Method of Analysis for the Determination of N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Content in Latanoprost (LTN/LTP) By GCMS/MS.

Sunil B. Lakhmapure[1], Sandip A. Telavane[1], Seema Kothri[1], Sushil Kumar Singh[2] , Manohar V. Lokhande [1, 2]*

[1] Department of Chemistry, PAHER University, Udaipur-303003, Rajasthan, India [2]Department of Chemistry, Sathaye College, Mumbai-400057, Maharashtra, India

Abstract:

It has been discovered that certain drug substances and drug products include N-nitrosodimethylamine (NDMA). Certain drugs have also been shown to contain N-nitroso diethylamine (NDEA). An approach that uses isotope dilution, a clean-up procedure, and gas chromatography-tandem mass spectrometry (GC-MS/MS) has been optimized for the simultaneous detection of NDEA and NDMA in drug substances and final products of Content in Latanoprost (LTN/LTP). High-performance liquid chromatography (HPLC) was selected as the analytical technique for the GCMS/MS determination of N-nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) content in latanoprost (LTN/LTP). ICH criteria validated this method. Its RSD's system precision is 2.12 and 2.73. The RSD method precision is 13.22 and 14. 13. The test procedure is verified to meet the predefined acceptance criteria regarding Specificity, Precision, and Linearity. Analysis was done on criteria including HPLC, Specificity, Precision, System Suitability, and Linearity.

Keywords: N-nitroso dimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), Latanoprost, GCMS/MS, LLOQ, LOD and Linearity

Introduction:

A class of carcinogens known as nitrosamines is frequently present in food processing, water treatment, and pharmaceutical industries. N-nitrosodimethylamine (NDMA) is typically classified as a class 2A carcinogen under the ICH M7 general guidelines for genotoxic impurities including nitrosamine compounds [1, 2]. It has the potential to cause direct or indirect damage to cells' DNA, which increases the likelihood of mutagenic and carcinogenic adverse effects. There are several ways that NDMA might enter a system, including through contaminated catalysts and solvents, contaminated raw materials obtained from other sources, or contamination during the manufacturing process [3,4]. It is general knowledge that several

items, including dairy, vegetables, cured or smoked meats, alcoholic beverages, and cereals, are contaminated with NDMA. As a result, the medical world has recently focused a lot of emphasis on its potential for carcinogenicity [5].

It was recently found that NDMA and NDEA were present in several medication items. It is thought that the manufacturing procedure of the drug substance brought it into the final items. The regulatory exposure limitations for drug items were greatly exceeded by this contamination. As a result, all implicated drug products were taken off the market by medical organizations in Europe and the US Food and Drug Administration (USFDA)[6]. Therefore, utilizing the appropriate analytical technique and developing a sensitive, accurate, robust, and reliable procedure is crucial [7].

To ensure the trustworthiness of the analytical data, the lower limit of quantification (LOQ) while creating an analytical method should generally be less than 1/10 of the permitted intake level. Consequently, in this instance, the LOQ for NDMA and NDEA must be less than 0.016 mg/kg and 0.004 mg/kg, respectively. Furthermore, a method that can analyze low µg/kg levels is needed because combining excipients reduces the analyte concentrations in pharmaceutical products by half [8].

This work set out to create a technique for the simultaneous GC-MS/MS analysis of NDEA and NDMA in drug compounds such as Latanoprost as well as their pharmaceutical derivatives. The goal of this work was to maximize the processes for removing and cleaning up NDMA and NDEA from Latanoprost. Additionally, by choosing the best multiple reactions monitoring (MRM) transition for low interference and high sensitivity of the analytes in the medium, a highly sensitive GC–MS/MS technique was constructed.

Material and Methods

Instruments

Instrument Name	Number	make
GCMS	RDS/ALIGCMS-01	Agilent
Analytical Balance	RDS/AL/BAL-10	Mettler Toledo

Column:

Column Make	Number	Column Dimension
Agilent	AMD/GC/C API77	VF- Wax ms (30 mX 0.25 mm,l.0 µm)

Standards/sample:

Chemical/Standards	Batch number	Source/Make
N- nitroso dimethylamine (NDMA)	ACPL-03-NDMA-43K2I	Advent
N- Nitroso diethylamine INDEA)	049K1613	Advent
Latanoprost	LTN/LTP/22/OO1	In-House

1)Limit:

Chemical	Limit
N- nitroso dimethylamine (NDMA)	NMT 200 ppm
N- Nitroso diethylamine INDEA)	NMT 200 ppm

2)Chromatographic conditions

Column	VF- Wax (30m x 0.25mm x l.0µm) or equivalent		
Oven temperature	Rate (in "C/min)	Temperature (in °C)	Hold time (in min)
	0.0	70	4.0
	20	240	3.5
Injector temperature	220 °C		
Carrier gas	Helium		
Helium flow rate	1.0 mL /min.		
Split Ratio/Spitless	Pulsed Spitless		
Injection Pulsed pressure	12.285 psi until 0.5 min		
Purge flow to split vent	50 mL /min at I min		
GS saver	On 20 mL/min after2mtn		
MSD Transfer line Temp.	250 ºC		
Thermal aux 2 Temp.	150 ºC		
Run Time	16.00		
Retention Time and Relative Retention Time	Component	RT (Min)	RRT
	N- nitroso dimethylamine	9.895	0.93
	N- Nitroso diethylamine	10.584	1.00
3)Headspace parameters			
Oven Temperature	120 ºC		
Loop Temperature	125 ºC		
Transfer line Temperature	130 ºC		
Vial equilibration time	15 min		
Injection time	1 min		
Vial Shaking	Level 9 (250 shakes/min)		
Fill pressure	15 psi		
Loop Size	1mL		
GC Cycle time	24 min		
4)Mass conditions			
Ionization mode	Electron Ionisation (EI)		
Electron energy	70 eV		
Source temp.	130 ºC		
Quad temp.	150 ºC		
Acquisition	MRM		
Dwell (ms)	150,150		
Collision energy	10,20		
MRM Transition	NDEA: 102.1 > 85, NDMA:74>42		
Quench Gas He	1.5 mL/ min		
Collision gas N_2	6 min		

Preparation of Solution:

Diluent : DMSO

Standard stock solution A: Weigh accurately 50 mg each of N-Nitroso dimethylamine(NDMA) and N-Nitroso diethylamine (NDEA) standard in a 50 mL Standard volumetric flask containing sufficient diluent in it, Mix and dilute with diluent" (NDMA and NDEA- 1000 ppm).

Standard stock solution B : Pipette out 0.5 mL of the above Standard stock solution A in 50 mL Standard volumetric flask & dilute up to mark with diluent. (NDMA and NDEA -10 ppm).

Standard solution: Pipette out 5.0 mL of above Standard stock solution B in 50 Standard volumetric flasks and diluted up to mark with diluent (NDMA and NDEA -1.0 ppm).

Objective: To validate the method for the determination of N-Nitrosodimethylamine (GDMA) and Nitrosodiethylamine (NDEA) content in Latanoprost (LTN/LTP) by GCMS/MS and to demonstrate that the method is appropriate for its intended use.

Scope: The protocol is applicable only for the determination of N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) content in Latanoprost (LTN/LTP) by GCMS/MS as per the method of analysis.

Background: As per the route of synthesis and risk assessment study, there is the possibility of the formation of N-Nitrosodimethylamine Q\DMA) and N-Nitrosodiethylamine (NDEA) impurity in Latanoprost. As per EDQM guideline EMA/40981512020 Rev.14 Dated 22 December 2022, NDMA & NDEA need to be controlled with an established acceptable intake limit. The acceptable intakes for NDMA & NDEA are 96 ng/day & 26.5 ng/day respectively. The maximum daily dose of Latanoprost is 1.5µg/ day. Based on this maximum daily dose and acceptable intake, the NDMA & NDEA need to be controlled in ITN/LTP at 32000 & 8833 ppm respectively. At FDC, the evaluation of both the Nitrosamine impurities i.e. NDMA & NDEA needs to be performed at a stringent limit i.e. 200 ppm. An In-house GCMSA4S method has been developed. The method needs to be validated per the current ICH guidelines to give evidence of its reliability and suitability at Supriya Life Sciences.

Result and Discussions:

System Suitability: Prepare a system suitability solution as per the method of analysis and analysis. System suitability was determined by performing six replicate injections of standard solution as per the method of analysis and analysis [9].

Table l: System suitability

Injection No	NDMA		NDEA	
	RT (Min)	Area	RT (Min)	Area
1	9.895	223286	10.585	48555
2	9.895	213847	10.584	49717
3	9895	231079	10.585	49477
4	9.895	221373	10.585	50512
5	9.895	223216	10.585	50268
6	9.895	224213	10.585	49877
Average	**9.895**	**219836**	**10.585**	**49734**
SD	**0.000**	**5027.38**	**0.000**	**688.08**
% RSD	**0.00**	**2.29**	**0.00**	**1.38**

Acceptance Criteria: The RSD of the area of six replicate injections for NDMA and NDEA peak from standard solution should be NMT 20.0%.

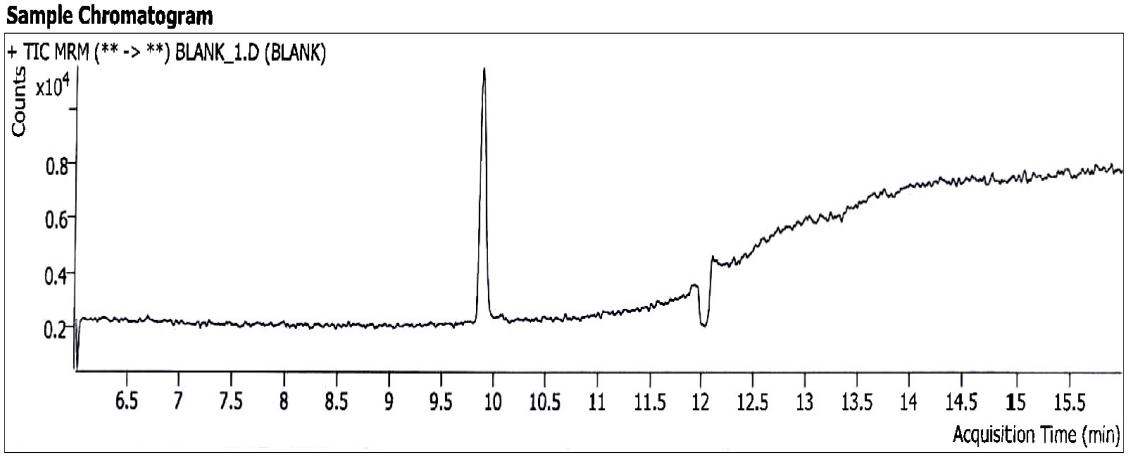
Specificity: Analyze NDMA and NDEA individually as per the method of analysis.

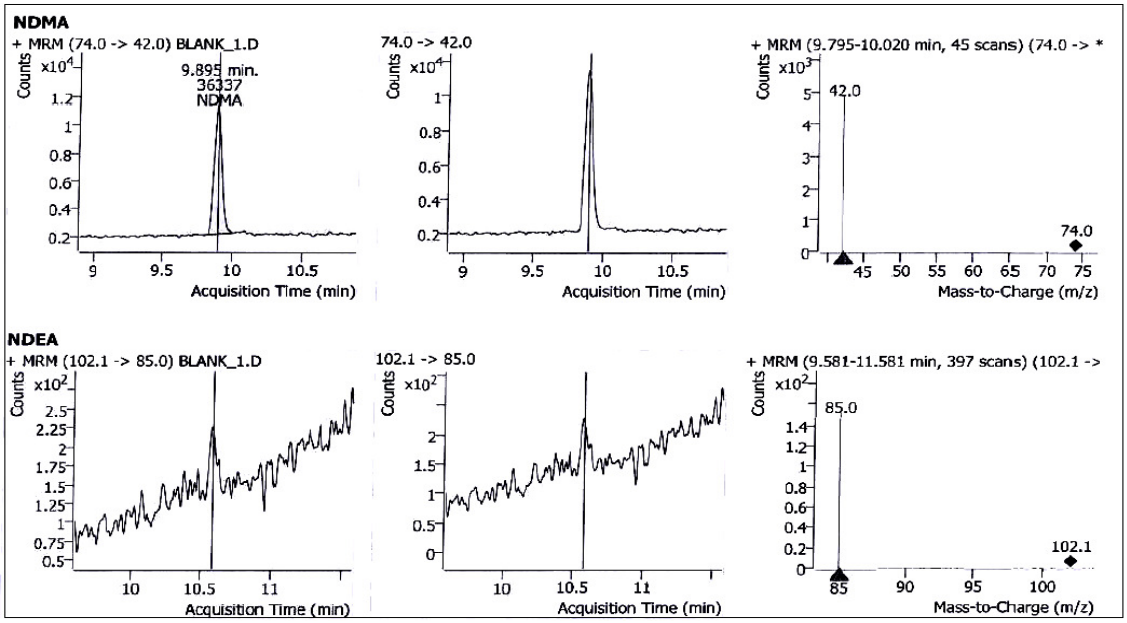
Table 2: Specificity

Component	RT(min)	RRT
NDMA	9.895	0.93
NDEA	10.585	1.00

Acceptance criteria: Peak due to NDMA & NDEA should be adequately resolved from each other.

Fig.1: Chromatogram of Specificity





Limit of Detection: The limit of detection[9] shall be determined by injecting a lower concentration of NDMA and NDEA and calculating the relative standard deviation of the analyte peak. The limit of detection and quantitation was determined by injecting low concentrations of NDMA and NDEA in six replicate injections. The Limit of detection and quantitation value and relative standard deviation obtained for NDMA and NDEA.

Table 3: RSD of Limit of Detection & Quantitation

Injection No.	Area			
	Limit of Detection		Limit of Quantification	
	NDMA	NDEA	NDMA	NDEA
1	8694	1956	16843	4629
2	7516	2176	15898	4129
3	8652	2161	17448	4502
4	7450	2328	16865	4718
5	8606	2301	15859	4492
6	8422	2206	17496	4909
Mean	**8223**	**2188**	**16735**	**4574**
SD	**581.30**	**132.20**	**718.89**	**241.15**
% RSD	**7.07**	**6.04**	**4.30**	**5.27**

Table 4:Limit of Detection and Quantitation

Component	LOD			LOQ		
	Conc.(ppm)		Conc(%)	Conc.(ppm)		Conc(%)
	As Such	w.r to Test		As Such	w.r to Test	
NDMA	0.0519	10.38	5	0.10.38	20.76	10
NDEA	0.0513	10.26	5	0.1025	20.76	10

Acceptance Criteria: RSD for the area of six replicate injections for NDMA and NDEA peak from a limit of detection solution should be NMT 30%.RSD of six replicate injections of a

limit of detection solution should be NMT 30.0%. RSD of six replicate injections of a limit of quantitation solution should be NMT 25.0%.

Limit of Quantitation: The limit of quantitation[9] is defined as the lowest concentration of NDMA and NDEA in a sample that can be determined with acceptable precision and accuracy. Determine the LOQ based on the LOD experiment.

Acceptance Criteria: RSD for area of six replicate injections of NDMA and NDEA peak from a limit of quantitation solution should be NMT 25%.

Linearity: The linearity shall be determined by injecting the solutions in triplicate, containing NDMA and NDEA ranging from LOQ to 150 % (i.e" LOQ, 30%, 50%, 80%, l00%, l20% and l50%) of the specification limit.

Table 5: Linearity for NDMA

Level	Conc(ppm)	Observed Area			Average Area
		Area-1	Area-2	Area-3	
I- (LOQ	0.1123	58078	59757	58673	58836
II- (30%)	0.3370	89522	88558	90169	89416
III- (50%)	0.5616	116548	116962	118129	117213
IV- (80%)	0.8986	191712	187562	193293	190856
V- (100%)	1.1233	231322	238897	229656	233292
VI- (120%)	1.3479	295926	298861	286521	293769
VII-(150%)	1.6849	349866	349647	355445	351653

Table 6: Linearity for NDEA

Level	Conc(ppm)	Observed Area			Average Area
		Area-1	Area-2	Area-3	
I- (LOQ	0.1037	10897	11112	11069	11026
II- (30%)	0.3112	15956	16438	16283	16226
III- (50%)	0.5187	26100	26495	26608	26401
IV- (80%)	0.8299	44326	43210	44639	44058
V- (100%)	1.0374	55024	56645	54374	55358
VI- (120%)	1.2449	68670	49142	66283	68032
VII-(150%)	1.5561	82806	87885	84376	85022

Table 7: Correlation coefficient (r) & % y-intercept

Component	Correlation coefficient(r)	% y- Intercept
NDMA	0.99	10.26
NDEA	1.00	2.81

Acceptance criteria: The plot of concentration in ppm versus average peak area for NDMA and NDEA should be linear with a correlation coefficient (r) NLT 0.99.

Solution I: Prepare a solution containing NDMA and NDEA at the LOQ level of Specification in the diluent.

Solution II: Prepare a solution containing NDMA and NDEA at a 30% level of specification in diluent.

Solution III: Prepare a solution containing NDMA and NDEA at 50%, 80%, 100%, 10%, and 150% levels of specification in the diluent.

Inject the above solutions in triplicate in the chromatographic system as mentioned in the method of analysis and note down the peak area" Perform the regression analysis and determine the correlation coefficient(r) and oh y intercept. Report the linearity as the range for determination of NDMA and NDEA.

Acceptance Criteria: The plot of concentration in ppm versus average peak area for NDMA and NDEA should be linear with a correlation coefficient (r) NLT 0.99.

y-intercept should be + 10 of response of I00% standard solution.

Accuracy: Prepare Latanoprost test solution spiked with NDMA and NDEA in triplicate at LOQ level, 50% level, 150% level, and six determinations for 100% level (in total 15 determinations) of the specified limit. The accuracy at LOQ Q0%), 50%, l00 %, and 150% a of specification level was determined by spiking NDMA and NDEA in Latanoprost at respective levels" The solutions were prepared individually in triplicate".

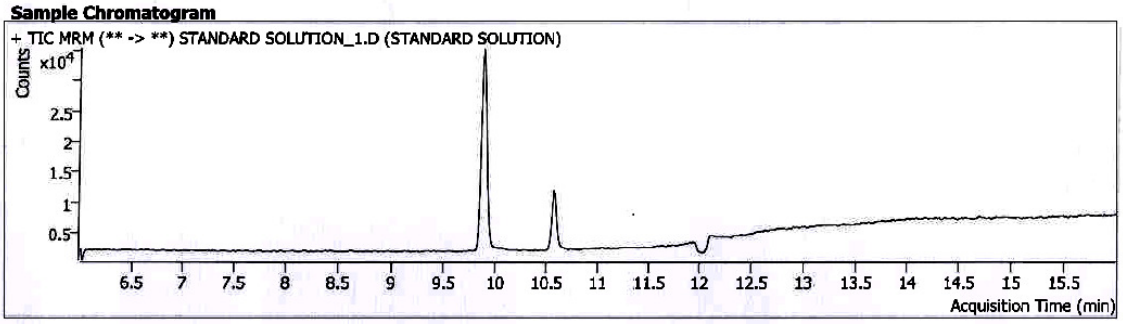
Solution I: Prepare a Test solution of Latanoprost spiked with NDMA and NDEA at LOQ level of specification in diluent.

Solution II: Prepare a Test solution of Latanoprost spiked with NDMA and NDEA at a 50% level of specification in a diluent.

Solution III: Prepare a Test solution of Latanoprost spiked with NDMA and NDEA at 100% level of specification in a diluent.

Solution IV: Prepare a Test solution of Latanoprost spiked with NDMA and NDEA at a 150 % level of specification in a diluent.

Fig.2: Chromatogram of Accuracy



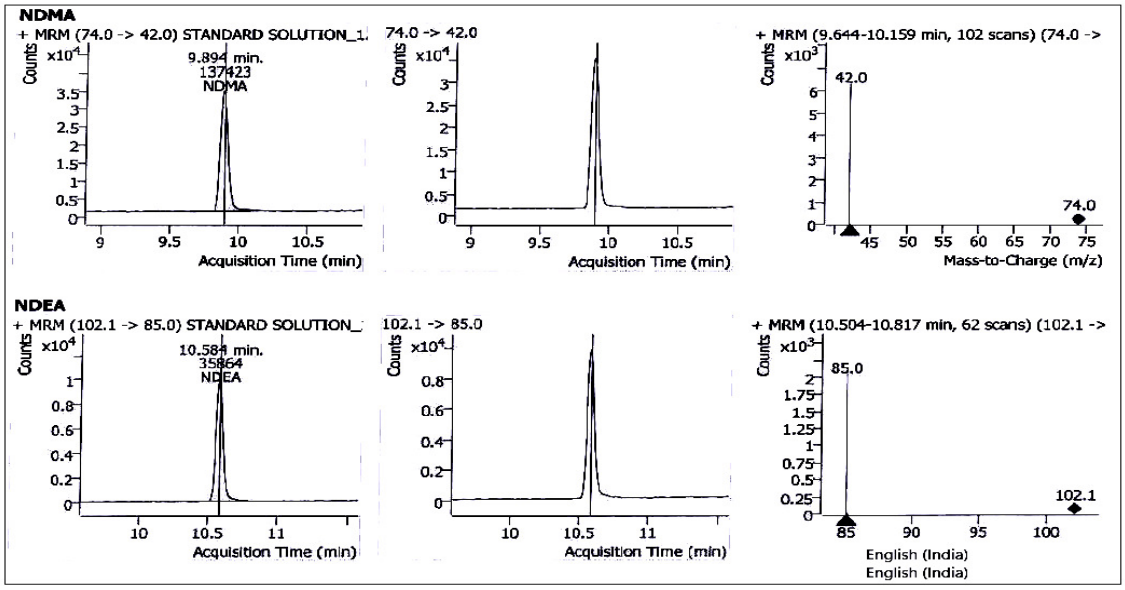


Table 8: Result of unspiked sample

Test Wt (mg)	NDMA in the unspiked sample		NDMA in the unspiked sample	
	Area	Content (ppm)	Area	Content (ppm)
5.87	196	0.26	Not Detected	Not Detected
5.54	253	0.36	Not Detected	Not Detected
Mean	**224.5**	**0.31**	**Not Detected**	**Not Detected**

Table 9: % Recoveries for NDMA

Level	**LOQ**	**50%**	**100%**	**150%**
Test Spike Wt(mg)	5.49	5.30	5.33	5.15
	5.78	5.25	6.02	5.20
	5.79	5.42	8.01	5.33
Amount Added (ppm)	18.9131	97.9558	194.8090	302.4268
	17.9642	98.8887	172.4802	299.5188
	17.9332	95.7871	129.6294	292.2135
Area in Test Spike	13937	77811	136688	222749
	15794	75856	138992	216454
	14600	75819	136489	222080
Amount found (ppm)	19.9625	115.4470	201.6604	340.1152
	21.4873	113.6183	181.5561	331.8621
	19.8286	110.0009	133.9932	327.6421
Amount received (ppm)	16.6525	19.6525	115.4470	339.8052
	21.1773	21.1773	113.6183	331.5521
	19.5186	19.5186	110.0009	327.3321
% Recovery	103.91	117.54	103.36	112.36
	117.89	114.58	105.08	110.69
	108.84	114.52	103.13	112.02
Mean % Rec	**110.21**	**115.55**	**103.86**	**111.69**
SD	**7.09**	**1.73**	**1.07**	**0.88**
% RSD	**6.43**	**1.50**	**1.03**	**0.79**

Table 10:% Recoveries for NDEA

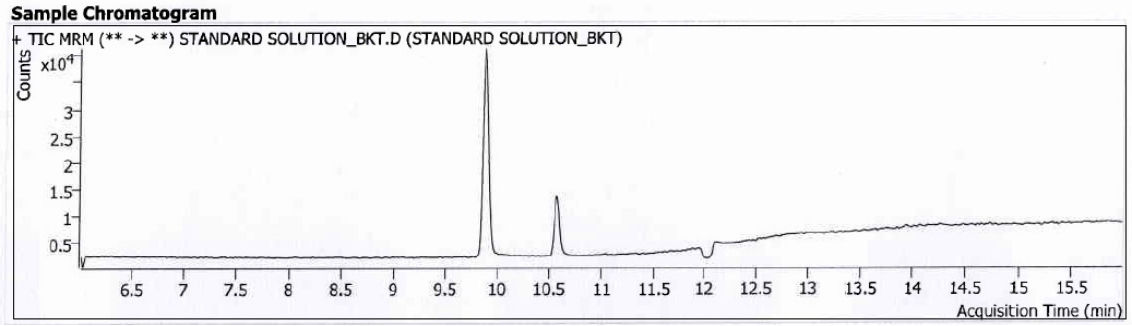
Level	LOQ	50%	100%	150%
Test Spike Wt(mg)	5.49	5.30	5.33	5.15
	5.78	5.25	6.02	5.20
	5.79	5.42	8.01	5.33
Amount Added (ppm)	18.6778	96.7358	192.3827	298.6602
	17.7405	97.6571	170.3322	295.7885
	17.7098	94.5941	128.0150	288.5741
Area in Test Spike	4013	22597	38771	61994
	4395	21544	39807	59702
	4135	21916	37789	61702
Amount found (ppm)	20.3186	118.5145	202.1977	334.6100
	21.1362	114.0679	183.8059	319.1405
	16.8515	1123980	131.1382	321.7870
Amount received (ppm)	20.3186	115.5145	202.1977	334.6100
	21.1362	114.0679	183.8059	319.1405
	19.8515	112.3980	131.1382	321.7870
% Recovery	108.79	118.51	105.10	112.04
	119.14	116.80	107.91	107.89
	112.09	118.82	102.44	111.51
Mean % Rec	**113.34**	**119.38**	**105.15**	**110.48**
SD	**5.29**	**2.90**	**2.74**	**2.26**
% RSD	**4.67**	**2.43**	**2.61**	**2.05**

Acceptance Criteria: % recoveries obtained for NDMA and NDEA should be in the range of 70% to 130%o at LOQ Level. RSD of the %recoveries obtained at LOQ should be NMT 25.0%. recoveries obtained for NDMA and NDEA should be in the range of 80% to l20% at 50% to I50% level. RSD of the recoveries obtained at 50% to I50% should be NMT 20.0%.

Precision:

System Precision: Inject the Standard solution at the specification level six times and determine the RSD of the peak area of NDMA and NDEA. System precision was determined by performing six replicate injections of standard solution as per the method of analysis.

Fig.3: Chromatogram of System Precision



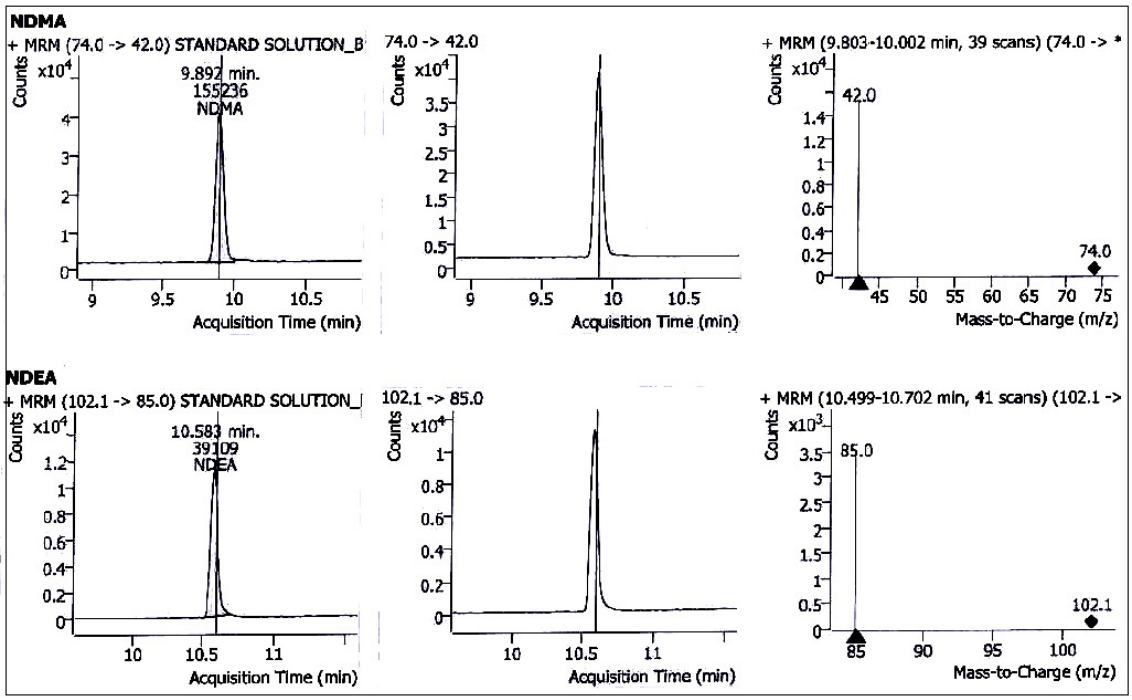


Table 11: System precision

Injection no.	Area	
	NDMA	**NDEA**
1	130178	35864
2	131985	36761
3	127761	35557
4	133726	37456
5	132837	38052
6	135776	37646
Mean	132044	36889
SD	2800.55	1008.80
%RSD	2.12	2.73

Acceptance Criteria: The RSD for the six replicate injections for NDMA and NDEA peak should be NNIT 15.0%.

Method Precision: Prepare a solution of Latanoprost spiked with NDMA times (six individual preparations) and analyze it as per the precision. A solution of Latanoprost spiked with NDMA and NDEA at specification level (100%) was prepared six times (six individual preparations) and analyzed as per the analytical method for determining the method precision.

Table l2: Summary of NDMA for Method precision (ppm)

Test Spike Wt(mg)	**Amount added (ppm)**	**Area in Test Spike**	**Amount Found (ppm)**	**Amount Received (ppm)**	**Mean (ppm)**	**SD**	**%RSD**
5.33	194.8090	136688	201.6604	201.3504			
6.02	172.4804	138992	181.5561	181.2461			
8.01	129.6294	136489	133.9932	133.6832	**179.1532**	**23.6810**	**13.22**

5.51	188.4450	134980	192.6351	192.3251			
5.82	178.4075	138830	187.5762	187.2662			
6.19	167.7434	141187	179.3583	179.0483			

Table 13: Summary of NDEA for Method precision (ppm)

Test Spike Wt(mg)	**Amount added (ppm)**	**Area in Test Spike**	**Amount Found (ppm)**	**Amount Received (ppm)**	**Mean (ppm)**	**SD**	**%RSD**
5.33	192.3827	38771	202.1977	202.1977			
6.02	170.3322	39807	183.8059	183.8059			
8.01	128.0150	37789	131.1382	131.1382	**180.0600**	**25.4416**	**14.13**
5.51	186.0980	38465	194.0486	194.0486			
5.82	176.1856	40145	191.7365	191.7365			
6.19	165.6543	39512	177.4332	177.4332			

Acceptance Criteria: RSD of ppm of the amount recovered for NDMA and NDEA should be NMT 15%.

Conclusion:

Validation shall be repeated whenever there is any significant change in the method used for the determination of the content of NDMA and NDEA in Latanoprost by GCMS/MS. Validation data shall be compiled based on the evaluation of test results and chromatograms Based on the results obtained, compile a validation report and conclude the suitability of the analytical method for regular use. The simultaneous detection of NDMA and NDEA in Latanoprost and pharmaceutical items including eight pharmacological components has been documented using an isotope dilution, GC–MS/MS approach. Precipitation and affinity differences of SPE active charcoal were used to purify NDMA and NDEA from drug substances and products. This approach was extremely effective in eliminating all interfering components, notably ranitidine. As a result, NDMA and NDEA in ranitidine could be measured using GC-MS/MS. This technique, which used the isotope dilution approach to lessen process mistakes, was sensitive because it used SPE to concentrate the sample extract and GC-MS/MS to analyze the results. In conclusion, the developed method can be used as a routine method for evaluating the presence of NDMA and NDEA Content in Latanoprost (LTN/LTP) By GCMS/MS.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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