT 15.0.

Calculation: Content of N-Nitrosodimethylamine / N-Nitrosodiethylamine in Olmesartan Medoxomil

$$= \frac{A_T}{A_S} \times \frac{C_S}{C_T} \times \frac{P}{100} \times 10^6$$

Where,

A_T = Peak area of N-Nitrosodimethylamine /N-Nitrosodiethylamine obtained in sample solution.

A_S = Average peak area of N-Nitrosodimethylamine /N-Nitrosodiethylamine obtained with standard solution

C_S = Standard concentration

C_T = Sample concentration

P = Purity of Standard

Specification: Content of N-Nitrosodiethylamine should be NMT 0.66µg/g and N-Nitrosodimethylamine in Olmesartan Medoxomil should be NMT 2.39µg/g.

RESULT AND DISCUSSION:

Observations & Results of experiement 1: No blank interference is observed at the retention time of standard in MDC diluent. Blank interference was observed in DMSO diluent. No blank interference was observed at NDMA standard RT-7.6 in Methanol diluent. Blank interference was observed at NDEA standard RT-8.4. NDMA standard Rt at 3.5min. NDEA standard RT at 4.5 min. Recovery results was not meeting the criteria. Further optimization changed to split ratio.

Observations & Results of experiement 2: No blank interference is observed at the retention time of NDMA & NDEA. System suitability found satisfactory. NDMA peak eluted at 7.5min, NDEA peak eluted at 8.6min. Correlation coefficient for linearity of NDMA is found: 0.99 and Correlation coefficient for linearity of NDEA is found: 0.99. Based on below mentioned recovery results method was meets the system suitability, Linearity, Accuracy criteria. Method is suitable for proceeding to validation. Based on the above experiments, the following method parameters are finalised for Content estimation of N-Nitrosodimethyl amine and N-Nitrosodiethyl amine in Olmesartan medoxomil.

Recovery Results (10%) :

Table 5: Recovery of 10% Spike solution

S.No.	Sample Name	% Recovery of NDMA	% Recovery of NDEA
1	Spiked spl-1	128.4	112.3
2	Spiked spl-2	125.0	122.0
3	Spiked spl-3	125.0	127.9

Recovery Results (100%) :

Table 6: Recovery of 100% Spike solution

S.No.	Sample name	%Recovery of NDMA	%Recovery of NDEA
1	Spiked spl-1	100.2	106.6
2	Spiked spl-2	105.7	113.9
3	Spiked spl-3	105.7	113.9
4	Spiked spl-4	99.3	106.7
5	Spiked spl-5	107.2	117.6
6	Spiked spl-6	106.1	114.5

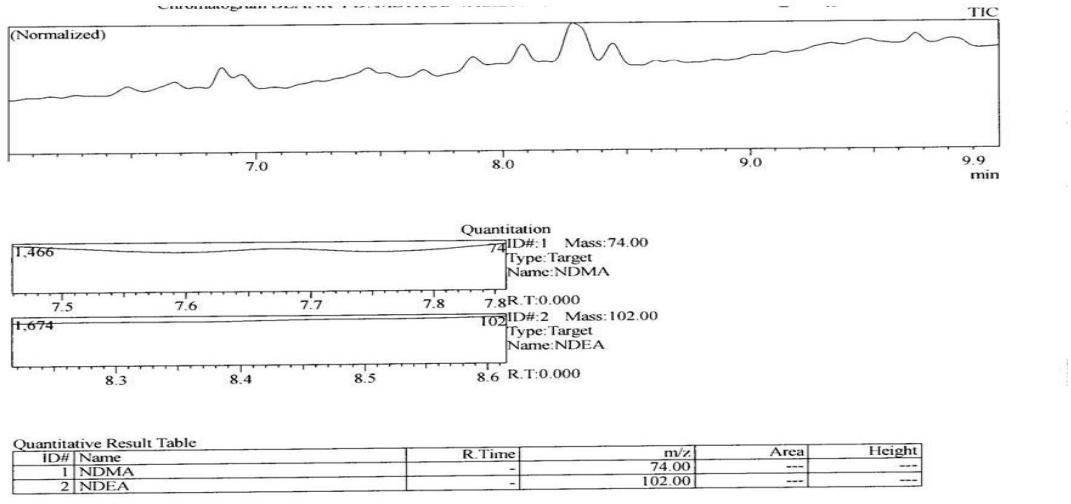
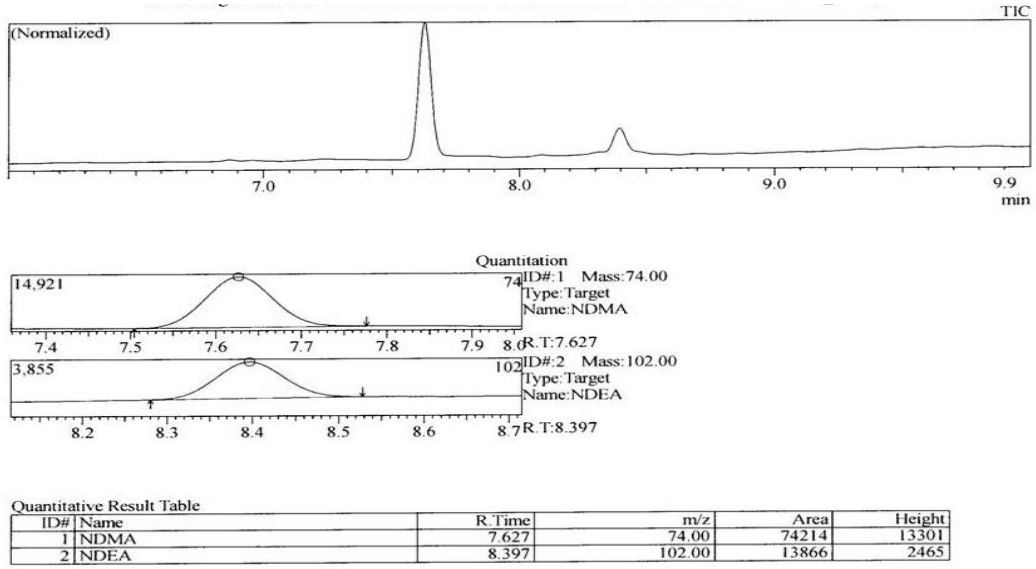


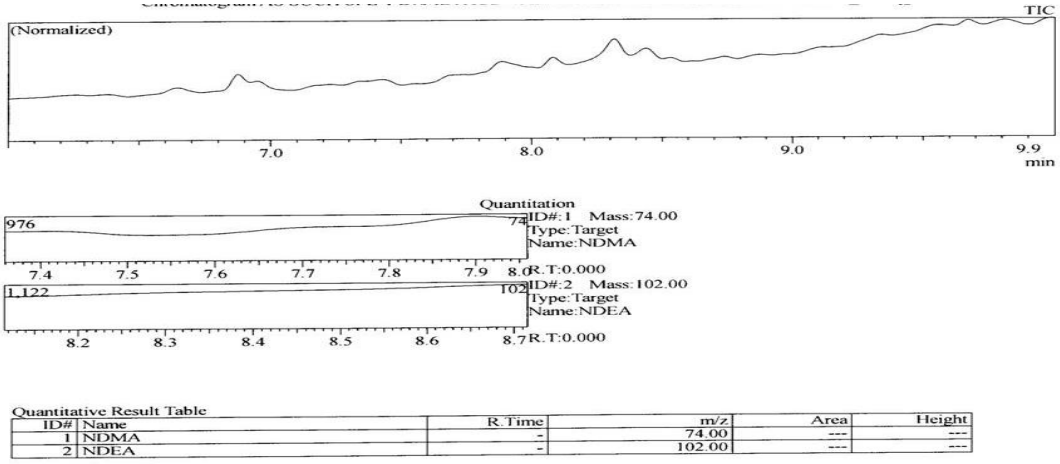
Figure 2: Typical chromatogram of Blank



Quantitative Result Table

ID#	Name	R.Time	m/z	Area	Height
1	NDMA	7.627	74.00	74214	13301
2	NDEA	8.397	102.00	13866	2465

Figure 3: Typical chromatogram of Standard



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

CONCLUSION:

The test method for the estimation of NDMA & NDEA in Olmesartan Medoxomil by GCMS has been developed. 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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CONFLICT OF INTEREST:

Author has no conflict of interest during preparation of this research manuscript.

Abbreviations:

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

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