Trace Level Quantification of Genotoxic Impurities through Atmospheric Pressure Chemical Ionization (APCI) Coupled with Triple Quardrapole Analyzer in Combination Diabetic Drug Product

MEHUL PATHAK[1], DHARA D. PATEL[1,*], DALIP SHARMA[2], AVINEESH SINGH[2] and SURESH AGRAWAL[2]

[1]Department of Chemistry, Smt. S.S. Patel Nootan Science & Commerce College, Sankalchand Patel University, Visnagar-384315, India
[2]Analytical Research Laboratory, Cadila Pharmaceuticals Ltd., Dholka, Ahmedabad-382225, India

*Corresponding author: E-mail: dr.dhara29@gmail.com

N-Nitrosamine impurities have been found in several drug products, showing a concern for regulatory aspects. The study presents the development and validation of a sensitive LC-MS/MS method for detecting eight nitrosamines in vildagliptin and metformin drug products. The developed method was specific and linearity was ranged 3.53-55.92 ppb for NDMA, 3.18-50.37 ppb for NMBA, 0.97-15.41 ppb for NDEA, 0.98-15.52 ppb for NEIPA, 1.00-15.86 ppb for NDIPA, 0.98-15.46 ppb for NDBA, 0.98-15.58 ppb for NMPA and 0.7-15.32 ppb for NDPA with correlation coefficient (r) was more than 0.98 and square of correlation coefficient (r^2) was found to be greater than 0.96. The LOQ were obtained in the range of 0.97-3.53 ppb with %RSD in the range of 2.5-10.9% for eight nitrosamines showed good sensitivity. Accuracy was found in the range of 98.63 ± 5.77-125.17 ± 3.68% at LOQ level, 74.77 ± 0.83-88.17 ± 0.15% at 50% level, 79.93 ± 8.47-89.00 ± 0.61% at 100% level and 74.67 ± 2.31-84.03 ± 0.93% at 150% level for the eight nitrosamine impurities that were well within acceptance criteria of not less than 70% and not more than 130%. The method validation results demonstrated that the method is precise, accurate and linear, can be applied to quantify the nitrosamines in vildagliptin and metformin drug products.

Keywords: Nitrosamines, Vildagliptin, Metformin, Method validation.

INTRODUCTION

N-Nitrosamines, potential carcinogenic compounds, were first identified in July 2018 [1]. The nitrosamine impurities are classified as a probable or possible carcinogen to humans, which can lead to formation of tumours even at a nanogram per liter (ng/L) concentration level. The International Agency for Research on Cancer (IARC) has recognized two nitrosamine impurities, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), as possibly carcinogenic to humans, placing them in Group 2A. Meanwhile, the European Union has categorized these substances as allegedly carcinogenic (Category 1B). The United States Environmental Protection Agency (EPA) has classified NDMA and NDEA as possibly carcinogenic to humans (Category B2) [2]. Following the ICH guideline M7 (R1), nitrosamines are considered as "cohort of concern" due to carcinogenicity and mutagenicity of NDMA and NDEA in rodents [3]. In 2019, Singapore Health Sciences Authority (HSA) recalled the metformin due to contamination of NMDA nitrosamine [4]. The FDA's Center for Drug Evaluation and Research (CDER) has also raised awareness of NMDA contamination in metformin [5]. Subsequently, in 2020, the USFDA disclosed that NDMA contamination in various metformin extended-release products exceeded recommended acceptable threshold [6].

Metformin, a structurally classified guanidine derivative lacks a nitro group in its chemical structure and requires critical co-existing exogenous factors such as nitrosating agent, moisture, or heat for the contamination or formation of nitrosamine. Some studies have reported that incomplete oxy-cracked metformin can generate amine byproducts like *N,N*-dimethylformamide (DMF), *N,N*-dimethylurea, dimethylguanidine, hydroxyacetonitrile including NDMA. Additionally, a few investigations have proposed that the manufacturing process of metformin drug products might also be a source of NDMA generation. Nitrites and nitrates existing in multiple excipients can result in nitrosamine contamination. The introduction of moisture and high temperatures, particularly during wet granu-

lation and drying in the manufacturing process of metformin tablets, may induce nitrosation reactions, thereby potentially causing the formation of nitrosamines [7,8].

These studies show that nitrosamines, even at low concentrations, have a strong potential to cause cancer and DNA mutations. Identifying and screening these compounds in drug substances and products requires highly sensitive and advanced analytical methods [9,10]. Therefore, the present study aims to develop and validate a simple and robust LC-APCI-MS/MS based for detecting and quantifying nitrosamines in metformin and vildagliptin drug products.

EXPERIMENTAL

A nitrosamine reference standard (N-nitrosodimethylamine (NDMA) was obtained from Sigma-Aldrich and N-nitroso-N-methyl-4-aminobutyric acid (NMBA), N-nitrosodiethylamine (NDEA), N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), N-nitrosomethylphenylamine (NMPA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA) were purchased from Saitraders, India. The active pharmaceuticals ingredient (API), vildagliptin was obtained from Lee Pharma Ltd. and metformin was purchased from Granules India Ltd. The placebo formulation was same as formulation of vildagliptin and metformin tablets except vildagliptin and metformin drug substance. LCMS-grade methanol and formic acid were obtained from Honeywell for the mobile phases, while HPLC-grade Milli-Q water was sourced from Qualigens, India.

Instrumentation conditions

Mass spectrometry: The experiment was performed on the LC-MS/MS system (Agilent, US) using atmospheric pressure chemical ionization (APCI) source with positive mode. The quantitation was performed in multiple reactions monitoring (MRM) mode (also known as selective reaction monitoring or SRM) and the MRM parameters are described in Table-1. The gas temperature, APCI heater temperature, gas flow, nebulizer pressure and capillary were maintained at 325 ℃, 350 ℃, 7 L/min, 45 PSI and 4000 V, respectively with APCI needle position 5. All the analytes were identified on the basis of their mass-to-charge (*m/z*) ratio and the precursor and product ions were identified based on their mass abundance.

TABLE-1
MRM PARAMETERS OF EIGHT NITROSAMINES

Impurity	Precursor ion	Product ion	Fragment. (V)	Collision energy (V)	Cell acc. (V)
NDBA	159.1	41.1	70	24	5
NMBA	147.1	44.2	50	7	5
NMPA	137.1	66.1	70	20	5
NDPA	131.1	43.1	80	20	5
NEIPA	117.1	75.1	70	7	5
NDIPA	131.1	89.1	50	7	5
NDMA	75.1	43.1	90	15	5
NDEA	103.1	75.1	85	8	5

Liquid chromatographic conditions: The chromatographic separation was performed using the LCMS column Allure BiPh (250 mm × 4.6 mm), 5 mm (Thames Restek, U.K. Ltd.) and the column temperature was maintained at 25 ℃. The separation was performed in gradient mode of the mobile phase A consisted of 0.1% formic acid in water and mobile phase B with 0.1% formic acid in methanol at a constant flow rate of 0.6 mL/min. The chromatographic separation was performed with a varied gradient program as follows: 0.0-20.0 min, 2-60% B; 20.0-37.0 min, 60-98% B; 37.0-41.0 min, 98% B; 41.0-42.0 min, 98-2% B; and 42.0-50.0 min, 2% B for equilibration with a total run time of 50 min. An injection volume of 20 µL was used for whole analysis. The sampler temperature was maintained at 10 ℃. For samples preparations, water and methanol in the ratio of 50:50% v/v was used as a diluent.

Preparation of *N*-nitrosamine mix standard: A primary stock solution of 1000 ppm (stock-I) was prepared by weighing 10 mg each of NNMP, NMBA, NDEA, NEIPA, NDIPA, NMPA, NDPA and NDBA into separate 10 mL volumetric flask in methanol and mixed well. Diluted each of 0.05 mL of NDEA, NEIPA, NDIPA, NMPA, NDPA, NDBA stock solution-I, 0.18 mL of NMBA stock solution-I and 0.9 mL NDMA (from 200 ppm stock solution of Sigma-Aldrich) to 10 mL with diluent (stock-II) to achieve the concentration of 18000 ppb for NMBA and NDMA, while 5000 ppb concentration for NDEA, NEIPA, NDIPA, NMPA, NDPA and NDBA. 0.2 mL of stock-II solution was diluted to 10 mL with diluent and mixed well. Further, 0.2 mL of this solution was diluted to 10 mL with diluents to achieve the final concentration of 7.2 ppb for NDMA and NMBA, while 2 ppb for NDEA, NEIPA, NDIPA, NMPA, NDPA and NDBA for diluted standard preparation. The specification limit of NDMA (38 ppb), NMBA (38 ppb), NDEA (10 ppb), NEIPA (10 ppb), NDIPA (10 ppb), NDPA (10 ppb), NMPA (10 ppb) and NDBA (10 ppb) was finalized based on maximum daily dose of metformin (2550 mg/day) and acceptable intake (AI) of nitrosamine impurities (26.5 ng/day or 96 ng/day).

Composite impurity stock solution: A 0.19 mL each of 1000 ppm stock of NDEA, NEIPA, NDIPA, NMPA, NDPA, NDBA (stock solution-I) and 0.582 mL of NMBA stock solution-I was diluted to 10 mL with diluent into separate volumetric flasks. Further, 0.527 mL each of above NDEA, NEIPA, NDIPA, NMPA, NDPA, NDBA solutions, 0.653 mL of NMBA solution and 0.19 mL NDMA (from 200 ppm stock solution of Sigma-Aldrich) was diluted to 10 mL with diluent to prepare composite impurity stock solution.

Sample preparation: The average weight of 20 tablets was determined and crushed into the fine powder. The powder equivalent to 1000 mg of metformin was transformed into a 25 mL volumetric flask and added 10 mL of diluent. The flask was swirled and sonicated for 10 min with intermittent shaking. Further, it was centrifuged for 20 min at 4000 rpm and the solution was filtered through 0.45 µm syringe PVDF filter by discarding first few mL of filtrate. The collected filtrate was used as a sample solution (metformin 100 mg/mL) and the percentage of each nitrosoamine impurities peak in the sample was calculated as per eqn. 1. All the calculations in this research were conducted using metformin due to its higher maximum daily dosage and the expected lower nitrosamine limit compared to vildagliptin.

$$\% \text{ Nitrosamine impurity (ppb)} = \frac{Au}{As} \times Cs \times \frac{P}{100} \times 100000 \quad (1)$$

where Au = Area of peak of impurity obtained with the sample preparation; As = Average area of impurity peak obtained with the diluted standard preparation; Cs = Concentration of the impurity in the diluted standard solution (mg/mL); P = Potency of impurity standard (%).

Spiked sample preparation: The average weight of 20 tablets was determined and then crushed into the fine powder. The powder equivalent to 1000 mg of metformin was weighed and transferred into a 25 mL volumetric flask. A 0.1 mL of composite impurity stock solution and 9.9 mL of diluent was added. The flask was swirled and sonicated for 10 min with intermittent shaking. Further, it was subjected to centrifugation for 20 min at 4000 rpm, followed by filtering the solution through 0.45 µm syringe PVDF filter by discarding first few mL of filtrate.

RESULTS AND DISCUSSION

Specificity/selectivity: Single injection of 38.00 ppb of NMBA, NDMA impurity solution and 10.01 ppb of NDEA,

NEIPA, NDIPA, NDBA, NMPA and NDPA impurities prepared from individual impurity stock solutions was injected into LC-MS/MS and retention times of peaks were recorded. To check the interference due to placebo, single injection of placebo solution was prepared as per described procedure for sample preparation and injected into LC-MS/MS. While to check the spectral homogeneity, test solution was injected and sample solutions were spiked into LC-MS/MS and recorded the chromatograms. All the peaks were well resolved from each other and no interference was observed with analyte, blank and placebo peaks (Fig. 1). The MRM interpreted in individual impurity solution and spiked sample solution matched with the MRM of NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, NDPA and NDBA peaks, which indicates that NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, NDPA and NDBA peaks are spectrally homogeneous and all peaks were well resolved from each other (Table-2). Study proves that method is specific for quantification of NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, NDPA and NDBA peaks in vildagliptin and metformin tablets.

Linearity: The method validation linearity with respect to square of correlation coefficient (r^2) was calculated as per
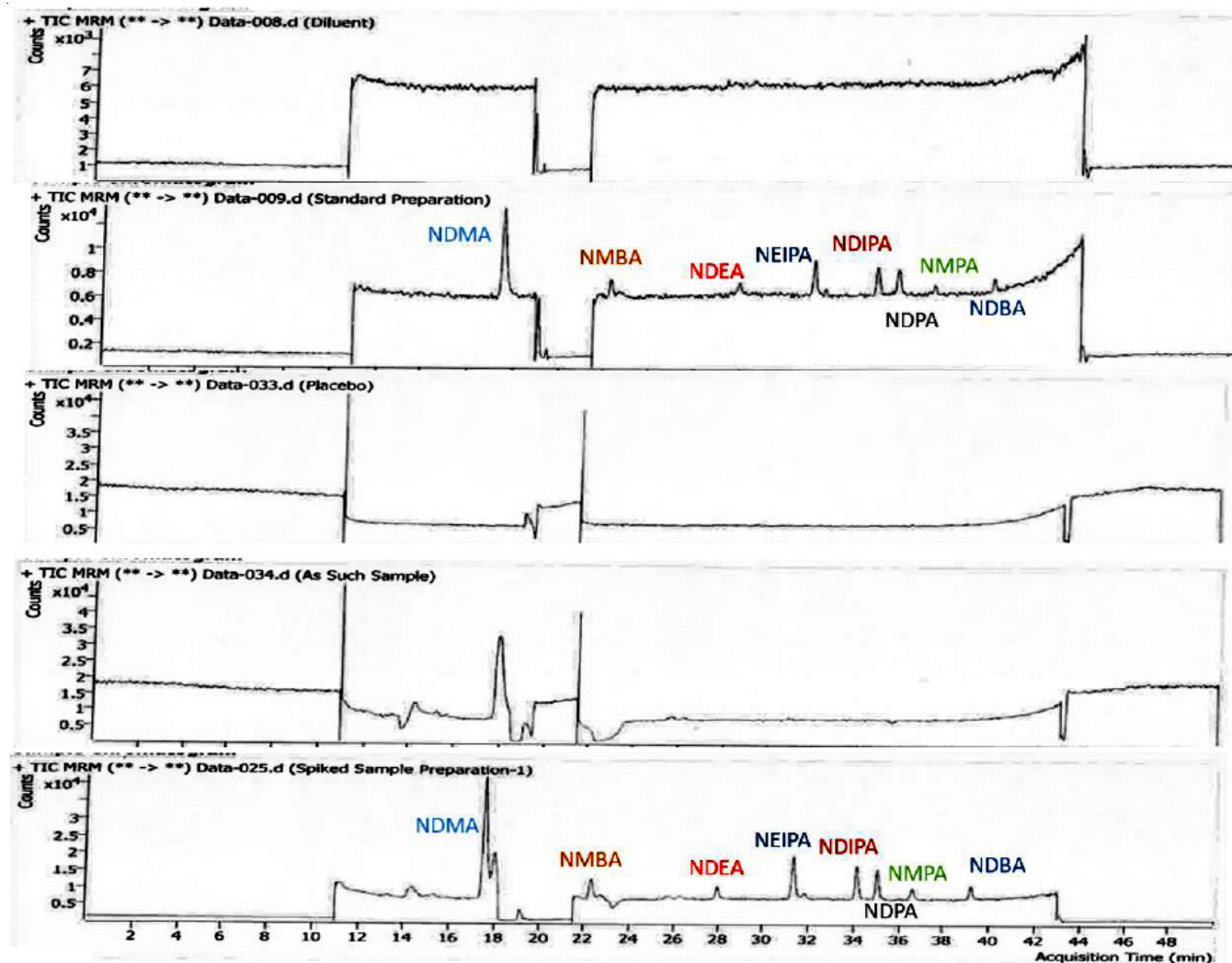

Fig. 1. Representative chromatograms of diluent, sample standard, placebo, as such sample and samples spiked with known impurities

Impurity	Peak identification solution		Spike sample preparation	
	RT (min)	MRM	RT (min)	MRM
NDMA	17.513	75.1 > 43.1	17.547	75.1 > 43.1
NMBA	22.247	147.1 > 44.2	22.270	147.1 > 44.2
NDEA	27.931	103.1 > 75.1	27.965	103.1 > 75.1
NEIPA	31.276	117.1 > 75.1	31.317	117.1 > 75.1
NDIPA	34.063	131.1 > 89.1	34.110	131.1 > 89.1
NDBA	39.167	159.1 > 41.1	39.186	159.1 > 41.1
NMPA	36.563	137.1 > 66.1	36.588	137.1 > 66.1
NDPA	34.988	131.1 > 43.1	35.020	131.1 > 43.1

ICH guidelines [11,12]. The linearity was constructed from LOQ to 150% level for 8 nitrosamine impurities at the concentration range shown in Table-3. The linearity plot was drawn by plotting peak area (Y-axis) against concentration (X-axis) of nitrosamines using a linear least-squares regression method and determined the correlation coefficient, square of correlation coefficient, slope of regression, RSD of response factor from 50% to 150% linearity level, Y-intercept and Y-intercept bias at 100% linearity level (Fig. 2). As per the United States Pharmacopeia general chapter <1469>, the linearity of the method is acceptable when the correlation coefficient should not be less than 0.98, square of correlation coefficient should

TABLE-3
LC-APCI-MS/MS LINEARITY METHOD VALIDATION PARAMETERS FOR NITROSAMINES

Impurity	Range (ppb)	r	r^2	Slope	*RSD	Y-intercept	**Y-intercept bias	LOQ (ppb)	LOD (ppb)
NDMA	18.64-55.92	0.9992	0.9984	13524.686	2.4	3517.757	0.7	3.53	1.20
NMBA	16.79-50.37	0.9927	0.9855	1824.871	6.1	-1823.614	-3.4	3.18	1.20
NDEA	5.14-15.41	0.9882	0.9765	4557.068	8.1	-1575.691	-4.0	0.97	0.33
NEIPA	5.18-15.52	0.9879	0.9759	13971.173	9.4	-4554.344	-3.0	0.98	0.33
NDIPA	5.29-15.86	0.9879	0.9759	3871.969	8.4	100.768	0.2	1.00	0.33
NDBA	5.15-15.46	0.9950	0.9900	4140.465	6.9	-1809.807	-4.1	0.98	0.33
NMPA	5.19-15.58	0.9942	0.9884	2673.621	6.3	488.445	1.6	0.98	0.33
NDPA	5.11-15.32	0.9908	0.9817	6859.556	7.8	740.748	0.9	0.97	0.33

*RSD of response factor from 50% to 150% linearity level, **Y-intercept bias at 100% linearity level; ppb = parts per billion.
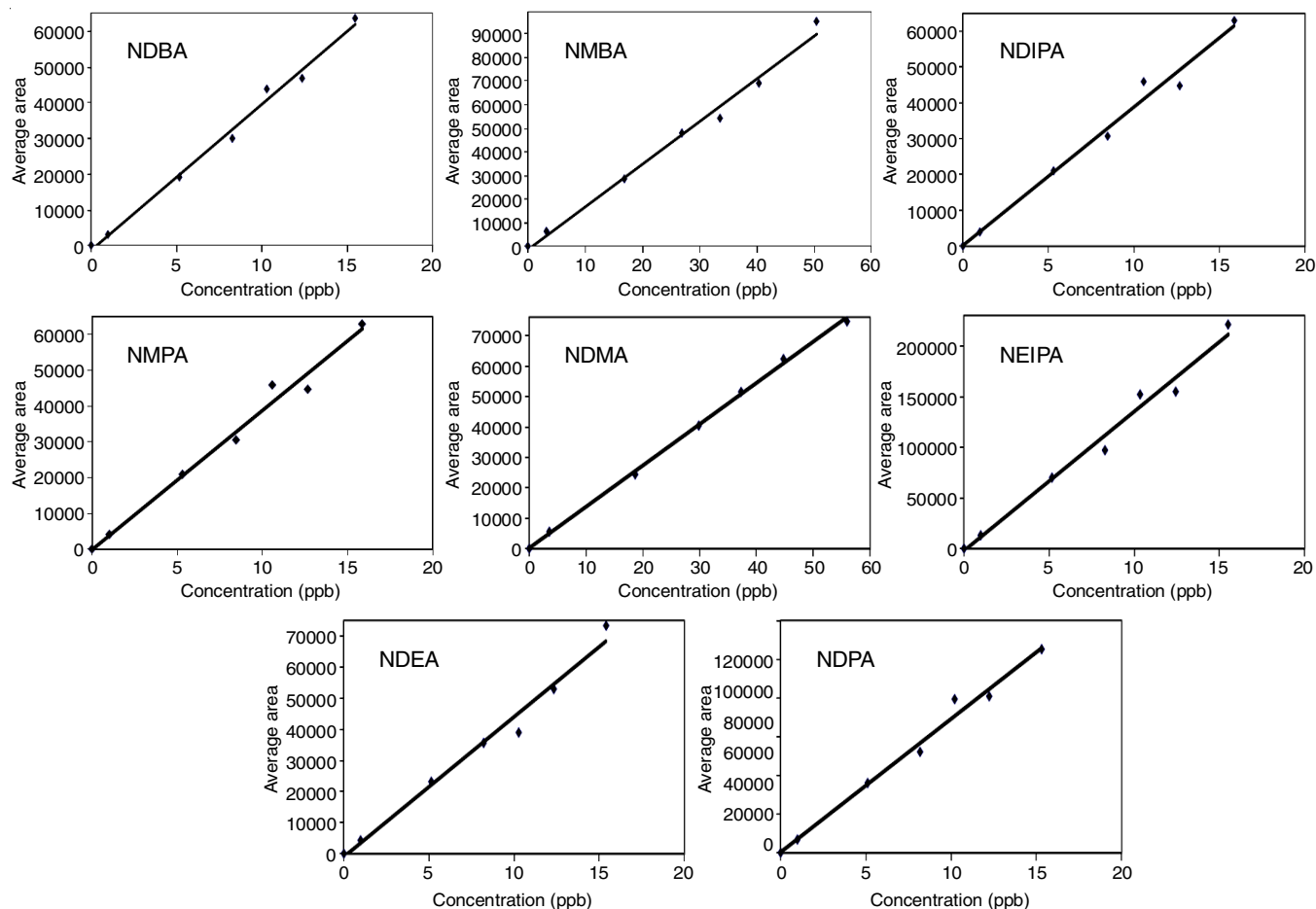

Fig. 2. Linearity curves of tested N-nitrosamines

be greater than or equal to 0.96, RSD of response factor from 50% to 150% linearity level should not be more than 20.0% and Y-intercept bias at 100.0% linearity level should be within ± 20.0%. This study proves that area response of NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, NDPA and NDBA is linear in range of LOQ to 150% of specified limit.

Sensitivity (LOD and LOQ): The sensitivity of the method can be determined on the basis of LOD and LOQ preparations. To evaluate sensitivity of the method, six replicate injections of the LOQ and three replicate injections of the LOD were performed. Subsequently, the RSD of the area for the six LOQ solution injections was determined. For LOD preparation, the peak should be detected and signal to noise ratio should be equal to or greater than 3:1 while, for LOQ, the RSD of area of six replicate injections should not be more than 20.0% and signal to noise ratio should be equal to or greater than 10:1. As a result, the LOD and LOQ of the present method were obtained in the range of 0.33-1.2 ppb and 0.97-3.53 ppb, respectively (Table-4). From the data, it was concluded that test method is capable of detecting and quantifying NDMA, NMBA, NDEA, NEIPA, NDIPA, NDBA, NMPA and NDPA if present in the sample.

TABLE-4
LC-APCI-MS/MS METHOD SENSITIVITY FOR NITROSAMINES

Impurity	LOQ			LOD	
	Conc. (ppb)	S/N	% RSD	Conc. (ppb)	S/N
NDMA	3.53	191	3.8	1.2	97
NMBA	3.18	982	5.9	1.2	195
NDEA	0.97	368	8.1	0.33	50
NEIPA	0.98	357	5.6	0.33	166
NDIPA	1.00	480	7.5	0.33	168
NDBA	0.98	318	6.2	0.33	99
NMPA	0.98	425	2.5	0.33	200
NDPA	0.97	253	10.9	0.33	92

The specification limits of NDMA, NMBA, NDEA, NEIPA, NDIPA, NDBA, NMPA and NDPA were finalized based on the MDD of metformin (2550 mg/day) and acceptable intake (AI) of nitrosamine impurities, *i.e.* 96 ng/day for NDMA and NMBA and 26.5 ng/day for the remaining nitrosamine impurities. The impurity limits were calculated in ppb by employing eqn. 2:

$$\text{Limit (ppb)} = \frac{AI}{MDD \text{ (g)}} \qquad (2)$$

For instance, the NDMA specification limit $= \dfrac{96}{2.550} =$ 37.65 ≈ 38 ppb.

Hence, as per above calculation, the concentration of NDMA will be 38 ng/g and the LOQ will be 3.8 ng/g. Here, the sample concentration is 100 mg/mL, hence, the concentration of NDMA with respect to the sample concentration will be 3.8 ng/100 mg and the LOQ will be 0.38 ng/100 mg of sample. The proposed LOQ was calculated against 1000 mg of samples. If we consider the sample concentration at 100 mg/mL, then the LOQ will be 0.38 ppb (ng), which is 10 times lower than the specification.

Method precision: To demonstrate the precision of test method, single preparation of test solution (for 50 mg and 1000 mg strength) as described in sample preparation and six preparations of spiked sample solution were prepared. Single injection of test solution and each preparation of spiked sample solution were injected into LC-MS/MS, chromatograms and peak response of NDBA, NDPA, NDIPA, NEIPA, NDEA, NMPA and NDMA, NMBA were recorded. Precision of test method was demonstrated by calculating average and RSD of all the nitrosamine peaks for six preparations of spiked sample solution. The %RSD for six results should not be more than 20%. The %RSD was found in well within the acceptance limit in the range of 0.7% to 11.6% (Table-5).

Accuracy: To demonstrate the accuracy of test method, accuracy was performed in the range of LOQ to 150% (LOQ, 50%, 100% and 150%) of target concentrations of eight nitrosamine impurities. To determine the recovery, single preparations of test solution as described in sample preparation method and triplicate preparation of accuracy sample solution at each accuracy level were prepared. Single injection of each preparation of test solution and accuracy sample solution were injected into LC-MS/MS and chromatograms were recorded. Accuracy was found in the range of 98.63 ± 5.77-125.17 ± 3.68% at LOQ level, 74.77 ± 0.83-88.17 ± 0.15% at 50% level, 79.93 ± 8.47-89.00 ± 0.61% at 100% level and 74.67 ± 2.31-84.03 ± 0.93% at 150% level for the eight nitrosamine impurities (Table-6). Recovery at each accuracy level should not be less than 70.0% and not more than 130%. As recovery results are within acceptable limit of recovery, study proves that test method is accurate in range of LOQ to 150% of specified limit.

TABLE-5
LC-APCI-MS/MS METHOD PRECISION FOR NITROSAMINES

Number of preparations	Concentration (ppb)							
	NDMA	NDBA	NDPA	NDIPA	NEIPA	NDEA	NMPA	NMBA
1	33.32	7.64	6.92	7.43	8.17	7.01	8.26	32.15
2	32.90	9.43	8.76	9.02	9.08	8.06	9.82	30.48
3	33.29	7.57	7.59	7.69	8.58	7.73	8.75	31.43
4	33.15	9.05	9.24	9.21	9.31	8.56	10.28	29.51
5	33.50	8.90	8.40	8.93	9.33	8.25	9.60	32.45
6	33.54	7.10	7.39	7.57	8.20	8.38	8.68	30.67
Average	33.30	8.30	8.10	8.30	8.80	8.10	9.20	31.10
SD	0.24	0.96	0.89	0.83	0.53	0.61	0.78	1.11
%RSD	0.7	11.6	11.0	10.0	6.0	7.5	8.5	3.6

TABLE-6
PERCENT RECOVERY AND PRECISION FOR NITROSAMINE

Impurity	Accuracy LOQ level		Accuracy 50% level		Accuracy 100% level		Accuracy 150% level	
	Average ± SD	%RSD	Average ± SD	%RSD	Average ± SD	%RSD	Average ± SD	%RSD
NDMA	104.13 ± 4.76	4.58	88.17 ± 0.15	0.17	89.00 ± 0.61	0.68	84.03 ± 0.93	1.11
NMBA	125.17 ± 3.68	2.94	74.77 ± 0.83	1.11	81.23 ± 2.15	2.65	74.67 ± 2.31	3.10
NDEA	108.10 ± 5.77	5.33	83.27 ± 4.57	5.49	82.60 ± 8.46	10.24	82.37 ± 5.32	6.46
NEIPA	101.97 ± 3.76	3.69	82.60 ± 6.66	8.06	85.20 ± 4.56	5.35	83.63 ± 6.63	7.93
NDIPA	108.77 ± 1.53	1.40	81.77 ± 8.22	10.06	79.93 ± 8.47	10.60	80.87 ± 7.05	8.72
NDBA	111.57 ± 16.66	14.93	82.97 ± 8.13	9.80	87.97 ± 10.08	11.46	79.53 ± 6.73	8.47
NMPA	114.80 ± 7.28	6.34	84.13 ± 6.18	7.35	88.30 ± 7.82	8.86	79.97 ± 3.17	3.97
NDPA	98.63 ± 5.77	5.85	84.77 ± 10.78	12.72	82.80 ± 9.92	11.98	83.53 ± 8.18	9.79

TABLE-7
SOLUTION STABILITY FOR NITROSAMINES STANDARD AND TEST SOLUTION

Impurity	% RSD for standard solution				Spiked solution			
					Initial		25 h	
	Initial	25 h	34 h	40 h	Conc. (ppb)	%RSD	Conc. (ppb)	%RSD
NDMA	1.5	1.5	1.7	1.9	33.3	Not applicable	32.1	2.6
NMBA	2.5	2.9	5.0	4.4	32.1	Not applicable	28.6	8.1
NDEA	6.6	6.1	6.7	7.0	7.0	Not applicable	7.8	7.7
NEIPA	8.3	7.7	7.6	8.2	8.2	Not applicable	8.9	5.7
NDIPA	10.3	9.6	6.7	7.3	7.4	Not applicable	8.3	8.1
NDBA	6.2	6.8	6.2	8.3	7.6	Not applicable	8.5	7.9
NMPA	5.4	5.0	5.7	6.1	8.3	Not applicable	8.3	0.0
NDPA	10.2	10.0	7.6	8.3	6.9	Not applicable	8.2	12.1

Solution stability: Standard solution and single preparation of spiked sample solution were prepared and analyzed at 10 ºC and for minimum 12 h with maximum time interval of 24 h by injecting single injection of standard solution and single injection spiked sample solution into LC-MS/MS and chromatograms were recorded. The content of nitrosamine impurities as described in test method, spiked sample solution against freshly prepared standard solution at each time interval were recorded. The %RSD of content of nitrosoamine impurities in standard solution and spiked sample solution should not be more than 20.0%. Thus, it is concluded that standard preparation is stable for 40 h while test preparation is stable for 25 h at 10 °C (Table-7).

Filter compatibility: Filter compatibility study was carried out using two different filters namely 0.45 µm PVDF filter and 0.45 µm nylon filter. Single preparation of centrifuged and filtered spiked sample solution was prepared and single injection of filtered spiked sample solution was injected into liquid chromatograph and chromatograms were recorded. The content of NDMA, NDBA, NDPA, NEIPA, NDIPA, NDEA, NMPA and NMBA for 0.45 µm PVDF filtered spiked sample solution and 0.45 µm nylon filtered spiked sample solution was calculated. The %RSD in result of content for NDMA, NDBA, NDPA, NEIPA, NDIPA, NDEA, NMPA and NMBA was determined to 0.45 µm nylon filtered spiked sample solution against respective 0.45 µm PVDF spiked sample solution. The %RSD of results for content of nitrosamine impurities should not be more than 20% (compared with 0.45 µm PVDF filter, Table-8). Thus both 0.45 µm PVDF filter and 0.45 µm nylon filter are compatible for filtration of sample solution.

The developed LC-MS/MS system using APCI source method is specific, accurate and precise for screening and quali-

TABLE-8
FILTER COMPATIBILITY STUDY FOR NITROSAMINES

Impurity	0.45 µm PVDF filter		0.45 µm nylon filter	
	Conc. (ppb)	%RSD	Conc. (ppb)	%RSD
NDMA	33.3	NA	33.2	0.2
NMBA	32.2	NA	28.0	9.7
NDEA	7.0	NA	8.6	14.5
NEIPA	8.2	NA	9.2	8.2
NDIPA	7.4	NA	9.0	13.8
NDBA	7.6	NA	8.2	5.3
NMPA	8.3	NA	9.7	11.0
NDPA	6.9	NA	8.4	13.8

fication of eight nitrosamines NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, NDPA and NDBA in the vildagliptin and metformin drug products. The method was specific in MRM interpreted and all peaks were well resolved from all the eight nitrosoamine impurities peaks and there was no interference observed from blank and placebo solution. The present method was validated in terms of linearity from LOQ level to 150% level ranged 3.53-55.92 ppb with coefficient of determination (r^2) 0.9984 for NDMA, 3.18-50.37 ppb ($r^2 = 0.9855$) for NMBA, 0.97-15.41 ppb ($r^2 = 0.9765$) for NDEA, 0.98-15.52 ppb ($r^2 = 0.9759$) for NEIPA, 1.00-15.86 ppb ($r^2 = 0.9759$) for NDIPA, 0.98-15.46 ppb ($r^2 = 0.9900$) for NDBA, 0.98-15.58 ppb ($r^2 = 0.9884$) for NMPA, 0.97-15.32 ppb ($r^2 = 0.9817$) for NDPA. The correlation coefficient (r) was more than 0.98 and square of correlation coefficient (r^2) was found to be greater than 0.96. The LOD and LOQ of the present method were obtained in the range of 0.33-1.2 ppb and 0.97-3.53 ppb, respectively. The percentage relative standard deviation of area of six replicate injections of LOQ solution was found in the range of 2.5-10.9% for eight nitrosamines showed method was sensitive enough to determine the

nitrosamine impurities. The % RSD of area of six preparations of spiked sample solution was found in the range of 0.7% to 11.6% for eight nitrosamines showed method was precise. Accuracy was found in the range of $98.63 \pm 5.77\text{-}125.17 \pm 3.68\%$ at LOQ level, $74.77 \pm 0.83\text{-}88.17 \pm 0.15\%$ at 50% level, $79.93 \pm 8.47\text{-}89.00 \pm 0.61\%$ at 100% level and $74.67 \pm 2.31\text{-}84.03 \pm 0.93\%$ at 150% level for the eight nitrosamine impurities that were well within acceptance criteria of not less than 70% and not more than 130%. From the solution stability study, it was observed that the standard solution was stable for 40 h while sample solution was stable for 25 h at 10 °C. Filter compatibility study showed that both 0.45 µm PVDF and 0.45 µm nylon filters are compatible for the test method. The method validation results demonstrated that the method is precise, accurate and linear for all the eight nitrosamine impurities and can be applied to determine and quantify the nitrosamines in vildagliptin and metformin drug products.

Conclusion

The developed and validated LC-APCI-MS/MS method is accurate, sensitive, specific and precise for the screening and qualification of eight nitrosamines in metformin and vildagliptin drug products. The method was validated for linearity (0.97-55.92 ppb) with coefficient of determination (r^2) (0.9759-0.9984), LOD (0.33-1.2 ppb), LOQ (0.97-3.53 ppb) and average recovery in the range of 74.67-125.17% for metformin and vildagliptin drug products with good precision. The method validation results demonstrated that the method is precise, accurate and linear; therefore, the method can be applied for the screening and quantification of nitrosamines in metformin and vildagliptin drug products.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. U.S. Food and Drug Administration, FDA Updates and Press Announcements on Angiotensin II Receptor Blocker (ARB) Recalls (Valsartan, Losartan, and Irbesartan). Available from https://www.fda.gov/drugs/drug-safety-and-availability/fda-updatesand-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan (Accessed 13 October 2023).
2. European Medicines Agency, Nitrosamine Impurities. Available from https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities (Accessed 13 October 2023).
3. J.B. Byrd, G.M. Chertow and V. Bhalla, *N. Engl. J. Med.*, **380**, 1589 (2019); https://doi.org/10.1056/NEJMp1901657
4. HSA Recalls Three out of 46 Metformin Medicines; https://www.hsa.gov.sg/announcements/news/hsa-recalls-three-out-of-46-metformin-medicines (Accessed 13 October 2023).
5. FDA Updates and Press Announcements on NDMA in Metformin. Available from https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-metformin (Accessed 13 October 2023).
6. A. Zmyslowski, I. Ksiazek and A. Szterk, *Molecules*, **25**, 5304 (2020); https://doi.org/10.3390/molecules25225304
7. I. Badran, A.D. Manasrah and N.N. Nassar, *RSC Adv.*, **9**, 13403 (2019); https://doi.org/10.1039/C9RA01641D
8. N.E.H. Nasr, G.M. Metwaly, E.O. Ahmed, A.R. Fares and A.N. ElMeshad, *Expert Opin. Drug Saf.*, **21**, 285 (2022); https://doi.org/10.1080/14740338.2021.1983312
9. C. Ripolles, E. Pitarch, J.V. Sancho, F.J. Lopez and F. Hernandez, *Anal. Chim. Acta*, **702**, 62 (2011); https://doi.org/10.1016/j.aca.2011.06.024
10. W. Wang, S. Ren, H. Zhang, J. Yu, W. An, J. Hu and M. Yang, *Water Res.*, **45**, 4930 (2011); https://doi.org/10.1016/j.watres.2011.06.041
11. ICH Guidelines, Federal Register, **62**, No. 96, pp. 27464-27467 (1997).
12. S. Walfish, *Biopharm Int.*, **19**, 28 (2006).