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Evaluation of Various Potential Genotoxic Nitrosamine Impurities by Using Validated Ultra - Sensitive LC/MS/MS Analytical Method in Anti-Hypertensive Drug Product.

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KEYWORDS

Nitrosamine;
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Genotoxic impurities;
Drug product;
Method validation.

ABSTRACT:

Currently, genotoxic impurities are a prominent concern among other impurities, and regulatory authorities are focused more on identifying, quantifying, and controlling these undesired chemicals in drug substances and drug products. ICH M7 provides an overview of the class of genotoxic impurities based on the potency category. To meet the regulatory requirement for the concerned impurity, a sensitive analytical method capable of quantifying these impurities at a lower level with accuracy and precision is required. This article focuses on the development of an analytical method for NDMA (N-Nitroso dimethylamine), NDEA (N-Nitroso diethylamine), NEIPA (N-Nitrosoethylisopropylamine), NDIPA (N-Nitroso diisopropylamine), NDPA (N-Nitroso dipropylamine) and NDBA (N-Nitroso dibutylamine) nitrosamine impurities in a single chromatography method by LC-MS/MS positive mode of atmospheric pressure chemical ionization (APCI), employing multiple reaction monitoring (MRM) with a limit of quantification (LOQ) of 29 parts per billions (ppb) for NDEA, NEIPA, NDIPA, NDPA & NDBA against the acceptance limit of 331 ppb and for NDMA, the LOQ is 107.77 ppb against the acceptance limit of 1200 ppb. The chromatographic separation was accomplished using an Agilent Poroshell 120EC- C18 (150 mm × 4.6 mm), 2.7 mm utilizing a gradient mode elution program including mobile phase A (0.1% formic acid in water) and mobile phase B (4mM ammonium acetate solution in methanol). The method was challenged for accuracy, precision, and linearity in accordance with ICH guidelines to ensure its suitability for the intended usage.

1. Introduction

Impurity is defined as any component of the new drug product that is neither the drug substance nor an excipient that is present in the drug product. In other words, this chemical is undesirable and remains in the active pharmaceutical ingredient (API) or may develop during the ageing process of the drug product [1]. These impurities are classified into various categories as per the International Council for Harmonization- ICH Q3 guideline, namely, organic impurities (process- and drug-related), inorganic impurities, and residual solvents. Drug synthesis involves the use of reactive chemicals, solvents, reagents, and other excipients. As a result of the process of chemical synthesis or subsequent decomposition, impurities can appear in all drug substances and their corresponding products. While ICH Q3A (R2) and ICH Q3B (R2) give the direction of capability and control for the larger part of the impurity, a constrained direction is given for those chemicals or impurities that can modify DNA arrangement or respond at the gene level [1]. The ICH S2 (R1) and ICH M7 define mutagenic/genotoxicity as "a broad term that refers to any adverse change within the genetic material by a chemical mechanism". These impurities are characterized as "impurities that have been indicated to be genotoxic in a relevant genotoxicity test model, such as the Ames test [2][3]. Genotoxic impurities are categorized based on a risk analysis that involves conducting database and literature searches to gather information on carcinogenicity and bacterial mutagenicity data. This categorization results in classifying genotoxic impurities into Class 1, 2, or 5

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based on available data. In cases where categorization data is unavailable, structure-activity relationships (SAR) are utilized to predict bacterial mutagenicity, resulting in classification as Class 3, 4, or 5 [4].

Nitrosamine impurities are classified as Class 1 genotoxic impurities by ICH M7 (R1), based on rodent carcinogenicity and mutagenicity evidence [5]. Nitrosamine impurities cause mutations in genetic material through various mechanisms like chromosomal breakage, rearrangements, covalent binding, or insertion into the DNA during replication. These modifications in the genetic elements caused by relatively low quantities of nitrosamine contaminants have been linked to the development of cancer [6][7]. Thus, it is critical to detect nitrosamine contaminants in drug products at extremely low quantities to safeguard public safety.

Nitrosamine and N-Nitrosamine compounds, characterized by the presence of the nitroso functional group (N-NO), are uncharged, highly polar, hydrophilic molecules commonly found in alkyl, alkaryl, aryl, and cyclic amines. These moieties are known for their mutagenic, teratogenic, and carcinogenic properties, causing significant DNA damage as depicted in Fig. 1. Within the human body, nitrosamines undergo enzymatic degradation facilitated by the cytochrome P-450 enzyme. This process results in the conversion of nitrosamines into unstable dealkylated primary amines, which subsequently transform into diazonium ions. These diazonium ions serve as DNA alkylating agents, inflicting damage to the DNA structure, ultimately contributing to the development of cancer [8][9].

In June 2018, the United States Food and Drug Administration (USFDA) was alerted to the presence of the impurity N-nitrosodimethylamine (NDMA) in valsartan, an Angiotensin Receptor Blocker (ARB) used as an anti-hypertensive drug [8]. Subsequent thorough investigations by the agency revealed inadequate levels of nitrosamines in numerous sartans and other drug molecules from various manufacturers [10]. In response, multiple manufacturers voluntarily recalled affected batches, resulting in shortages of certain drug products. Additionally, in December 2019, nitrosamine contamination was also identified in some anti-diabetic drugs, such as Metformin [11][12]. Through an extended investigation, it was observed that nitrosamine formation occurs via a nitrosating reaction involving different types of amines (secondary, tertiary, or quaternary amines) and nitrous acid (nitrite salt in acidic conditions), as illustrated in Fig. 2.

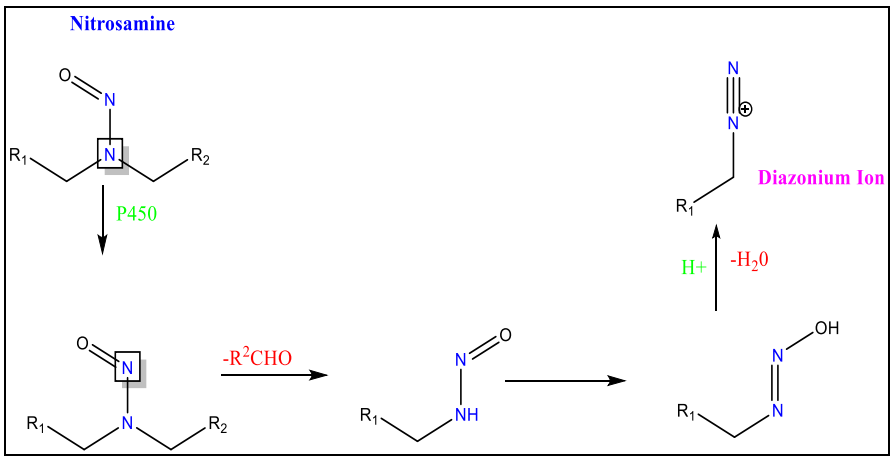

Fig. 1. Transformation of nitrosamine into diazonium via enzymatic alpha-hydroxylation.

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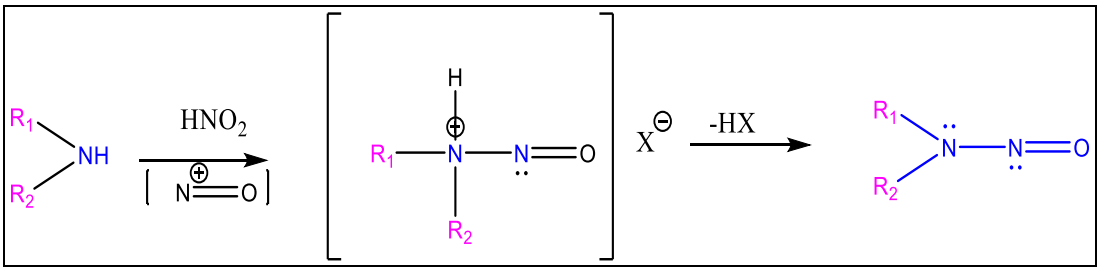

Fig. 2. Nitrosamine formation mechanism in presence of nitrosating agent.

The FDA has discovered seven nitrosamine impurities that conceptually could be present in drug products: NDMA, N-nitrosodiethylamine (NDEA), N-nitroso-N-methyl-4-aminobutanoic acid (NMBA), N-nitrosoisopropylethyl amine (NIPEA), N-nitrosodiisopropylamine (NDIPA), N-nitrosodibutylamine (NDBA), and N-nitrosomethylphenylamine (NMPA). Fig. 3 illustrates the structure and molecular weight of these impurities. Five of these (NDMA, NDEA, NMBA, NIPEA, and NMPA) have been uncovered in drugs or drug products [13].

FDA recommends the following acceptable intake (AI) limits for the nitrosamine impurities NDMA, NDEA, NMBA, NMPA, NIPEA, and NDIPA as tabulated in the Table 1 [14]. FDA recommend manufacturers to use below listed AIs when determining limits for nitrosamine impurities in drug substances and drug products.

These limitations apply only if a drug product carries a single nitrosamine. If the manufacturer detects more than one of the nitrosamine impurities listed in Table 1 and the total amount of nitrosamine impurities exceeds 26.5 ng/day (the AI for the most powerful nitrosamines) based on the maximum daily dosage (MDD), the manufacturer should contact the agency for assessment. A suggested limit for total nitrosamines of 0.03 ppm is not more than 26.5 ng/day and is regarded as appropriate for drug products having an MDD of less than 880 mg/day. For medicinal products with an MDD greater than 880 mg/day, the total nitrosamine limit should be reduced so that it does not exceed the recommended limit of 26.5 ng/day.

Table 1 AI limit for all nitrosamines in the drug products.

Nitrosamine Impurity	AI limit (ng/day) [1,2]
NDMA	96
NDEA	26.5
NMBA	96
NMPA	26.5
NIPEA	26.5
NDIPA	26.5

[1]The AI limit is a daily exposure to a compound such as NDMA, NDEA, NMBA, NMPA, NIPEA, or NDIPA that approximates a 1:100,000-cancer risk after 70 years of exposure.

[2]The conversion of AI limit into parts per million (ppm) varies by product and is calculated based on a drug's maximum daily dose (MDD) as reflected in the drug label (ppm = AI (ng)/MDD (mg)).

The leading worldwide avoidable risk factor for cardiovascular disease (CVD) and overall mortality is hypertension. Defined by a systolic blood pressure (BP) of 140 mmHg and/or a diastolic BP of 90 mmHg, hypertension is a prevalent condition. According to the World Health Organization's report of 2023, an estimated 1.28 billion adults aged 30–79 years worldwide are affected by hypertension [15]. Due to population aging, increased exposure to lifestyle risk factors such as bad diets (high salt and low potassium intake), and a lack of physical activity, the prevalence of hypertension is increasing worldwide. The prevalence of hypertension has changed, but not consistently, around the world. High-income nations (HICs) had a little decline in the prevalence of hypertension during the past two decades, whereas low- and middle-income nations (LMICs) saw notable rises. These differences in hypertension prevalence patterns indicate that LMIC health care

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systems may be at risk of dealing with a significant burden of infectious diseases in addition to a fast-growing burden of hypertension and related CVDs [16]. The most often used medications for CVDs and hypertension are angiotensin receptor blockers (ARBs), and angiotensin-converting enzyme inhibitors (ACEI) which also function as renin-angiotensin-aldosterone system (RAAS) blockers. It is crucial to screen for nitrosamine impurities in drug products, notably in life-saving pharmaceuticals like telmisartan and other members of the sartan class of medications, primarily employed as ARB inhibitors.

This article presents a novel analytical method designed for the simultaneous detection and quantification of multiple nitrosamine impurities in Telmisartan tablets within a single analysis using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in positive mode atmospheric pressure chemical ionization (APCI). This newly developed analytical method was rigorously evaluated for accuracy, precision, and linearity in accordance with the established guidelines outlined by the ICH. These validation tests ensure the method's suitability for its intended application, reaffirming its robustness and reliability in the analysis of nitrosamine impurities. This research represents a significant advancement in analytical techniques, offering a powerful tool for pharmaceutical quality control and safety assessments in drug manufacturing processes.

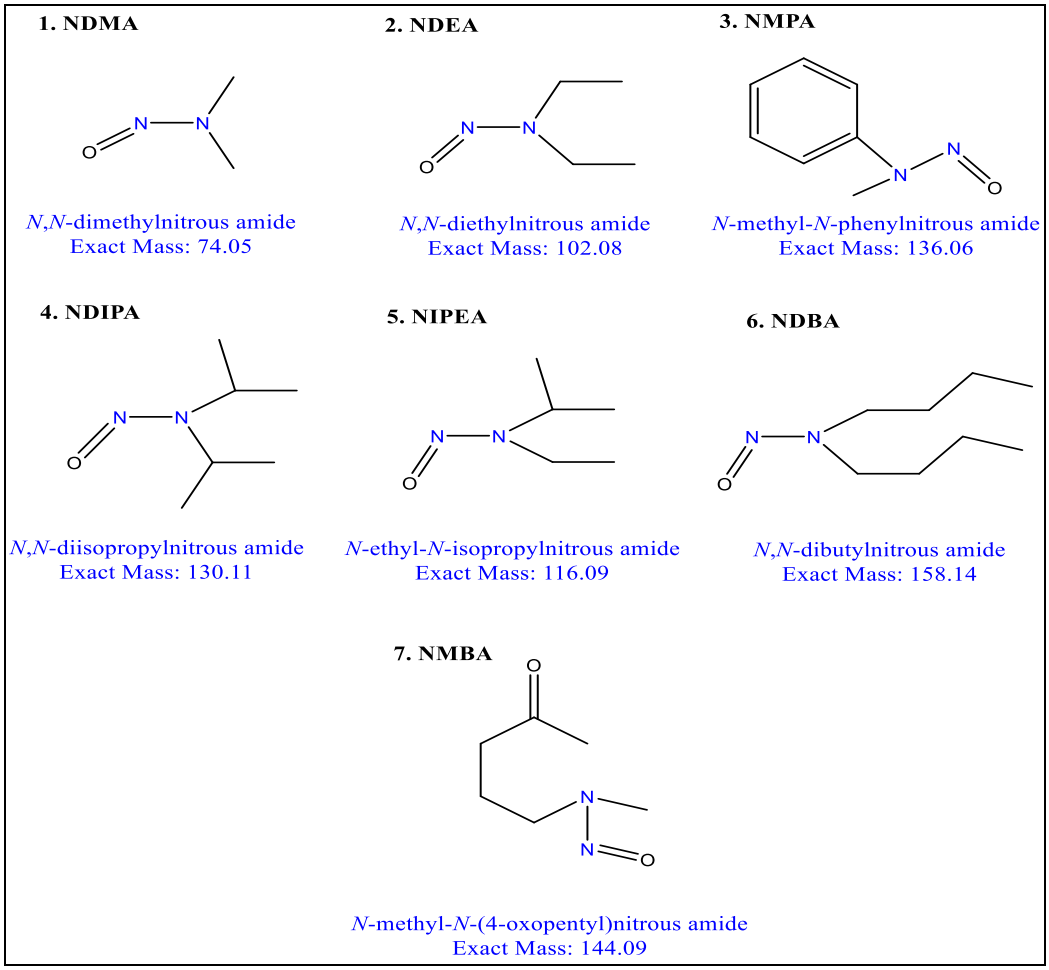

Fig. 3. Chemical structure and exact mass of the nitrosamine impurities.

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2. Materials and Methods

2.1. Materials

Methanol and formic acid (>98%) with LCMS grade were obtained from Honey Well, New Jersey, United States. Water with an HPLC grade was obtained from Qualigens, India. Ammonium acetate extra pure was purchased from Merck, Mumbai, India. All nitrosamine impurity standards; NDMA (N-Nitroso dimethylamine) (>98%), NEIPA (N-Nitrosoethylisopropylamine) (>98%), NDEA (N-Nitroso diethylamine) (>98%), NDIPA (N-Nitroso diisopropylamine) (>99%), NDPA (N-Nitroso dipropylamine) (>98%) and NDBA (N-Nitroso dibutylamine) (>98%) were purchased from Sai traders, Mumbai, India. Telmisartan tablets were obtained from Cadila Pharmaceuticals Limited, Ahmedabad, India.

2.2. Preparation of solutions

2.2.1. Preparation of diluent and mobile phase

All solutions were prepared using a diluent that was a 90:10 ratio of water to methanol. Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.3084 g of ammonium acetate in 1000 mL of methanol. Through 0.45 µm nylon membrane filter paper, both mobile phases were filtered.

2.2.2. Preparation of standard solutions

2.2.2.1. Preparation of impurities standards stock solution I

5.0 mg of each impurity standard (NDMA, NDEA, NEIPA, NDIPA, NDPA, and NDBA) was precisely measured and transferred into a 5 mL volumetric flask each, to which 2 mL of methanol was added. These substances were subsequently dissolved using a sonicator, the volume was brought up to the required level with methanol, and the mixture was thoroughly mixed (Stock-I). About 1000 ppm of the specified impurities are present in the stock solution I.

2.2.2.2. *Preparation of impurity stock solution II*

0.050 mL of NDEA, NDIPA, NDPA, NDBA, and 0.185 mL of NDMA stock solution-I were diluted in 10 mL of volumetric flask, made up with diluent, and thoroughly mixed. For NDMA, the impurity concentration was around 18500 parts per billions (ppb) while it was approximately 5000 ppb for NDEA, NEIPA, NDIPA, NDPA, and NDBA.

2.2.2.3. Preparation of mix impurity diluted standard solution

In a 10 mL volumetric flask, 0.400 mL of the NDMA, NDEA, NEIPA, NDIPA, NDPA, and NDBA common stock solution-II were diluted with diluent and mixed throughly. In a volumetric flask with a 10 mL capacity, 0.044 mL of the aforementioned solution was added and diluted further with the diluent. For NDMA, the impurity concentration was around 3.26 ppb, while it was approximately 0.88 ppb for NDEA, NEIPA, NDIPA, NDPA, and NDBA.

2.2.3. Preparation of sample solutions

Ten tablets were randomly chosen, weighed, and crushed into a fine powder. Subsequently, 10 mL of diluent was added to a 50 mL volumetric flask after transferring tablet powder equal to 300 mg of telmisartan from the tablets. The flask was shaken occasionally while being sonicated for 5 min. Following this, the solution was centrifuged for 10 min at 4500 rpm. The solution was then passed through a 0.45 µm nylon filter, with the first few mL of filtrate being discarded. The filtrate collected served as the sample solution, with a telmisartan concentration of 30 mg/mL.

2.2.4. Preparation of validation solutions

Suitable dilutions from the impurity standard stock solutions were used to produce limit of detection (LOD), limit of quantification (LOQ), and linearity solutions for each of the six nitrosamine impurities (NDMA, NDEA, NEIPA, NDIPA, NDPA, and NDBA). Aiming at target concentrations of 36 ppb for NDMA and 9.93 ppb for NDBA, NDPA, NDIPA, NDEA, and NEIPA in the sample, repeatability solutions were prepared by spiking impurity standard solutions into the sample. In order to validate the test method's accuracy, target concentrations of NDBA, NDPA, NDIPA, NDEA, NEIPA (9.93 ppb) and NDMA (36.00 ppb) were assessed in the range of LOQ to 150% (LOQ, 50%, 100%, and 150%). A solution of placebo and individual impurities standard at concentrations of 9.94 ppb of NDEA, NDIPA, NDPA,

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NDBA, NEIPA, and 36.78 ppb of NDMA impurity was prepared to make specificity solutions. Standard solution and sample concentrations were tuned to attain the appropriate S/N and peak shapes. All solutions, as mentioned earlier, were made via dilution with diluent, sonication, and final filtration through 0.45 µm nylon filters before use.

2.3. LC/MS/MS methods

2.3.1. Chromatographic parameters

The NDMA, NDEA, NDIPA, NDPA, NDBA, and NEIPA impurities were identified and quantified using a high-efficiency liquid chromatograph Agilent Infinity II with an MS detector (6545 QTOF). Agilent poroshell 120EC- C18 (150 mm × 4.6 mm), 2.7 µm was used. The gradient elution parameters, chromatographic separation parameters, retention time of analyte, MS parameters and settings for MS detection are presented in Table 2, Table 3, Table 4, Table 5, and Table 6 respectively.

Table 2 Gradient elution parameters for separation of desired analytes.

Time (min)	Flow (mL/min)	% of mobile phase A	% of mobile phase B
0	0.6	95	5
3	0.6	95	5
5	0.6	90	10
9	0.6	60	40
15	0.6	50	50
30	0.6	5	95
32	0.6	5	95
33	0.6	95	5
35	0.6	95	5

Table 3 HPLC chromatographic parameters.

HPLC parameters	Unit
Column temperature	40º C
Sample temperature	10° C
Flow rate	0.6 mL/min
Injection volume	20 µL
Runtime	35 min

Table 4 Retention time of the individual analytes.

Name of the peak	Retention time
NDMA (N-nitrosodimethylamine)	~5.19 min
NDEA (N-Nitrosodiethylamine)	~11.66 min
NEIPA (N-	~13.72 min
NDIPA (N-Nitrosodiisopropylamine)	~16.42 min
NDPA (N-Nitroso-di-n-propylamine)	~17.93 min
NDBA (N-Nitrosodibutylamine)	~24.40 min

Table 5 Selected MS parameters for the detection of the analytes.

MS parameters	Unit
Source	APCI+
Gas Temperature (°C)	300
APCI Heater	350
Gas Flow (l/min)	5
Nebulizer (psi)	45
Capillary (V)	4000
APCI Needle Pos.	4

Table 6 Single ion monitoring parameter for triple quadrupole.

Impurities	Precursor ion	Product ion	Fragment (V)	Collision	Cell acc. (V)
NDBA	159.1	57.1	86	12	4
NDIPA	131.1	89.1	50	7	5
NDPA	131.1	43.1	80	20	5
NEIPA	117.1	75.1	70	7	5
NDEA	103.1	75.1	85	8	5
NDMA	75.1	43.1	90	15	5

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2.4. Statistics

The data analysis, involving the calculation of mean values and standard deviations (SDs), was performed using Microsoft Excel (Microsoft® Office® 2019, Microsoft Corp., USA).

3. Results and Discussion

3.1. Method development

Several development trails were conducted in order to optimize the final chromatographic parameters, which can provide improved separation and peak form. Different combinations of mobile phase pH, gradient, and column were explored in order to get better separation and shape; finally, formic acid was chosen for mobile phase with methanol as it provided better separation. Because NDBA has a lower polarity than other nitrosamines, it presented some difficulties during development, but they were addressed after some experiments by adjusting the gradient of the mobile phase. Column chemistry and other column parameters were also optimized during development trials, as column chemistry plays a significant role in molecule retention and resolution. Agilent poroshell 120EC- C18 (150 mm × 4.6 mm), 2.7 mm, was chosen as the final column.

3.2. Method validation

The method was validated for specificity, accuracy, precision, linearity, LOD-LOQ and solution stability. The method validation parameters were evaluated according to ICH guidelines.

3.2.1. Specificity

The specificity study was performed to confirm the interference or co-elution of nitrosamine impurities with blank, placebo, and telmisartan peaks and all representative chromatogram are displayed in the Fig. 4.

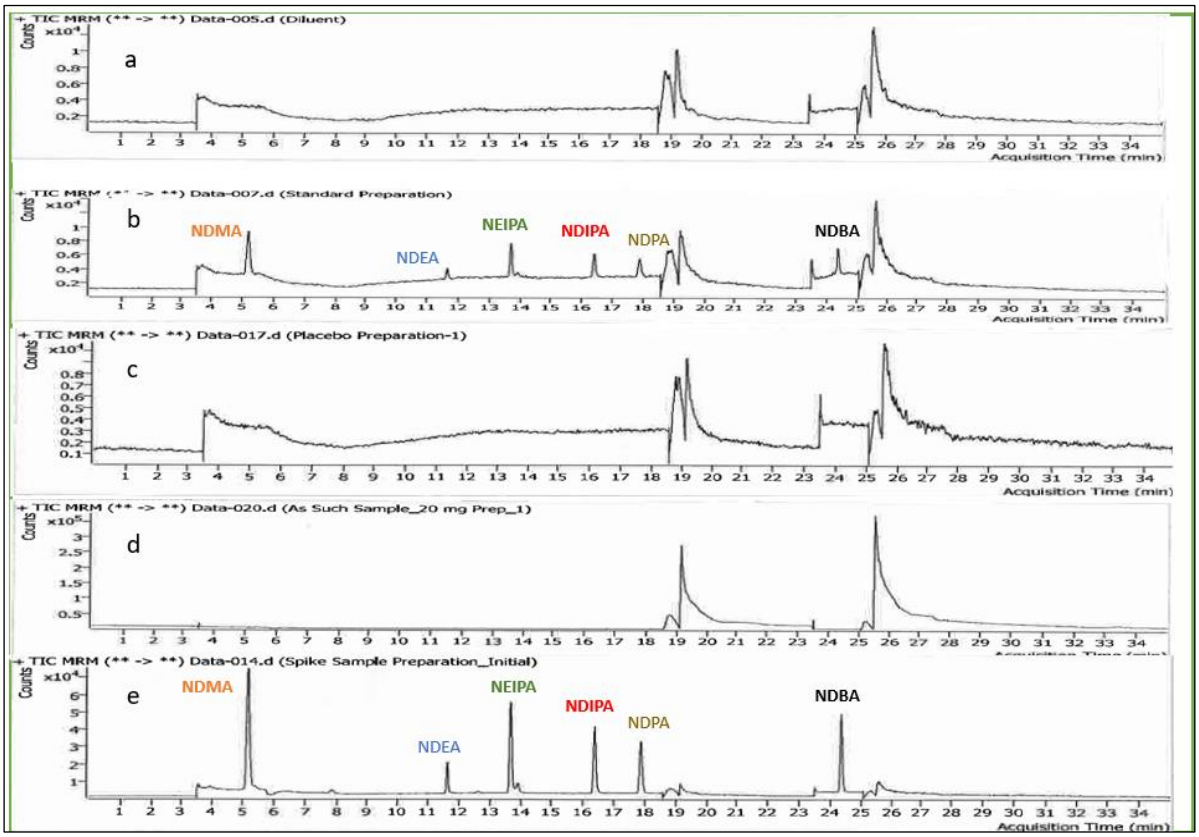

Fig. 4. Specificity chromatogram (a). Diluent chromatogram; (b). Standard chromatogram; (c). Placebo chromatogram; (d). Sample preparation chromatogram; and (e). Chromatogram of sample spiked with all impurities.

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Diluent, placebo, and a sample spiked with all nitrosamine impurities and individual impurity solutions were prepared and injected into chromatography, and mass spectra were evaluated. The MRM (multiple reaction monitoring) interpreted in individual impurity solutions and spiked sample solutions matched the MRM of NDMA, NEIPA, NDEA, NDIPA, NDPA, and NDBA peaks, indicating that NDMA, NEIPA, NDEA, NDIPA, NDPA, and NDBA peaks are spectrally homogeneous and all peaks are resolved from each other. The study proves that the method is specific for the quantification of NDMA, NEIPA, NDEA, NDIPA, NDPA, and NDBA peaks in telmisartan tablets USP by LC-MS/MS.

3.2.2. Accuracy

To demonstrate the accuracy of the test method, accuracy was performed in the range of LOQ to 150% (LOQ, 50%, 100% and 150%) of the target concentration of NDBA, NDPA, NDIPA, NDEA, NEIPA (9.93 ppb) and for NDMA & NMBA (36.00 ppb). Recovery and average recovery at each accuracy level were determined and results obtained from the accuracy study as described in the Table 7.

Table 7 % Recovery of each impurity at LOQ, 50%, 100% and 150% levels of the targeted concentration.

Nitrosamine	Accuracy level	Preparation	Amount added (ppb)	Amount recovered (ppb)	% Recovery	% Avg. recovery	%RSD
NDMA	LOQ	1	107.8	77.6	72.0	85.9	15.2
		2	107.8	94.4	87.6		
		3	107.8	105.6	98.0		
	50%	1	609.7	556.0	91.2	93.8	6.5
		2	609.7	545.6	89.5		
		3	609.7	614.9	100.8		
	100%	1	1217.0	1031.9	84.8	93.8	16.8
		2	1217.0	1029.8	84.6		
		3	1217.0	1362.7	112.0		
	150%	1	1826.8	1544.9	84.6	92.6	13.3
		2	1826.8	1950.6	106.8		
		3	1826.8	1578.7	86.4		
NDBA	LOQ	1	29.5	21.3	72.2	74.8	6.2
		2	29.5	21.3	72.1		
		3	29.5	23.7	80.2		
	50%	1	166.8	139.6	83.7	80.2	4.1
		2	166.8	133.2	79.8		
		3	166.8	128.8	77.2		
	100%	1	333.1	288.1	86.5	84.8	4.9
		2	333.1	266.6	80.0		
		3	333.1	292.3	87.8		
	150%	1	499.9	383.6	76.7	79.6	9.8
		2	499.9	442.4	88.5		
		3	499.9	368.6	73.7		
NDPA	LOQ	1	29.9	23.7	79.3	75.2	4.7
		2	29.9	21.7	72.7		
		3	29.9	22.0	73.7		

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	50%	1	169.2	142.0	83.9	83.3	2.7
		2	169.2	144.3	85.2		
		3	169.2	136.8	80.8		
	100%	1	337.8	291.5	86.3	90.5	5.0
		2	337.8	303.7	89.9		
		3	337.8	321.4	95.2		
	150%	1	507.0	492.7	97.2	94.7	4.3
		2	507.0	491.3	96.9		
		3	507.0	456.3	90.0		
NDIPA	LOQ	1	30.0	25.1	83.8	81.3	3.7
		2	30.0	23.3	77.9		
		3	30.0	24.6	82.1		
	50%	1	169.5	136.4	80.5	81.6	1.3
		2	169.5	140.1	82.6		
		3	169.5	138.5	81.7		
	100%	1	338.3	284.6	84.1	85.5	6.2
		2	338.3	273.9	81.0		
		3	338.3	309.2	91.4		
	150%	1	507.8	481.0	94.7	95.1	4.2
		2	507.8	503.9	99.2		
		3	507.8	463.5	91.3		
NDEA	LOQ	1	29.4	27.2	92.3	91.1	4.8
		2	29.4	27.9	94.7		
		3	29.4	25.4	86.2		
	50%	1	166.5	153.6	92.2	93.7	1.4
		2	166.5	157.3	94.5		
		3	166.5	157.1	94.4		
	100%	1	332.4	331.5	99.7	97.8	2.2
		2	332.4	317.1	95.4		
		3	332.4	326.6	98.3		
	150%	1	498.9	517.1	103.6	101.1	2.2
		2	498.9	494.8	99.2		
		3	498.9	500.7	100.4		
NEIPA	LOQ	1	29.6	24.3	82.0	80.0	2.3
		2	29.6	23.2	78.5		
		3	29.6	23.5	79.4		
	50%	1	167.5	146.2	87.2	90.1	3.2
		2	167.5	151.2	90.2		
		3	167.5	155.8	93.0		
	100%	1	334.4	313.4	93.7	95.3	5.9
		2	334.4	303.1	90.6		
		3	334.4	339.6	101.6		
	150%	1	501.9	498.0	99.2	100.0	2.8
		2	501.9	517.7	103.2		
		3	501.9	490.2	97.7		

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3.2.3. Precision

To demonstrate the precision of the test method, six preparations of spiked sample solution were prepared and injected into the LC-MS/MS. The precision of test method was demonstrated by calculating the average and relative standard deviation (RSD) of NDBA, NDPA, NDIPA, NDEA, NEIPA, and NDMA for six preparations of spiked sample solution. The primary acceptance criteria for precision was RSD ≤15%. RSD of impurities values indicate that the test method is precise. %RSD of the six preparations are given in the Table 8.

3.2.4. Linearity

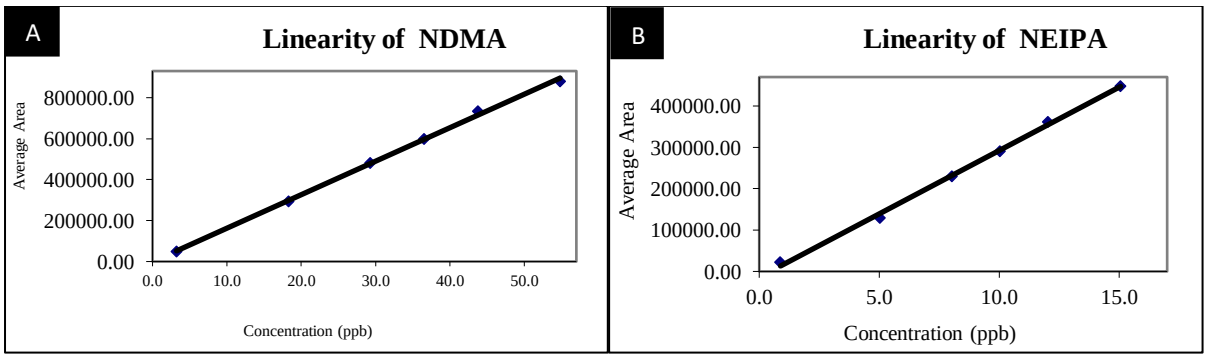
To demonstrate the linearity of the detector response, linearity was performed from LOQ to 150% for NDMA, NEIPA, NDEA, NDIPA, NDPA, and NDBA (LOQ, 50%, 80%, 100%, 120%, and 150%) (Fig. 5 (A-F)). The LOQ was established as per ICH guideline Q2, the signal-to-noise ratio method, and on visual evaluation. Linearity graphs were plotted of concentration in µg/mL (X-axis) versus average area (Y-axis). The correlation coefficient, square of correlation coefficient, slope of regression, relative standard deviation of response factor from LOQ to 150% linearity level, Y-intercept, and Y-intercept bias at 100% linearity level were calculated and tabulated in Table 9. The study proves that the area response of NDMA, NEIPA, NDEA, NDIPA, NDPA, and NDBA is linear in the range of LOQ to 150% of the specified limit. Linearity graphs for all impurities are presented in the Fig. 5.

3.2.5. LOD-LOQ

To evaluate the LOD and LOQ of the method, sequentially reduced solutions were injected, and their S/N ratios were determined. Concentrations of LOD and LOQ correspond to S/N of 3:1 and 10:1, respectively. LOD and LOQ solutions were prepared, and six replicate injections of LOQ concentration and LOD concentration were injected and peak responses recorded. The RSD of the area of six replicate injections was calculated for the LOQ solution, and the S/N ratio of the LOD solution and the LOQ solution was determined (Table 10).

Table 8 %RSD of six preparations of spiked sample solutions where the values are in ppb.

Preparati	NDMA	NEIPA	NDEA	NDPA	NDIPA	NDBA
1	1031.9	310.4	331.5	291.5	284.6	288.1
2	1029.8	300.2	317.1	303.7	273.9	266.6
3	1362.7	336.4	326.6	321.4	309.2	292.3
4	1378.0	309.8	323.7	274.4	273.2	243.8
5	1151.1	313.4	321.2	281.5	280.1	252.0
6	1215.1	324.7	332.3	299.3	295.2	245.9
Average	1194.8	315.8	325.4	295.3	286.0	264.8
SD	153.54	12.80	5.91	16.79	13.93	21.27
%RSD	12.9	4.1	1.8	5.7	4.9	8.0



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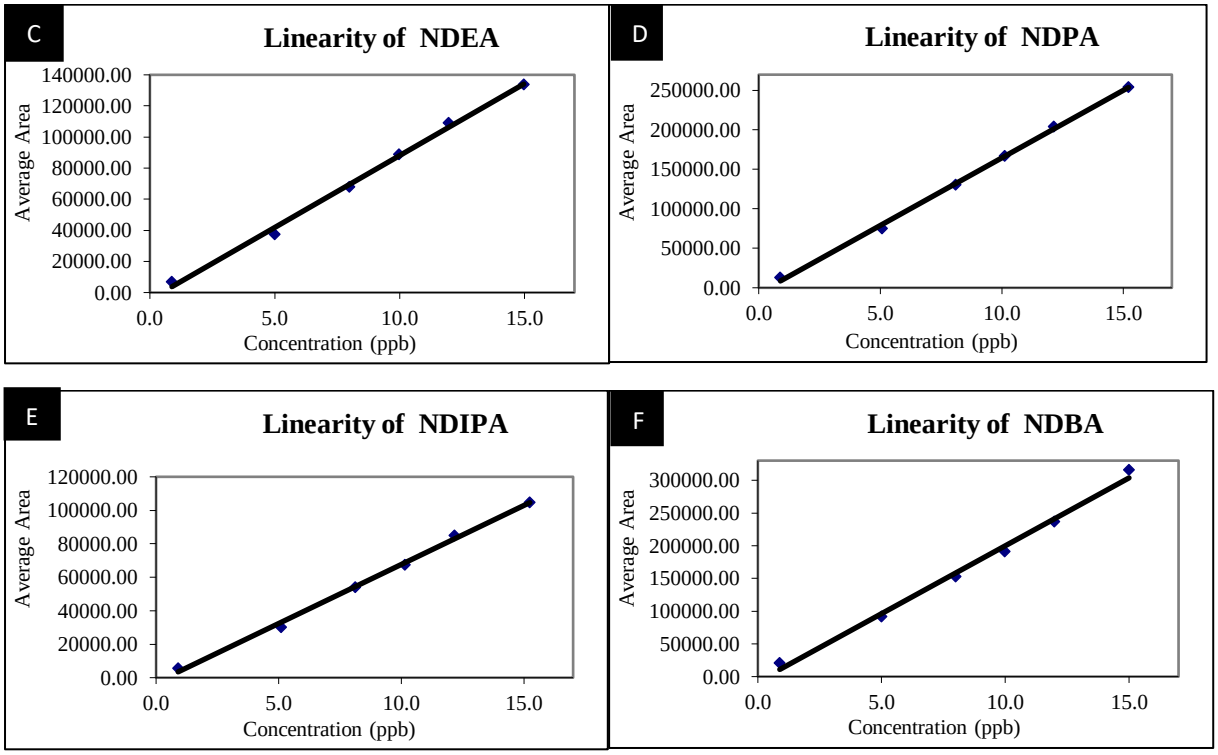

Fig. 5. Linearity graph of (A) NDMA, (B) NEIPA, (C) NDEA, (D) NDPA, (E) NDIPA, and (F) NDBA.

Table 9 Outcome from the linearity study in terms of correlation coefficient, regression, and intercept.

Nitrosamine impurities	Y-intercept	Slope of regression	Square of correlation coefficient (r^2)
NDMA	-870.927	16358.493	0.9986
NEIPA	-14031.835	3036.805	0.9980
NDEA	-4393.788	9249.847	0.9962
NDPA	-6721.023	17122.485	0.9986
NDIPA	-2880.454	7055.503	0.9972
NDBA	-7461.334	20740.564	0.9928

Table 10 Impurities along with their respective LOD and LOQ values in ppb.

Impurity name	LOD in ppb	LOQ in ppb
NDMA	35.3	107.8
NDEA	9.7	29.4
NEIPA	9.8	29.6
NDIPA	9.9	30.0
NDPA	9.9	29.9
NDBA	9.7	29.5

3.2.6. Solution stability

Standard solution and single preparation of spiked sample solution were prepared and analysed at 10 °C at different time intervals up to 46 hours for standard and 40 hours for sample preparation. The content of NDMA, NDBA, NDPA, NDIPA, NDEA, and NEIPA was calculated as described in the test method for spiked sample solutions against freshly prepared standard solutions at each time interval. From the above study, the the standard preparation demonstrated stability for 46 hours, whereas the sample preparation exhibited stability for 21 hours at 10 °C.

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3.2.7. Testing of samples

The developed method was applied for the determination of nitrosamine impurities (NDMA, NDBA, NDPA, NDIPA, NDEA, and NEIPA) on three different lots of 80 mg of telmisartan tablets, and no detectable levels of any nitrosamine impurities were found.

4. Conclusion

The analytical method developed for the estimation of nitrosamine impurities (NDMA, NDBA, NDPA, NDIPA, NDEA, and NEIPA) in telmisartan tablets was validated for all critical parameters such as precision, linearity, accuracy, specificity, LOD-LOQ, and solution stability as per ICH guidelines. Selectivity was determined with the lack of foreign peaks in the area of nitrosamine detection. The linearity of NDMA, NDBA, NDPA, NDIPA, NDEA, and NEIPA was studied from LOQ to 150% of the target concentration, and the correlation coefficient was over 0.99. Method accuracy was assessed using recovery, and all values were within 70% to 130%. Method precision complies with the acceptance criterion of RSD <15%. The LOQ is about 3 ppb for NDMA and less than 1 ppb for NDBA, NDPA, NDIPA, NDEA, and NEIPA, which is ten times lower than the current acceptable limit. It shows that the method is very sensitive and can detect nitrosamine impurities at very low levels. The method can be used for routine quality control analysis of nitrosamine impurities in telmisartan tablets.

CRediT authorship contribution statement

Mehul Pathak, Dalip Sharma, and Suresh Agrawal: Conceptualization, Methodology, Investigation, Visualization, and Writing - original draft; Dhara Patel: Conceptualization, Supervision.

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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