ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 11, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

ADVANCED LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR SELECTIVE QUANTIFICATION OF NITROSAMINE IMPURITIES WITH PARTS PER BILLION LEVELS IN SACUBITRIL VALSARTAN TABLETS: ENHANCING DRUG SAFETY

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Received: 28 June 2025, Revised and Accepted: 21 August 2025

ABSTRACT

Objectives: Nitrosamine impurities may be present at low levels in various products to which people are routinely exposed. Regulatory agencies have determined that nitrosamines can form in any drug substance or drug product in which secondary, tertiary, or quaternary amines were present along with nitrosating agents. The objective of this current work is to develop a sensitive and robust method that outputs a trace-level quantification and simultaneous detection of the nitrosamine impurities (N-Nitrosodimethylamine [NDMA] and N-Nitrosodiethylamine [NDEA]) in the drug product of Sacubitril valsartan using a triple quadrupole liquid chromatography tandem mass spectrometry (LC-MS/MS) system to ensure the patient safety and compliance with regulatory expectations.

Methods: A liquid chromatograph system with a triple quadrapole mass spectrometry detector (Shimadzu LC-MS/MS). During the development of a method, the conditions for chromatographic separation with Shim-pack GST C8 Column (150 mm*4.6 mm, 3.0 µm) elution with formic acid and methanol as a mobile phase (separation achieved with gradient program with run time of 25 min) using gradient elution with formic acid and methanol as a mobile phase. Peak shape and area response optimized with diluent methanol and water in the ratio of 80:20 v/v with an injection volume of 50 µL. For ionization, atmospheric pressure chemical ionization mode with Multiple Reaction Monitoring (MRM) transition has been used for the quantification of NDMA and NDEA with a program run time of 25 min. The method utilizes a flow rate of 0.500 mL/min and at a column temperature of 35°C±0.5°C, sample temperature of 15°C±0.5°C. The resulting method was validated for specificity, linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, and precision.

Results: The method was specific as there is no interference was observed at the retention time of each analyte, and the results were well within the limits. The LOD and LOQ of Sacubitril and valsartan for NDMA and NDEA were reported with 7 parts per billion (ppb) and 21.2 ppb, and 1.9 ppb and 5.8 ppb levels, respectively. The Linearity curve was generated by plotting the area ratios against the drug concentration and reported the R^2 values for NDMA, NDEA, is 0.997 and 0.999, respectively, and the method can quantify NDEA linear from 10 ppb to 120 ppb and NDMA linear from 20 ppb to 420 ppb. Recovery results (NDEA) reported for LOQ are 117.24%, 50% is 115.69%, 100% is 109.58, and 200% is 108.72, and recovery results (NDMA) reported for LOQ are 110.27%, 50% is 107.84%, 100% is 102.58, and 200% is 94.83 with % relative standard deviation <15.0%.

Conclusion: Based on the results, it can be concluded that the method is reliable for the detection and quantification of NDMA and NDEA nitrosamine impurities in Sacubitril valsartan tablets.

Keywords: Nitrosamine impurities, Sacubitril, Valsartan, Validation, Liquid chromatography tandem mass spectrometry, N-nitrosodimethylamine, N-nitrosodiethylamine, Parts per billion.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i11.55806. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Heart failure represents a significant public health issue, particularly affecting the elderly population due to its high prevalence [1]. Heart failure is a complex clinical syndrome resulting from the heart's inability to effectively pump blood throughout the body [2]. This condition significantly contributes to global morbidity, hospitalization, and mortality rates [3]. One of the main therapeutic approaches involves blocking the renin-angiotensin-aldosterone system (RAAS) through the use of angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and mineralocorticoid receptor antagonists [4]. Sacubitril/valsartan, a combination drug classified under angiotensin receptor-neprilysin inhibitors, is used in the management of chronic heart failure with reduced ejection fraction in patients classified under NYHA Class II, III, or IV [5,6].

Representing a significant advancement in the management of chronic heart failure, Sacubitril and valsartan, administered as a fixed-dose combination. As part of ongoing regulatory and quality assurance efforts, particular attention has been directed toward the detection and control of Nitrosamine impurities in pharmaceutical products. Nitrosamines, such as N-Nitrosodimethylamine [NDMA] and N-Nitrosodiethylamine (NDEA), are classified as probable human carcinogens, and their presence in valsartan-based formulations has prompted global scrutiny. Therefore, nitrosamine testing for Sacubitril and valsartan is a critical component of product safety evaluation, ensuring compliance with international guidelines and safeguarding patient health. Analytical methods such as reversed-phase – high-performance liquid chromatography [7,8] and liquid chromatography tandem mass spectrometry (LC-MS/MS) have been developed and validated for the quantification of these impurities.

The presence of nitrosamine [9] impurities in drug products has been a significant safety concerns, falling under the category of "cohort of concern" according to International Council for Harmonization (ICH)

M7 guidelines [10]. Nitrosamine impurities have been classified as probable human carcinogens for decades. These impurities were reported in beverages [11], tobacco [12], food [13], personal care products [14], chlorinated and chloraminated water. Consequently, the United States Food and Drug Administration [15] and the European Medicines Agency [16] in July 2018 announced the presence of a new class of carcinogenic impurities, NDMA and (NDEA), in generic active pharmaceutical ingredients and products of angiotensin receptor blockers (ARBs) [17]. To limit the potential carcinogenic risk, regulatory agencies have published acceptable intakes (AIs) to control nitrosamine impurities in pharmaceutical products. The nitrosamine-specific AIs were established by linear extrapolation from a dose producing a 50% tumor incidence (TD50) over background in a carcinogenicity study with experimental animals to a 1 in 100,000 excess risk of cancer, namely, NDMA (96 nanogram [ng]/day); NDEA (26.5 ng/day) [18]. These developments underscore the urgency of understanding nitrosamine formation and implementing effective control strategies to safeguard public health.

Sacubitril is a neprilysin inhibitor with the chemical name 4-{[(1S,3R)-1-([1,10-Biphenyl]-4-ylmethyl)-4-ethoxy-3-methyl-4-oxobutyl] amino}-4-oxobutanoic acid [19] represented in Fig. 1. It acts as a prodrug that undergoes activation through de-ethylation by esterases, ultimately inhibiting neprilysin, an enzyme responsible for the degradation of vasoactive peptides such as atrial natriuretic peptide and bradykinin [20]. Valsartan, chemically N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-L-valine, represented in Fig. 1 [21,22], functions as an angiotensin II receptor blocker (ARB), preventing vasoconstriction and reducing blood pressure [23-25]. Sacubitril is used in combination with valsartan in a drug with brand name Entresto. This combination commonly used to treat Heart failure [26]. The main aim of the presented study was to develop and validate [27-30] a routine method for the identification and quantification of two possible Nitrosamine impurities (NDMA and NDEA) in Sacubitril Valsartan with high-performance LC-MS/MS [31-33] in accordance with ICH guidelines [34].

Drug identification

CAS Description**:** 936623-90-4

Generic Name: Sacubitril/Valsartan

Brand Name: Entresto

ATC Code: C09DX04.

METHODS

Chemicals and reagents

Formic acid (for LC-MS Gradient, Honeywell), Methanol (for LC-MS Gradient, JT Baker), and Water (Milli-Q, Milli Pore), nitrosamine impurity standards NDMA and NDEA from Lee Pharma Limited, Sacubitril Valsartan Tablets, and Sacubitril Valsartan Tablets (Placebo Powder) from Lee Pharma Limited, respectively.

Instruments/equipment

The below instrument's/equipment's used for the analysis are tabulated below (Table 1).

Analytical methodology

The Liquid chromatography method was carried in Shimadzu Model 8045 with a quaternary pump and Lab Solutions software SP-118. The experimental work was performed on Shimadzu pack GIST, C8 (150 × 4.6 mm, 3.0 µ) column to retain slightly non-polar impurities of NDMA and NDEA, respectively, and to get better resolution and selectivity from diluent, placebo matrix, and Sacubitril and valsartan components. To reduce the run time and to get better selectivity, the C8 boned-phase column was optimized for the separation. Method optimized using a mobile phase consisting of a volatile buffer of 0.1% formic acid in water as a mobile phase-A and 0.1% formic acid in methanol as a mobile phase-B with a gradient elution and a flow rate of 0.5 mL/min. Area response and asymmetric peak shape of impurities optimized with a diluent of methanol and water in the ratio of 90: 10 v/v and injection volume of 50 µL with wavelength detection at 254 nm as tabulated in Tables 2-6.

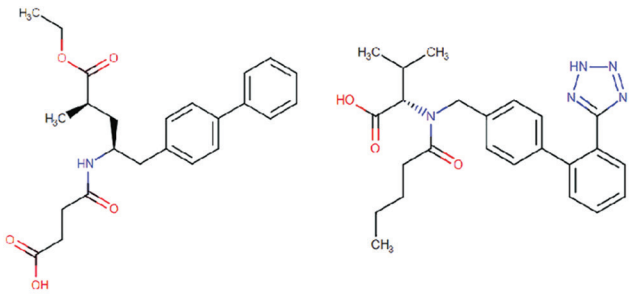


Fig. 1: Chemical structure of sacubitril and valsartan. (a) Sacubitril (b) Valsartan

Table 1: Instruments/equipment's used

S. No.	Instruments	Make	Model
1.	Liquid chromatograph mass spectrometer	Shimadzu	8045
2.	Analytical balance	Sartorius	SECURA225D-10IN
3.	Vortex	REMI	CM-101PLUS
4.	Sonicator	PCI	30L5001D1SPL
5.	Micro pipette	Dragon	YE169AA0040149

Table 2: Chromatographic conditions (LC conditions)

HPLC Parameters	Details
Column	Shim-pack GIST C8 (150 mm×4.6 mm, 3.0 µm)
Column oven temperature	35°C
Sampler cooler temperature	15°C
Injection volume	50µL
UV wavelength	254 nm
Flow rate	0.500 mL/min
Total run time	25.00 min
Needle wash	Methanol: Water (90:10) v/v
Detector	Mass detector
LC system	Shimadzu LC

Table 3: Gradient program

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.00	65	35
2.00	65	35
2.10	50	50
4.00	50	50
4.10	37	63
14.00	37	63
14.60	25	75
20.60	25	75
21.00	65	35
25.00	65	35

Table 4: LC time program

Time	Module	Command	Value
0.01	Column Oven	Oven Valve 2	0
4.20	Column Oven	Oven Valve 2	1
5.80	Column Oven	Oven Valve 2	0
7.20	Column Oven	Oven Valve 2	1
8.50	Column Oven	Oven Valve 2	0
25.00	Controller	Stop	-

LC: Liquid chromatography

Table 5: Mass conditions

Mass Parameters	Details
Interface	APCI
Interface temperature	350°C
Desolvation temperature	602°C
DL temperature	200°C
Heat block temperature	200°C
Nebulizing gas flow	3.00 L/min
Drying gas flow	5.00 L/min
Acquisition mode	MRM
Polarity	+ve

Table 6: MRM transition

Precursor (m/z)	Product (m/z)	Dwell time (m sec)	Q1 Pre bias (V)	CE (Volts)	Q3 Pre bias (V)		
NDMA	75.20	43.10	200.0	−5.0	−19.0	−45.0	NDMA
NDEA	103.20	29.15	17.0	−10.0	−15.0	−30.0	NDEA

NDMA: N-nitrosodimethylamine, NDEA: N-nitrosodiethylamine

Table 7: Results for retention time confirmation and identification

Name of the impurity	Retention time (min)				
	Standard solution	Placebo solution	Sample solution	Spiked test solution	Identification solution
NDMA	4.966	ND	ND	4.960	4.973
NDEA	7.851	ND	ND	7.857	7.854

NDMA: N-nitrosodimethylamine, NDEA: N-nitrosodiethylamine

Table 8: Results for LOD and LOQ establishment

S. No.	Name of the solution	Area	S/N ratio	Concentration (ppb)
NDMA				
1.	LOQ solution	9359	17.24	21.2
2.	LOD solution	3377	3.58	7.0
NDEA				
1.	LOQ solution	9029	19.76	5.8
2.	LOD solution	5350	11.43	1.9

Ppb: Parts per Billion, NDMA: N-nitrosodimethylamine, NDEA: N-nitrosodiethylamine, LOQ: Limit of quantification, LOD: Limit of detection, S/N: Signal to noise

Table 9: Results for LOQ precision

S. No.	Name of the solution	Area	
		NDMA	NDEA
1.	LOQ Precision-1	6038	1823
2.	LOQ Precision-2	5039	1804
3.	LOQ Precision-3	5062	1727
4.	LOQ Precision-4	5385	1633
5	LOQ Precision-5	6591	1811
6.	LOQ Precision-6	6313	1493
Average		5738	1715
SDEV		666.10	130.35
RSD (in %)		11.61	7.6

NDMA: N-nitrosodimethylamine, NDEA: N-nitrosodiethylamine, LOQ: Limit of quantification, SDEV: Standard deviation, RSD: Relative standard deviation

Mass spectrometry

Triple quadrupole mass spectrometry has been used to provide higher selectivity, sensitivity, and high degree of signal-to-noise levels to detect the trace levels of nitrosamine impurities in parts per billion (ppb) levels than a single quadrupole mass detector. Mass spectrometry experimental was performed with triple quadrupole mass spectrometry from Shimadzu with atmospheric pressure chemical ionization (APCI) interface operating in positive mode, set up in MRM transition. APCI mode chosen over electron spray ionization mode, since both NDMA and NDEA nitrosamine impurities are having lower molecular weight and low polar in nature. In APCI mode, both the impurities are easily ionized and sensitivity has been achieved.

Different gradient and mobile phase ratio trials are evaluated to achieve the separation of impurities from blank, placebo, and from the main analytes of sacubitril and valsartan. NDMA and NDEA are eluted and separation at more than 50% of the higher organic ratio. Better selectivity and area response achieved with following gradient elution given in Table 2.

Mobile phase preparation

- Preparation of mobile phase–A: Pipette out 1.0 mL of Formic acid to a bottle containing 1000 mL of water, mix well sonicate to degas
- Preparation of Mobile phase–B: Pipette out 1.0 mL of Formic acid to a bottle containing 1000 mL of methanol, mix well sonicate to degas
- Preparation of diluent: Prepare the mixture of water and methanol in the ratio of 80:20 v/v
- Preparation of blank solution: Same as diluent.

Preparation of standard stock solutions

Preparation of NDMA standard stock solution

Weigh and transfer accurately 5.00 mg of NDMA standard into a 100 mL volumetric flask, add 50 mL of methanol, sonicate to dissolve. Dilute to volume with methanol and mix well.

Preparation of NDEA standard stock solution

Weigh and transfer accurately 5.00 mg of NDEA standard into a 100 mL volumetric flask, add 50 mL of methanol, sonicate to dissolve. Dilute to volume with methanol and mix well.

Preparation of intermediate standard solution-1

Pipette out 1.70 mL of NDMA and 0.46 mL of NDEA standard stock solutions into a 50 mL volumetric flask. Dilute to volume with methanol and mix well.

Preparation of intermediate standard solution-2

Pipette out 0.5 mL of intermediate standard solution-1 into a 20 mL volumetric flask. Dilute to volume with methanol and mix well.

Preparation of standard solution

Pipette out 1.0 mL of intermediate standard solution-2 into a 10 mL volumetric flask. Dilute to volume with diluent and mix well.

Preparation of sample solution

Considering solubility and complexity of the formulations crushing technique chosen over the intact method for the sample extraction procedure, and diluent optimized with methanol and water to achieve the complete dispersion and extraction of impurities form the sample solution. Keeping in view of thermal stability of impurities vortex technique chosen for the recovery procedure, and polypropylene tubes are used for the sample preparation to avoid the leaching and instability of nitrosamines. Optimized extraction technique and finalized concentration levels of the sample solution are summarized as follows.

Transfer 10 tablets of Sacubitril and valsartan tablets into motor and pestle. Crush into fine powder, weigh and transfer test sample (Equivalent to 200 mg of Sacubitril and valsartan) into a 15 mL polypropylene centrifuge tube, add a few mL of diluent and mix well. Dilute to a volume up to 10 mL with diluent and mix well. Vortex for 1 min and filter through 0.22µ polyvinylidene fluoride (PVDF) (Make: Thermo Scientific) filter. (After filtration, the obtained clear solution is taken to inject into the sample vial).

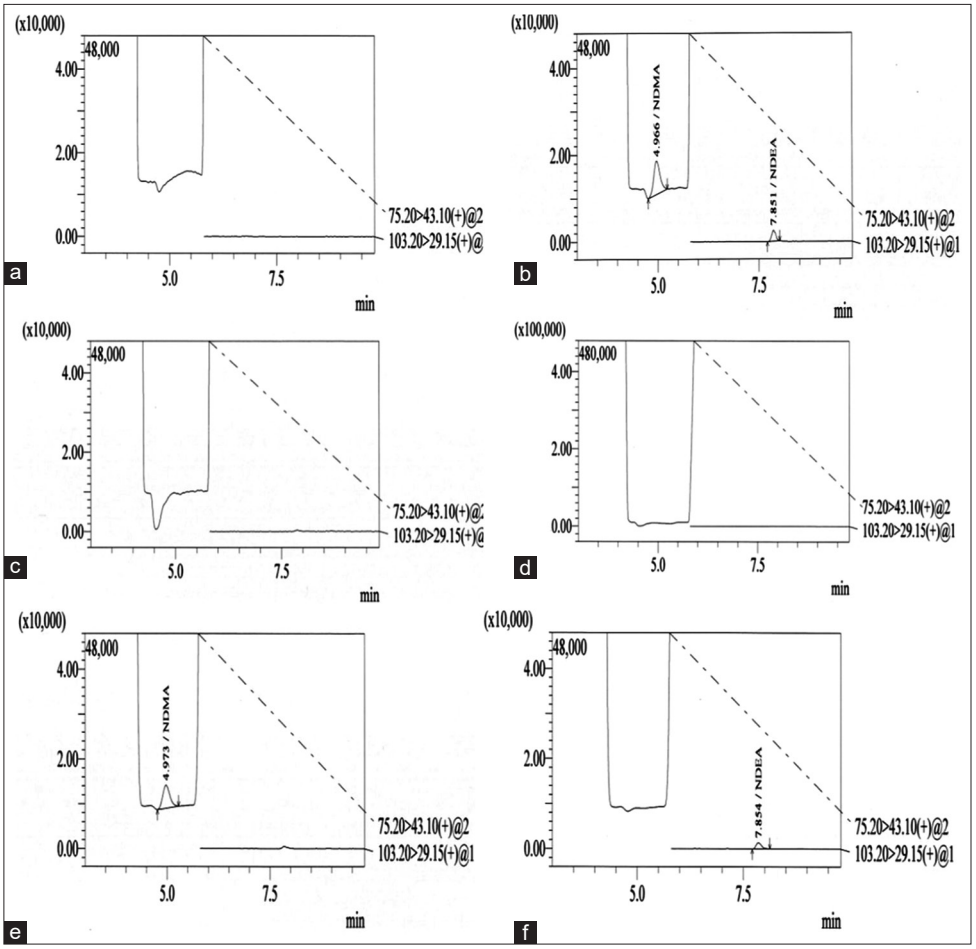


Fig. 2: Typical chromatograms. (a) Typical chromatogram for blank (b) Typical chromatogram for standard solution (c) Typical Chromatogram for placebo (d) Typical chromatogram for test solution (e) Typical chromatogram for N-Nitrosodimethylamine Identification (f) Typical chromatogram for N-Nitrosodiethylamine identification

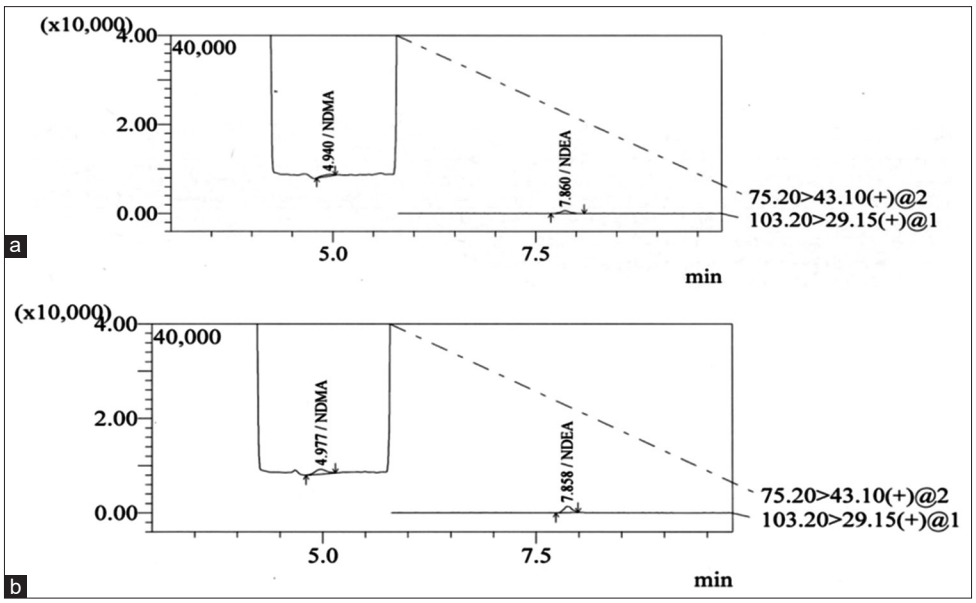


Fig. 3: Chromatogram for limit of detection (a) Solution and limit of quantification (b)

Table 10: Results for LOQ precision

Linearity levels	NDMA		NDEA	
	Concentration (ppb)	**Average peak Area**	**Concentration (ppb)**	**Average peak Area**
Linearity level-1 (LOQ)	21	5738	6	1715
Linearity level-2 (50%)	105	28465	29	7364
Linearity level-3 (100%)	211	56730	58	13803
Linearity level-4 (150%)	316	77540	86	19640
Linearity level-5 (200%)	421	103732	115	26831
Correlation coefficient	0.999		1.000	
Regression coefficient	0.997		0.999	
Slope	242053.14		227209.77	
Y-Intercept	2447.98		510.67	
% Y-intercept at 100% Level	4.32		3.70	

NDMA: N-nitrosodimethylamine, NDEA: N-nitrosodiethylamine, LOQ: Limit of quantification, ppb: Parts per billion

Table 11: Statistical analysis for NDMA

Regression statistics					
Multiple R	0.998651726				
R square	0.99730527				
Adjusted R square	0.996407027				
Standard error	2324.44818				
Observations	5				
ANOVA					
	Df	**SS**	**MS**	**F**	**Significance F**
Regression	1	5998931610.0	5998931610.0	1110.3	0.000
Residual	3	16209178.0	5403059.3		
Total	4	6015140788.0			
	Coefficients	**Standard Error**	**t-stat**	**p-value**	**Lower 95%**
Intercept	2447.98	1874.93	1.31	0.28	−3518.89
X Variable 1	242.05	7.26	33.32	0.00	218.93
			Upper 95%	**Lower 95.0%**	**Upper 95.0%**
			8414.86	−3518.89	8414.86
			265.17	218.93	265.17

ANOVA: Analysis of variance, NDMA: N-nitrosodimethylamine

Table 12: Statistical analysis for NDEA

Regression statistics					
Multiple R	0.999599568				
R square	0.999199297				
Adjusted R square	0.998932396				
Standard error	323.1927538				
Observations	5				
ANOVA					
	df	**SS**	**MS**	**F**	**Significance F**
Regression	1	391043608.5	391043608.5	3743.708	0.000
Residual	3	313360.6683	104453.56		
Total	4	391356969.2			
	Coefficients	**Standard Error**	**t-stat**	**p-value**	**Lower 95%**
Intercept	510.67	261.85	1.95	0.15	−322.67
X Variable 1	227.21	3.71	61.19	0.00	215.39
			Upper 95%	**Lower 95.0%**	**Upper 95.0%**
	1344.00	−322.67		1344.00	
	239.03	215.39		239.03	

ANOVA: Analysis of variance, NDEA: N-nitrosodiethylamine

Table 13: Results for method precision

S. No.	Preparation name	NDEA	NDMA
		In ppb	In ppb
1.	Spiked sample solution-1	51	207
2.	Spiked sample solution-2	61	210
3.	Spiked sample solution-3	60	209
4.	Spiked sample solution-4	61	206
5.	Spiked sample solution-5	57	200
6.	Spiked sample solution-6	59	210
Average		60	207
SDEV		0.0038	0.0038
RSD (in %)		6.56	1.83

ppb: Parts per billion, SDEV: Standard deviation, RSD: Relative standard deviation, NDMA: N-nitrosodimethylamine, NDEA: N-nitrosodiethylamine

Procedure
Inject blank solution and standard solution (six) into the LC-MS/MS system and check the system suitability parameters.

System suitability
The relative standard deviation (in %) for the peak areas of each analyte from six replicate injections of standard solution should be not more than 15.0.

$$\frac{AT}{AS}*\frac{WS}{DS}*\frac{DT}{WT}*\frac{AW}{LC}*\frac{P}{100}*100000000000$$

AT : Area of each analyte in sample preparation
AS : Average Area of analyte in standard preparation
WS : Weight of standard taken in mg
DS : Dilution for standard preparation
DT : Dilution for sample preparation
WT : Weight of sample taken in mg
AW : Average weight of tablet in mg
LC : Label claim in mg (200 mg)
P : (%) purity/potency.

RESULTS AND DISCUSSION

Current work was executed to establish a highly sensitive liquid chromatographic method for sensitive liquid chromatographic method for separation with LC-MS/MS detection. Initiated with various mobile phase pH conditions in both modes of elution

Specificity
The specificity of the analytical method for NDMA and NDEA was rigorously assessed by evaluating chromatograms of contaminant-free samples before and after fortification with the respective analytes. No matrix-derived peaks were observed to coelute at the retention times corresponding to NDMA and NDEA represented in Fig. 2, which were depicted in Table 7. With the absence of interference, it can be confirmed that the method is specific.

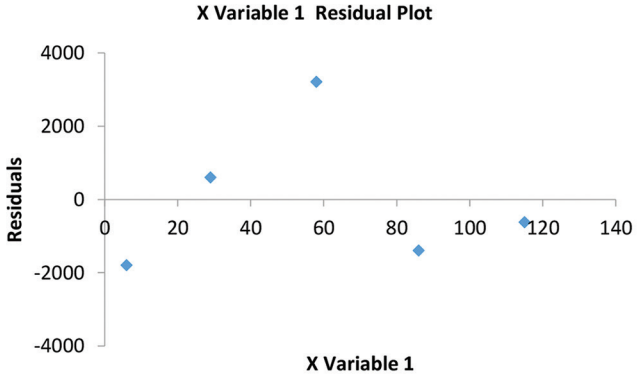
Estimation of limit of detection (LOD) and limit of quantification (LOQ)
According to signal-to-noise ratio technique, the limitations of LOD and LOQ represented in Fig. 3 that should not be <3.3 and should not be <10, respectively. The results revealed that the developed method is highly sensitive and capable of detecting and quantifying NDMA and NDEA nitrosamine impurities at ppb concentration levels. The results of LOD and LOQ of NDMA and NDEA are depicted in Table 8, respectively.

LOQ precision
The precision at LOQ was evaluated by preparing six standard solutions having impurity at about the limit of quantification level on diluent and injected. The results were found to be Precise and tabulated below in Table 9.

Linearity
A linearity experiment was performed to assess the response of each analyte by preparing five different aliquots. These solutions were prepared from the standard stock solution to achieve concentration levels ranging from LOQ to 200%. The concentration ranges were 6 ppb to 115 ppb for NDEA, 21 ppb to 421ppbfor NMEA. The experimental data obtained were analyzed using linear regression analysis represented in Fig. 4. Regression coefficient was observed as 0.999 and 0.997 for NDEA and NDMA, respectively, as given in Table 10. This indicates that the internal standards of NDEA and NDMA nitrosamine impurities are appropriate for accurate quantification of the samples using. The statistical analysis was depicted in Tables 11 and 12.

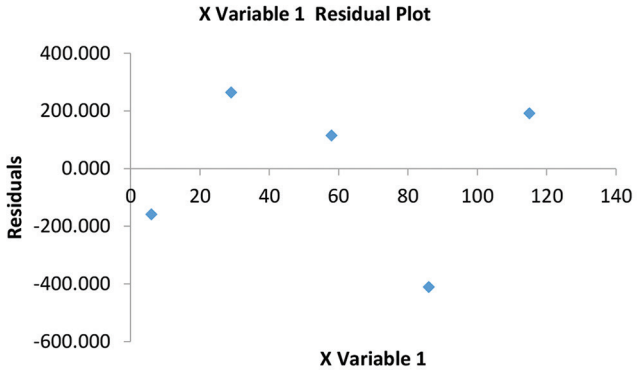
RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	Standard residuals
1	7531.101	−1793.101	−0.891
2	27863.565	601.435	0.299
3	53521.198	3208.802	1.594
4	78936.778	−1396.778	−0.694
5	104352.358	−620.358	−0.308



RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	Standard residuals
1	1873.924	−158.924	−0.568
2	7099.749	264.251	0.944
3	13688.832	114.168	0.408
4	20050.706	−410.706	−1.467
5	26639.789	191.211	0.683



Precision
Method precision was assessed by injecting six spiked samples and the results of NDMA and NDEA represented in Fig. 5, which was found

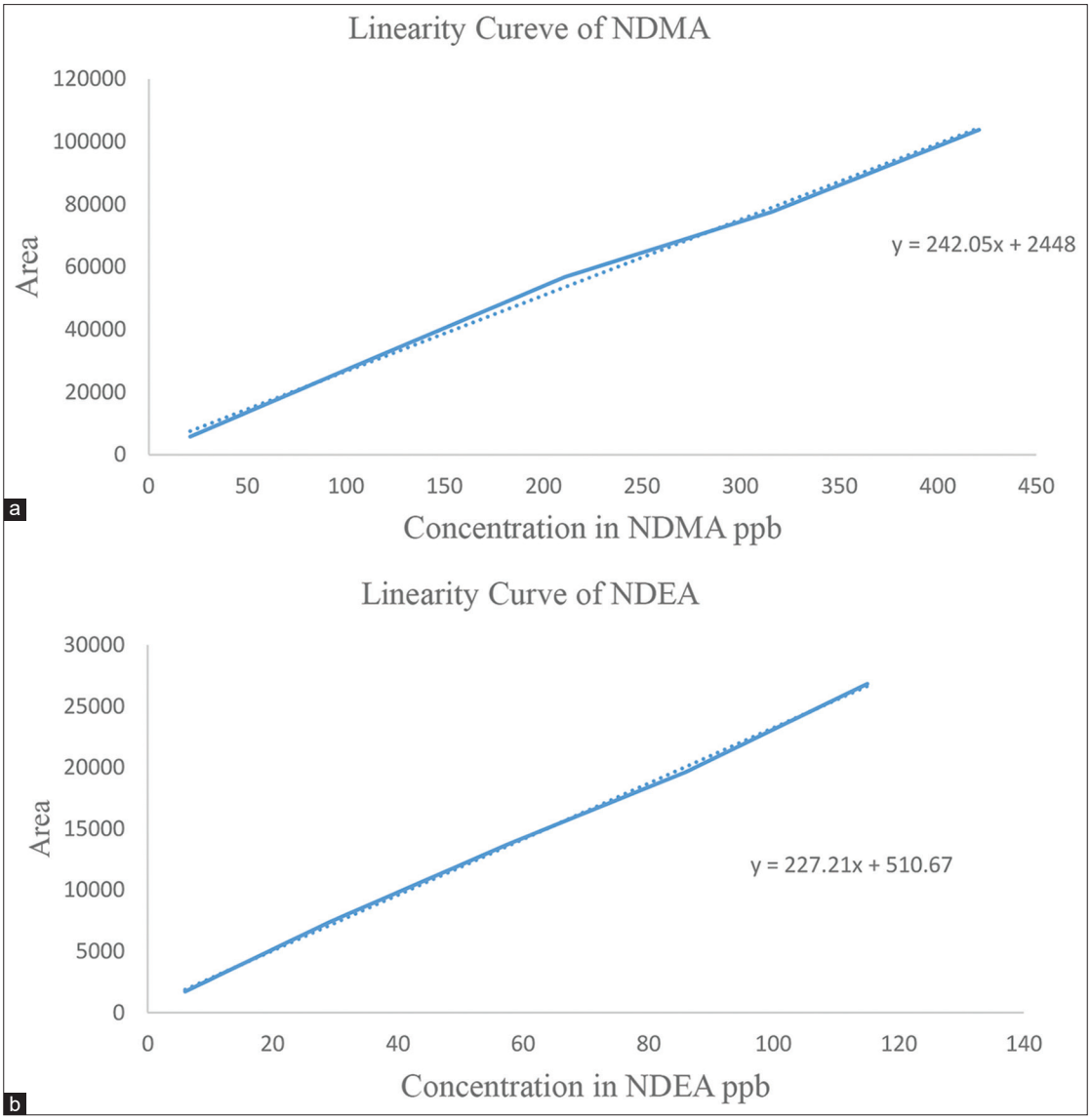


Fig. 4: Linearity curve of N-Nitrosodimethylamine (a) and N-Nitrosodiethylamine (b)

Table 14: Results for accuracy form LOQ to 200% level (NDEA)

Name of the solution	Measured Conc.in ppb	Theoretical Conc. In ppb	% Recovery	Average % Recovery	SDEV	%RSD
LOQ Preparation-1	6.9	6	118.97	117.24	4.3618	3.72
LOQ Preparation-2	6.6		113.79			
LOQ Preparation-3	6.5		112.07			
LOQ Preparation-4	6.9		118.97			
LOQ Preparation-5	7.2		124.14			
LOQ Preparation-6	6.7		115.52			
50% Preparation-1	33	29	113.40	115.69	1.9840	1.71
50% Preparation-2	34		116.84			
50% Preparation-3	34		116.84			
100% Preparation-1	63	58	108.43	109.58	1.9874	1.81
100% Preparation-2	63		108.43			
100% Preparation-3	65		111.88			
200% Preparation-1	124	116	106.71	108.72	3.7970	3.49
200% Preparation-2	131		112.74			
200% Preparation-3	119		102.41			
200% Preparation-4	126		108.43			
200% Preparation-5	128		110.15			
200% Preparation-6	130		111.88			

RSD: Relative standard deviation, LOQ: Limit of quantification, NDEA: N-nitrosodiethylamine

Table 15: Results for accuracy form LOQ to 200% level (NDMA)

Name of the solution	Measured Conc. in ppb	Theoretical Conc. in ppb	% Recovery	Average % recovery	SDEV	%RSD
LOQ Preparation-1	23	21	109.00	110.27	5.4834	4.97
LOQ Preparation-2	22.5		106.64			
LOQ Preparation-3	23.9		113.27			
LOQ Preparation-4	23.7		112.32			
LOQ Preparation-5	24.9		118.01			
LOQ Preparation-6	21.6		102.37			
50% Preparation-1	103	105	97.72	107.84	8.9674	8.32
50% Preparation-2	121		114.80			
50% Preparation-3	117		111.01			
100% Preparation-1	200	211	94.83	102.58	6.7446	6.58
100% Preparation-2	223		105.74			
100% Preparation-3	226		107.16			
200% Preparation-1	395	422	93.65	94.83	1.6357	1.72
200% Preparation-2	403		95.54			
200% Preparation-3	406		96.25			
200% Preparation-4	390		92.46			
200% Preparation-5	398		94.36			
200% Preparation-6	408		96.73			

RSD: Relative standard deviation, LOQ: Limit of quantification, NDMA: N-nitrosodimethylamine

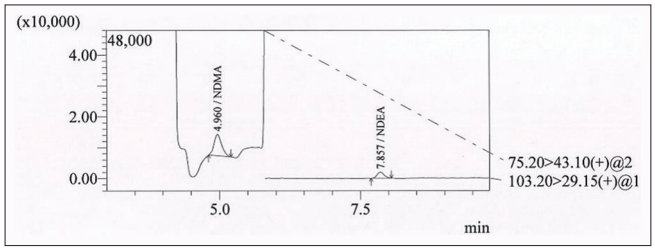


Fig. 5: Chromatogram for 100% spiked sample solution

to be within the acceptance criteria. The results were tabulated in Table 13.

Accuracy and recovery

By creating sample (i.e., spiking on test solution with each contaminant) at the level of LOQ, 50%, 100% and 200% of the target concentration, the test method's accuracy was examined. With the exception of the LOQ and 200% levels, the accuracy samples were made in replicate of three for each level (six preparations). Obtained recovery of NDEA and NDMA values was discussed in Tables 14 and 15. Individual %recovery of each impurity should be from 70.0% to 130.0%, indicating good accuracy of the method.

CONCLUSION

The LC-MS/MS analytical method for detecting nitrosamine impurities (NDMA and NDEA) in Sacubitril Valsartan Tablets was successfully developed and validated in compliance with ICH Q2 (R1) guidelines. Demonstrating high specificity, accuracy, linearity, and precision at trace levels, this approach offers a reliable and robust tool for routine monitoring. The method's ability to detect NDMA and NDEA in ppb ensures enhanced quality control, regulatory compliance, and a proactive commitment to patient safety in pharmaceutical manufacturing.

A validated LC-MS/MS method was successfully established for the quantification of nitrosamine impurities (NDMA and NDEA) in Sacubitril valsartan tablets, in accordance with ICH Q2 (R1) guidelines. The method exhibits high sensitivity, specificity, and precision, enabling accurate detection of target impurities at trace levels. Its reliability and linearity across the analytical range ensure suitability for routine quality control. By facilitating stringent monitoring of nitrosamines in finished dosage forms, the method reinforces regulatory compliance and underscores a proactive approach to patient safety in pharmaceutical development and manufacturing.

ACKNOWLEDGMENTS

The authors thank the Management of the Lee Pharma Limited, Visakhapatnam for providing laboratory facilities for performing experimental work.

AUTHOR'S CONTRIBUTIONS

We declare that it's an original research work which was carried out by G. V. Padmakar Rao and Bavisetti Lakshmi under the supervision of Dr. Meka Lingam. Dr. D. Suryakala proofread the manuscript, suggested the necessary corrections, and helped in writing the manuscript.

CONFLICTS OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

FUNDING DISCLOSURE

Nil.

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