DEVELOPMENT AND APPLICATION OF LC-MS/MS METHOD FOR DETERMINATION OF SIX NITROSAMINE IMPURITIES IN TWO SARTAN DRUG PRODUCTS AS PER PROCEDURE 3, USP GENERAL CHAPTER <1469>

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Abstract: *In this study, we investigated the suitability of chromatographic conditions, mass spectrometry conditions, and sample preparation procedures when applying Procedure 3, USP General Chapter <1469> under the existing equipment conditions of the National Institute of Drug Quality Control for six nitrosamine impurities: NDMA, NDEA, NDBA, NIPEA, NMBA and NDIPA. We also validated the method on two pharmaceutical product matrices: film-coated tablets containing Losartan potassium and tablets containing Telmisartan. The results showed that the validation criteria were met for both matrices, except for the recovery of NMBA. The quantification limits for the nitrosamines ranged from 0.08 to 0.44 µg/g. Through experimental procedures, it was observed that the sample matrix significantly affected the analysis of nitrosamine impurities. The method was applied to analyze the six nitrosamines in 12 finished product samples containing Losartan potassium and Telmisartan available on the market.*

Keywords: *Nitrosamine, Sartan, LC-MS/MS*

1. INTRODUCTION

Nitrosamines are a group of chemical compounds classified as potential carcinogens in various organs and tissues, such as the lungs, brain, liver, kidneys, bladder, stomach, esophagus, and nasal sinuses [1]. In recent years, nitrosamines have been detected in pharmaceutical products used for the treatment of hypertension, diabetes, and H2 histamine receptor antagonists… Currently, several analytical methods using mass spectrometry techniques have been developed worldwide to detect nitrosamine impurities. Some pharmacopeias have officially published general chapters (USP General Chapter <1469>, Appendix VIII V of the BP) outlining procedures for testing 7 nitrosamine impurities in Sartan substances but there is no official method for finished products. Based on available equipment, we conducted a study "*Development and Application of LC-MS/MS Method for Determination of Six Nitrosamine Impurities in two Sartan Drug Products as per Procedure 3, USP General Chapter <1469>*" to assess the suitability of applying this method to finished product matrices.

2. EXPERIMENTAL

2.1. Equipments, Instruments, Chemicals, and Reference Standards

2.1.1. Equipments and Instruments

Waters ACQUITY H-Class UPLC System with Xevo TQD MS/MS detector; Restek Raptor ARC-18 (150 x 3.0 mm, 2.7 µm) column; Mettler Toledo MS 105 analytical balance with 0.01 mg readability; Sigma 4 – 16 KS centrifuge; Multi-tube vortexer (Benchmark); micropipet Eppendorf: 10 – 100 µL, 100 – 1000 µL; class A volumetric flasks and glass pipettes ….

All equipments and instruments were periodically calibrated according to ISO/IEC 17025 and GLP requirements.

2.1.2. Chemicals and Reference Standards

- Reference standards are listed in Table 1

Table 1. *List of Reference Standards*

Name	Origin	Batch No.	Concentration
N-nitrosodimethylamine (NDMA)	USP	R15630	1.00 mg/mL
N-nitrosodiethylamine (NDEA)	USP	R15620	1.01 mg/mL
N-nitrosoethylisopropylamine (NEIPA)	USP	R155F0	1.00 mg/mL
N-nitrosodiisopropylamine (NDIPA)	USP	R155E0	1.01 mg/mL
4-[Methyl(nitroso)amino]butanoic acid (NMBA)	USP	R155W0	1.02 mg/mL
N-Methyl-N-nitroso-phenylamine (NMPA)	USP	F155U0	1.00 mg/mL
N-Butyl-N-nitroso-1-butanamine (NDBA)	USP	F145C0	1.00 mg/mL
NDEA-d10	TRC	FG-303	99.20%
NMBA-d3	TRC	10-EAW-152-2	99.97%
NDMA-d6	HPC	802853	99.96%
NDBA-d18	Clearsynth	CRC-2711-R&D-026	98.28%

- Methanol HPLC-grade (Merck).
- Formic acid: LC-MS-grade (Fisher chemical).

2.2. Subjects and Methods

2.2.1. Study subjects

- *Samples:* are listed in Table 2

Table 2. *List of Samples Used in the Study*

No.	Code	Dosage Form	Active ingredient	Concentration (mg)
1	M1	Film-coated tablet	Losartan potassium	50
2	M2	Tablet	Telmisartan	20
3	M3	Tablet	Telmisartan	80
4	M4	Tablet	Telmisartan	40
5	M5	Tablet	Telmisartan	40
6	M6	Film-coated tablet	Losartan potassium	50
7	M7	Film-coated tablet	Losartan potassium	50
8	M8	Film-coated tablet	Losartan potassium	50
9	M9	Tablet	Telmisartan	40
10	M10	Tablet	Telmisartan	40
11	M11	Film-coated tablet	Losartan potassium	50
12	M12	Tablet	Telmisartan	40
13	M13	Film-coated tablet	Losartan potassium	50
14	M14	Film-coated tablet	Telmisartan	40
15	M15	Film-coated tablet	Losartan potassium	50
16	M16	Tablet	Telmisartan	40
17	M17	Film-coated tablet	Losartan potassium	50

Note: M1 to M5 samples were used for method development and validation. M6 to M17 samples were used for method application to quantify nitrosamine impurities.

- *Spiked Samples*: The samples (drug products) are spiked with nitrosamine impurities solution at different concentrations.

2.2.2. Research Methods

Based on *Procedure 3, USP General Chapter <1469>* [2], chromatographic conditions, mass spectrometry parameters, and sample preparation procedures were investigated to optimize and adapt the method to the available laboratory conditions.

2.2.2.1. Analytical Procedure

** Chromatographic Conditions:*

Column: Restek Raptor ARC-18 (150 x 3.0 mm; 2.7 µm), maintained at 60°C. Mobile Phase A: 0.1% formic acid in water. Mobile Phase B: 0.1% formic acid in methanol. Gradient program: see Table 3. Flow rate: 0.5 mL/min. Injection volume: 50 µL. Auto-sampler temperature: 18°C.

Table 3. *Gradient Program*

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	97	3
1.5	97	3
4.0	50	50
7.0	25	75
8.1	15	85
9.2	5	95
12.0	5	95
12.1	97	3

** Mass Spectrometry Conditions:*

Use Triple quadrupole mass spectrometer detector (MS/MS). Ionization source: $APCI^+$. Source temperature: 350°C. Desolvation gas flow: 1000 L/h. Scan mode: MRM (Table 4).

Table 4. *MRM setting*

Nitrosamine	Cone (V)	Dwell (s)	MRM Transitions (m/z)			
			MRM-1[a]	CE (V)	MRM-2	CE (V)
NDMA	35	0.034	75.1 → 58.2	10	75.1 → 44.1	10
NDMA-d6	45	0.034	80.9 → 45.9	12	80.9 → 63.9	10
NDEA[b]	20	0.030	103.1 → 75.1	10	103.0 → 47.1	14
NDEA- d10	25	0.030	113.0 → 48.9	15	113.0 → 33.9	13
NMBA	10	0.030	146.9 → 116.9	6	146.9 → 43.9	12
NMBA-d3	14	0.030	150.1 → 47.1	12	150.1 → 120.2	6
NDBA	22	0.040	159.0 → 56.9	12	159.0 → 102.9	10
NDBA-d18	26	0.040	177.2 → 66.2	15	177.2 → 46.1	20
NEIPA[b]	20	0.030	116.9 → 74.9	9	116.9 → 46.8	15
NDIPA[b]	20	0.030	131.1 → 89.2	8	131.1 → 47.1	12
NMPA[b]	25	0.031	136.9 → 106.9	12	136.9 → 65.9	17

[a]: is used for quantitation; [b]: NDEA-d10 is used as internal standard for NDEA, NEIPA, NDIPA, NMPA.

* Preparation of solutions:

- Diluent: 1% formic acid in water.

- *Internal Standard Solution:* 10 µg/mL each of NDMA-d6 and NMBA-d3, 1 µg/mL each of NDEA-d10 and NDBA-d18 in water.

- *Nitrosamine standards stock solution mixture:* Prepare a mixture of 200 ng/mL each of NDMA, NDEA, NDBA, NMPA, NIPEA, NMBA and NDIPA by mixing appropriate volumes of the respective USP Reference Standards and dilute with water.

- *Calibration Standards:* Calibration solutions were prepared from the stock solution as shown in Table 5.

Table 5. *Preparation of Calibration Standards*

No.	Concentration of NDMA, NMBA, NDBA, NEIPA, NDIPA, NMPA, NDEA (ng/mL)	Volume of Nitrosamine standards stock solution mixture (µL)	Volume of Internal Standard Solution (µL)	Volume of Water (µL)	Total Volume (µL)
S1	1.33	8	12	1180	1200
S2	5	30	12	1158	1200
S3	15	90	12	1098	1200
S4	30	180	12	1008	1200
S5	60	360	12	828	1200
S6	90	540	12	648	1200
S7	120	720	12	468	1200
S8	150	900	12	288	1200

Note: Calibration solutions S2 - S6 were used for NDEA; S3 - S8 for NDMA; S1 - S6 for the remaining nitrosamines.

- *Sample Solution:* Weight and powder 20 tablets. Accurately weigh an amount of the sample powder equivalent to 80 mg of Losartan/Telmisartan into a 5 mL centrifuge tube. Add 1188 µL of sample diluent and 12 µL of internal standard solution. Shake to obtain a homogeneous mixture and vortex at 2500 rpm for 20 minutes (for Telmisartan products) and no more than 5 minutes (for Losartan products). Centrifuge at 10,000 rpm for 20 minutes (Telmisartan) and 10 minutes (Losartan), then filter through a 0.2 µm PTFE membrane filter.

2.2.2.2. Method Validation

The method was validated following USP guidelines for analytical procedures for impurities [3], including the following parameters: specificity/selectivity, limit of detection, limit of quantification, linearity, accuracy, repeatability, intermediate precision, and method robustness.

3. RESULTS AND DISCUSSION

3.1. Chromatographic Conditions

Chromatographic conditions were applied as specified in Procedure 3, USP General Chapter <1469>, described in section ***2.2.2.1. Analytical Procedure***. Due to limited sensitivity of the equipment, the injection volume was increased from 20 µL (as per USP GC <1469>) to 50 µL to enhance sensitivity. At these conditions, the peak signals of nitrosamine impurities in standards solution at concentration L1 (1.33 ng/mL) are observed clearly excepting NDMA required at concentration L5 (10 ng/mL).

3.2. Mass Spectrometry Conditions

- *Ion Source Parameters* (corona discharge current, source temperature, disolvation gas flow) and *analyte-specific parameters* (Cone voltage và Collision energy) were optimized to yield strong and stable signals of both nitrosamines and internal standards.

- *Precursor and Product Ion Masses*: Full scan and MRM modes were used to preliminary survey the signals of nitrosamines and internal standards. The results showed that the majority of precursor ion and products ion of nitrosamine impurities and internal standards consistent with the information in the Procedure 3, USP General Chapter <1469>. For NDMA, based on experimental results and reference [4], two product ions (m/z = 58.2 and 44.1) were selected as characteristic product ions because they provided stronger and less noisy signals than the m/z = 43.1 product ion listed in USP GC <1469> (see Figure 1). For NDBA, based on experimental data and reference [5], product ions (m/z = 56.9 and 102.9) were selected as characteristic product ion instead of the less sensitive product ions (m/z = 41.1 and 29.1) (see Figure 2). High-intensity and stable fragment ions of NMPA – is not included in Procedure 3, USP GC <1469> - were determined based on literature [5] and using full scan mode at Q1 quadrupole with positive mode to determine the precursor ion, then analyze at the Q3 quadrupole with full scan mode to determine product ions (results presented in Table 4, Figure 3).

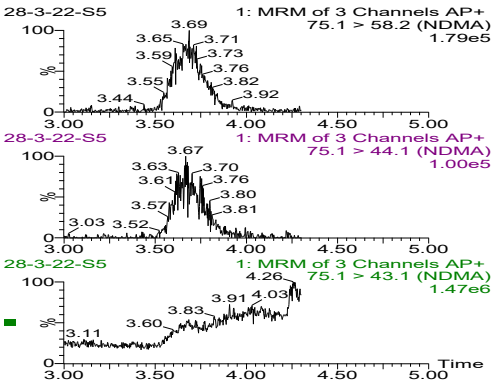

Figure 1. *NDMA signal in standard solution at 60 ng/mL*

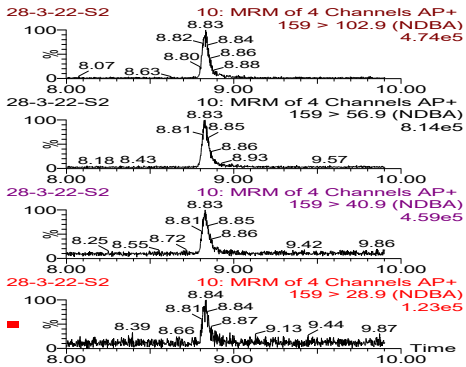

Figure 2. *NDBA signal in standard solution at 5 ng/mL*

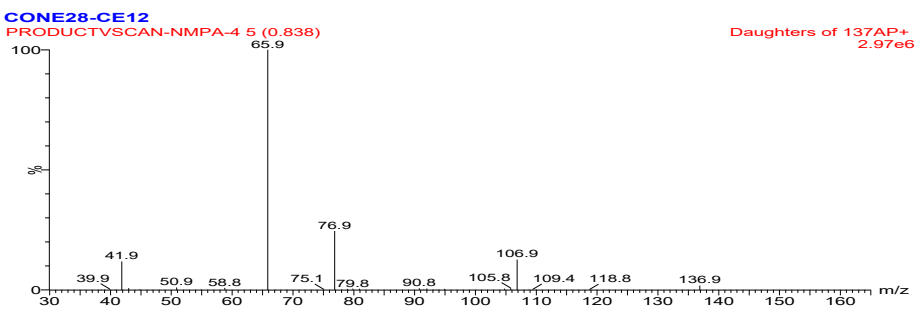

Figure 3*. MS/MS fragmentation ion mass spectrum [NMPA+ H]+*

3.3. Sample Preparation Procedure

When applying the sample preparation procedure described in Procedure 3, USP General Chapter <1469> to two finished pharmaceutical products film-coated tablets containing Losartan potassium (M1) and tablets containing Telmisartan, it was observed that the sample matrix significantly affected the analysis of nitrosamine impurities. In formulations with low active pharmaceutical ingredient content and high excipient levels (M2 sample), homogenization was not achievable using the standard procedure, and no supernatant could be obtained post-treatment. In some samples, component swelling occurred, making difficult homogenization, and the resulting sample was either not clear (M3 sample) or the supernatant volume was insufficient (M4, M5 samples). Especially, likely due to competition during the ionization process, signals of nitrosamines and internal standards in spiked samples were significantly reduced, as a results, some analytes were undetectable even when spiked above their respective specification limits (M3 sample). Matrices of M1 and M4 sample had the least impact on the analytical performance.

3.4. Method Validation

3.4.1. System Suitability

Six replicate injections of the standard solution S5 (as described in Table 5) were performed. The chromatograms were recorded, and key parameters were assessed (see Table 6).

Table 6. *System Suitability Results*

Nitrosamine	Retention Time (RSD (%), n = 6)	Response Ratio (RSD (%), n = 6)	Relative ion intensities (n = 6)	
			Mean ± SD	RSD
NMBA	0.2	3.0	0.57 ± 0.02	3.0
NDEA	0.1	2.8	0.25 ± 0.01	2.8
NDMA	0.8	3.0	0.44 ± 0.01	2.5
NEIPA	0.1	2.2	0.10 ± 0.01	2.6
NDIPA	0.1	3.9	0.64 ± 0.02	2.8
NMPA	0.1	7.2	0.97 ± 0.01	0.9
NDBA	0.1	3.7	0.58 ± 0.02	2.6

All parameters met the acceptance criteria (RSD of retention time < 1.0%; RSD of response ratio and relative ion intensities <20.0%). Therefore, the system is stable and suitable for the quantification of nitrosamine impurities.

3.4.2. Specificity

Blank samples, test samples, standard solutions, and spiked samples were analyzed following analytical procedure. The results showed:

- The blank chromatogram and sample without internal standard chromatogram do not show any peak which have specific mass fragment of nitrosamine impurities and internal standards at the retention time of those in the nitrosamine impurities standard solutions chromatogram.

- The spiked sample chromatogram show nitrosamine impurities peak and internal standards peak have retention time and relative ion intensities correspond to those of nitrosamine impurities and internal standards peak in the nitrosamine impurities standard solutions chromatogram.

Conclusion: The method is specific and selective for the determination of the target nitrosamines and internal standards.

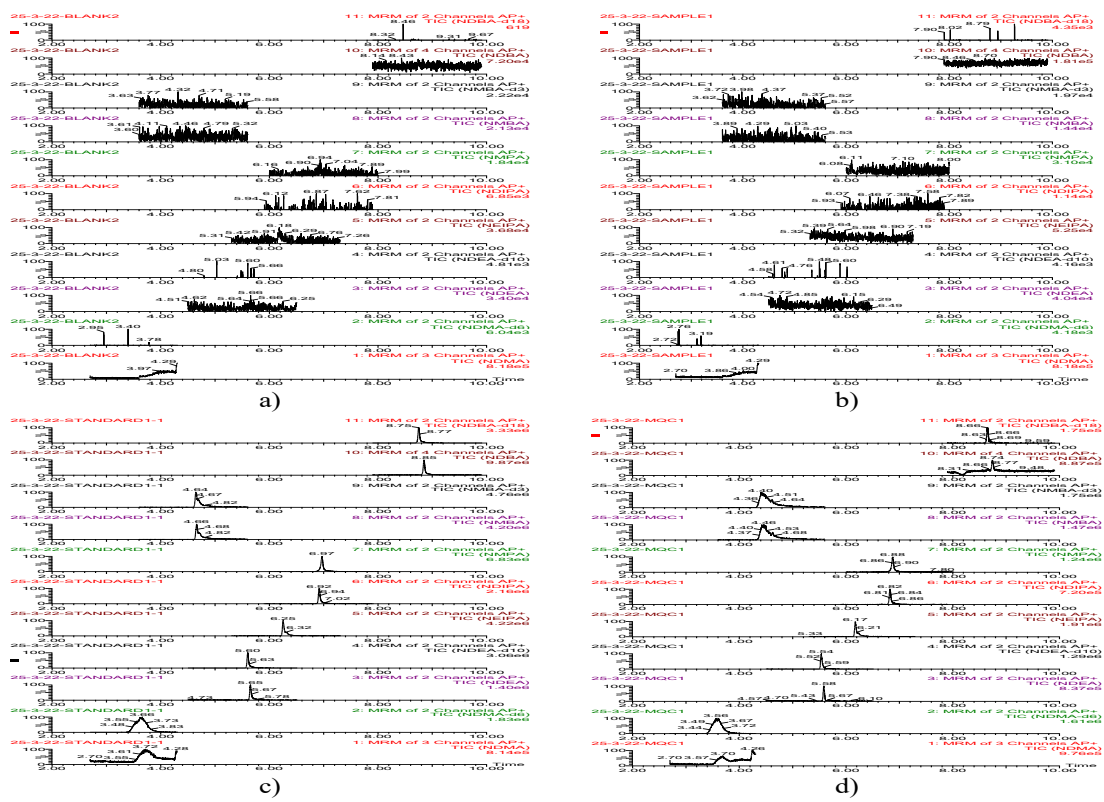

Figure 4. *Chromatograms of Specificity (a-Blank sample; b-Test sample M1; c-Standard solution; d-Spiked sample M1; e-Test sample M4; f-Spiked sample M4)*

3.4.3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Standard solutions at progressively lower concentrations were analyzed. The LOD was defined as the concentration at which the signal-to-noise ratio (S/N) was at least 3 : 1, and the LOQ was defined as the concentration at which the S/N was at least 10 : 1. To confirm the suitability of the determined LOD and LOQ values, spiked samples were analyzed at these concentration using the developed method. Results demonstrated that: At the LOD level, nitrosamine peaks were clearly detectable in both M1 and M4 sample matrices. At the LOQ level, in the M1 sample matrix, recovery (74.7% - 124.6%) and repeatability (RSD < 20%) of 7 nitrosamines meet the acceptance criteria. In the M4 sample matrix, recovery of 6 nitrosamines meet the acceptance criteria (99.9% - 111.8%), except for NMPA, which showed a recovery of only 48.4%, failing to meet the acceptance criteria.

Based on the experimental data, the LOD and LOQ values are summarized in Table 7:

Table 7. *LOD, LOQ of each nitrosamine*

Nitrosamine	M1 sample matrix		M4 sample matrix	
	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)
NMBA	40.8	81.6	10.2	20.4
NDEA	40.4	80.8	40.4	80.8
NDMA	220.0	440.0	80.0	160.0
NEIPA	40.0	80.0	10.0	20.0
NDIPA	10.1	20.2	10.0	20.0
NMPA	40.0	80.0	-	-
NDBA	80.0	160.0	80.0	160.0

3.4.4. Calibration Curve and Linearity

A series of standard solutions with concentrations as shown in Table 5 were analyzed. The results of the linearity assessment for each nitrosamine are summarized in Table 8.

Table 8. *Results of calibration curve and linearity*

Name	Concentration range (ng/mL)	Linear regression equation (y = ax + b)	Correlation coefficient (r)	% y-intercept
NMBA	1.36 – 122.40	y = 0.0139x – 0.0046	1.0000	1.1
NDEA	5.05 – 151.50	y = 0.0221x + 0.0006	0.9998	1.4
NDMA	10.0 – 150.0	y = 0.0016x + 0.0028	0.9997	1.5
NEIPA	1.33 – 120.0	y = 0.0944x – 0.0355	0.9998	1.3
NDIPA	1.35 – 121.20	y = 0.0334x – 0.0194	0.9999	2.0
NMPA	1.33 – 120.0	y = 0.0508x – 0.0341	0.9999	2.3
NDBA	1.33 – 120.0	y = 0.1339x – 0.0315	0.9999	0.8

Experimental results showed that all seven nitrosamines exhibited correlation coefficients greater than 0.990, and the % y-intercepts were within acceptable limits. These results confirm a linear correlation between analyte concentration and the peak area ratio across the tested concentration range.

3.4.5. Accuracy and Repeatability

Accuracy and repeatability of the method were assessed using spiked samples, in which a known volume of stock standard solution was added to the test samples to achieve three spiking levels: low (LOQ), medium (acceptable nitrosamine limit), and high (approximately 120% of the acceptable nitrosamine limit). At each level, six replicates were analyzed. The results are presented in Table 9 and Table 10.

Table 9. *Accuracy and Repeatability - M1 sample matrix*

Nitrosamine	LOQ concentration		Medium concentration		High concentration	
	Recovery (mean ± SD) (n = 6)	***RSD (%, n = 6)***	***Recovery (mean ± SD) (n = 6)***	***RSD (%, n = 6)***	***Recovery (mean ± SD) (n = 6)***	***RSD (%, n = 6)***
NMBA	112.2 ± 9.7	8.6	91.0 ± 5.1	5.6	94.6 ± 4.3	4.6
NDEA	103.0 ± 8.4	8.1	104.7 ± 3.1	2.9	95.7 ± 8.1	8.5
NDMA	93.2 ± 7.6	8.1	89.1 ± 6.3	7.1	93.9 ± 7.2	7.7
NEIPA	118.5 ± 6.2	5.2	103.5 ± 4.9	4.7	104.0 ± 6.3	6.1
NDIPA	124.6 ± 8.9	7.1	92.5 ± 6.3	6.8	110.8 ± 7.7	7.0
NMPA	74.7 ± 11.7	15.7	59.0 ± 3.8	6.4	67.0 ± 4.7	6.9
NDBA	96.7 ± 10.4	10.8	102.8 ± 4.7	4.6	103.9 ± 6.7	6.5

Table 10. *Accuracy and Repeatability – M4 sample matrix*

Nitrosamine	LOQ concentration		Medium concentration		High concentration	
	Recovery (mean ± SD) (n=6)	***RSD (%, n=6)***	***Recovery (mean ± SD) (n=6)***	***RSD (%, n=6)***	***Recovery (mean ± SD) (n=6)***	***RSD (%, n=6)***
NMBA	108.4 ± 8.3	7.6	99.8 ± 3.5	3.5	99.1 ± 3.3	3.4
NDEA	99.9 ± 6.6	6.6	105.4 ± 3.7	3.5	102.0 ± 4.0	4.0
NDMA	111.6 ± 11.9	10.7	113.2 ± 3.4	3.0	111.7 ± 4.7	4.2
NEIPA	111.8 ± 10.7	9.6	96.8 ± 2.9	3.0	93.5 ± 3.1	3.3
NDIPA	109.2 ± 8.2	7.5	89.3 ± 3.9	4.4	86.6 ± 4.7	5.4
NMPA	48.4 ± 5.4	11.2	36.8 ± 3.8	10.4	28.4 ± 1.6	5.6
NDBA	103.8 ± 11.2	10.8	95.5 ± 4.1	4.3	89.8 ± 4.2	4.7

The results show that for both matrices (M1 and M4), six nitrosamines (excluding NMPA) met USP criteria for recovery (70 - 130%) and repeatability (RSD ≤ 20%) across all concentration levels. NMPA only met acceptance criteria at the LOQ level in M1 matrix; recovery at higher levels and in M4 matrix was below acceptable limits. Thus, the method meets the requirements for accuracy and repeatability with 6 nitrosamines, except for NMPA.

3.4.6. Precision

Method precision includes both repeatability and intermediate precision. Intermediate precision was assessed using spiked samples at the medium concentration level, with the analyses performed on different days by different analysts. The results are presented in Table 11.

Table 11. *Result of method precision*

Name	M1 sample matrix		M4 sample matrix	
	Recovery (%) (mean ± SD) (n = 12)	*RSD (%) (n = 12)*	*Recovery (%) (mean ± SD) (n = 12)*	*RSD (%) (n = 12)*
NMBA	90.1 ± 4.0	4.5	101.5 ± 3.7	3.7
NDEA	109.1 ± 5.4	4.9	105.6 ± 3.2	3.0
NDMA	82.6 ± 9.8	11.8	106.4 ± 7.7	7.2
NEIPA	100.0 ± 5.6	5.6	96.4 ± 2.9	3.0
NDIPA	87.6 ± 7.4	8.5	89.0 ± 4.4	4.9
NMPA	**56.2 ± 4.8**	8.5	**34.6 ± 3.8**	10.9
NDBA	102.6 ± 5.4	5.3	92.7 ± 4.2	4.5

The results demonstrated that the method met the criteria for intermediate precision, with RSD (n = 12) below 25% for six nitrosamines across both matrices, except recovery of NMPA did not meet the recovery criteria.

3.4.7. Robustness

The robustness of the method was assessed by examining system suitability and recovery of medium-concentration spiked samples under slight variations of chromatographic conditions: Column temperature: ± 5°C, flow rate: ± 10%, mobile phase B ratio: ± 2%. Recovery results of nitrosamines were compared with recovery values in Section ***3.4.6. Precision.*** Acceptance criteria required the RSD to be ≤ 20%. The results are summarized in Table 12.

Table 12. *Method robustness*

Nitrosamine		NMBA	NDEA	NDMA	NEIPA	NDIPA	NDBA
Flow rate 0.5 + 10% (mL/min)	***System suitability***	Accept	Accept	Accept	Accept	Accept	Accept
	Recovery (%)	101.3	103.6	104.4	93.9	85.7	92.6
	RSD (%)	4.1	4.1	7.7	4.9	7.6	4.9
Flow rate 0.5 - 10% (mL/min)	***System suitability***	Accept	Accept	Accept	Accept	Accept	Accept
	Recovery (%)	99.7	102.9	101.3	94.0	85.0	91.1
	RSD (%)	4.1	4.1	7.7	4.9	7.6	4.9
Mobile phase ratio (+ 2%)	***System suitability***	Accept	Accept	Accept	Accept	Accept	Accept
	Recovery (%)	101.3	103.6	104.4	93.9	85.7	92.6
	RSD (%)	4.1	4.1	7.7	4.9	7.6	4.9
Mobile phase ratio (- 2%)	***System suitability***	Accept	Accept	Accept	Accept	Accept	Accept
	Recovery (%)	99.7	102.9	101.3	94.0	85.0	91.1
	RSD (%)	5.9	5.0	3.5	4.2	8.3	4.1
Column temperature (60 + 5^{0}C)	***System suitability***	Accept	Accept	Accept	Accept	Accept	Accept
	Recovery (%)	100.9	100.3	102.8	98.6	90.0	100.8
	RSD (%)	4.6	8.7	5.4	4.8	5.2	4.7
Column temperature (60 - 5^{0}C)	***System suitability***	Accept	Accept	Accept	Accept	Accept	Accept
	Recovery (%)	99.9	100.2	105.6	101.6	92.8	94.9
	RSD (%)	5.7	8.8	8.9	8.6	8.0	8.2

The results shown in Table 12 confirm the robustness of the method under changes. Thus, chromatographic conditions can change as follow: Column temperature: ± 5°C, flow rate: ± 10%, mobile phase B ratio: ± 2%.

3.5. Application

Following the development and validation of the analytical method, Procedure 3, USP General Chapter <1469>, with slight adjustments as described in Section **2.2.2**, was applied for the analysis of nitrosamine impurities in 12 commercially available finished pharmaceutical products containing Losartan potassium and Telmisartan. The results are presented in Table 13.

Table 13. *Analysis of nitrosamine impurities in several pharmaceutical products*

Sample	NMBA	NDEA	NDMA	NEIPA	NDIPA	NDBA
M6	ND	ND	ND	ND	ND	ND
M7	ND	ND	ND	ND	ND	ND
M8	ND	-	ND	-	-	ND
M9	-	-	-	-	-	-
M10	ND	ND	ND	ND	ND	ND
M11	ND	ND	ND	ND	ND	-
M12	ND	ND	-	ND	ND	ND
M13	ND	-	ND	-	-	ND
M14	ND	ND	ND	ND	ND	ND
M15	ND	ND	ND	ND	-	ND
M16	ND	ND	ND	ND	ND	ND
M17	ND	-	ND	ND	-	ND

*** Note:** *"ND": Not detected; "-": Unable to determine the nitrosamine content in the sample*

The results of nitrosamine analysis in 12 marketed finished products show that several nitrosamines were not detected in the tested samples (denoted as "ND"). For other nitrosamines (denoted as "–"), due to matrix effects, recovery in spiked samples did not meet criteria, preventing accurate determination of nitrosamine content in those cases.

4. CONCLUSION

Through the course of experimentation, this study assessed the suitability of chromatographic and mass spectrometric conditions for applying Procedure 3, USP General Chapter <1469> under the current equipment conditions at the National Institute of Drug Quality Control Vietnam. The method was implemented largely in accordance with Procedure 3, USP GC <1469>, with two key modifications: (1) injection volume was increased and (2) product ions were adjusted to enhance sensitivity. Regarding sample preparation, several challenges were encountered when using the procedure specified in Procedure 3, USP GC <1469>: insufficient solvent volume led to incomplete wetting of powdered samples, difficulty in homogenization, low volume of extractable solution, and due to matrix interference, peak was distorted, recovery of certain nitrosamines in some matrices did not meet acceptance criteria, even when spiked at impurity limit concentrations. Method validation was conducted in accordance with USP guidelines using two representative product matrices. Parameters evaluated included: specificity, LOD/LOQ, linearity, accuracy and precision (repeatability and intermediate precision), and robustness. The method met validation requirements for both matrices, except for NMPA, which showed poor recovery. Due to limited instrument sensitivity and strong matrix effects, the limits of quantification (LOQs) for most nitrosamines ranged from 0.08 to 0.44 µg/g, which are generally higher than the recommended LOQ thresholds by the FDA and EMA for nitrosamine impurity analysis.

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