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A Simple Liquid Chromatograph Method for Determination of Mass Spectrometric Method for Determination of Seven Nitrosamine Impurities in Cinacalcet Hydrochloride

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Abstract

For the identification of the seven Nitrosamine Impurities i.e. N-Methyl-N-Nitrosopropane-1,3-diamine (Nitroso MAPA), N-Nitrosomethyl phenylamine (NMPA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitroso-N-Methyl-4-Aminobutyric acid (NMBA), N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosodiisopropylamine (NDIPA) content in Cinacalcet Hydrochloride a sensitive, fast, linear, precise, and efficient liquid chromatography mass spectrometry method has been developed and validated. As a calcimimetics, cinacalcet hydrochloride is an active pharmaceutical ingredient that lowers blood calcium levels by telling the body to create less parathyroid hormone. It is known that amines react with nitrosating substances like nitrite to create nitrosamines. Residual amines in the APIs are the primary source of amines in the case of minor alkylnitrosamines like NDMA and NDEA. Although the degree of the risk of nitrosamine contamination has rarely been documented, residual amines in the APIs provide a possible problem. A simultaneous analytical method for amines that correspond to seven common small alkyl nitrosamines was developed and validated in this study. For every Nitrosamine Impurity, including N-Nitrosodimethylamine (NDMA), N-Nitroso-N-Methyl-4-Aminobutyric acid (NMBA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosomethylphenylamine (NMPA), and N-Methyl-N-Nitrosopropane-1,3-diamine (Nitroso MAPA), good linearity was obtained in the range of 0.009 ppm

to 0.0450 ppm. The detection and quantification limits were 0.009 ppm. The range of recovery rates was 70% - 130%. With % RSD < 15%, repeatability was likewise good. Despite this, each nitrosamine impurity has a correlation of 0.990. Seven nitrosamine impurities in Cinacalcet Hydrochloride APIs will be identified using the proposed analytical LC-MS/MS method.

Keywords

Liquid Chromatography with mass Spectrometer (LC-MS), N-Nitrosodimethylamine (NDMA), N-Nitroso-N-Methyl-4-Aminobutyric Acid (NMBA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosomethylphenylamine (NMPA), N-Methyl-N-Nitrosopropane-1,3-Diamine (Nitroso MAPA), LC-MS/MS, Cinacalcet Hydrochloride

1. Introduction

Based on research on animals, nitrosamine contaminants are probably carcinogenic to humans [1]. In 2018, many blood pressure medications known as "sartans" were found to have the nitrosamine contaminant N-nitrosodimethylamine (NDMA), necessitating their removal from the market. As a result, the European Union has strict production regulations for certain medications [2]. Since then, batches of ranitidine and a few batches of pioglitazone from one firm have been found to contain nitrosamine impurities. A review of ranitidine has been started for the entire EU [2].

Owners of marketing authorizations are in charge of making sure their products are manufactured in compliance with applicable laws. As a result, they are in charge of making sure that every batch of their final product is of a completely satisfactory quality, including the quality of the active ingredients and other components [3]. In accordance with Article 5(3) of Regulation (EC) No 726/2004, the European Union network's subject matter experts reviewed nitrosamine impurities in marketed products following an outbreak of their occurrence.

They are giving marketing authorization holders instructions on how to keep their medications free of seven nitrosamine contaminants [3]. The FDA learned at the end of December 2019 that several diabetic medications, including Metformin, had been found to have NDMA Nitrosamine impurity in other nations [4]. Based on sample testing, the Agency found that the quantity of NDMA Nitrosamine impurity in 2020 was more than what was considered acceptable. The USFDA and EMA required that the applicant recall the batch samples of Metformin as well as those drug goods and chemicals voluntarily [4].

Nitrosamine impurity is a class of compounds that have the chemical structure of a nitroso group bonded to an amine (R1N(-R2)-N=O), as shown in **Figure 1**. The compounds can be formed by the reaction between secondary amines, tertiary

amines, or quaternary amines and nitrous acid, nitrite salts under acidic conditions [4].

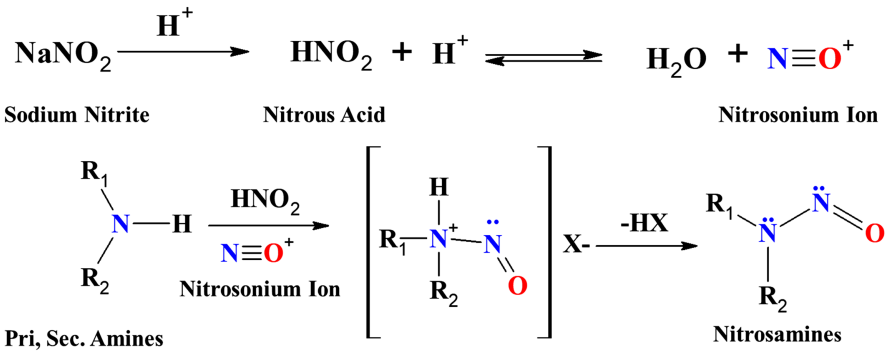


Figure 1. Representative reaction to form nitrosamines.

Various analytical methods are developed and published for the different products as drug products as well as drug substances. The Nitrosamine impurities are screened based on their manufacturing reaction scheme, process and route of synthesis key starting materials, reagents, catalysts, and solvents [5] [6].

Cinacalcet hydrochloride is a calcimimetic drug approved by the US Food and Drug Administration for the management of secondary hyperparathyroidism associated with end-stage kidney disease and primary hyperparathyroidism in cases where surgical intervention is not feasible [7].

The objective of this research study is to quantify the seven potential nitrosamine impurities in Cinacalcet Hydrochloride by LC-MS/MS in a single method. During the literature survey, no method has been published to date for the determination of seven nitrosamine impurities in a single analytical method using LC-MS/MS for Cinacalcet Hydrochloride.

According to "Control of Nitrosamine Impurities in Human Drugs", Guidance for Industry, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), the nitrosamine acceptable limit under the cohort of concern, therefore all seven nitrosamine levels should be considered 0.03 ppm in Cinacalcet Hydrochloride.

In this research paper, we are going to present a simple and most reliable LC-MS method that has been developed and validated for the determination of seven nitrosamine impurities as shown in **Figure 2**, and these are N-Nitrosodimethylamine (NDMA), N-Nitroso-N-Methyl-4-Aminobutyric acid (NMBA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosomethylphenylamine (NMPA), and N-Methyl-N-Nitrosopropane-1,3-diamine (Nitroso MAPA).

A mass spectrometer typically works in two modes: full scan or selected ion monitoring (SIM). The typical LC-MS instrument is capable of performing both functions either individually or concomitantly, depending on the setup of the particular instrument. The benefit of SIM is that the detection limit is lower since the instrument only looks at a small number of fragments during each scan. For SIM

mode the mass spectrometer is 'targeting the selected mass value'; the number of scans has increased as a result in a good peak shape is observed. This is an easy solution for getting better quantitation for early eluting peaks. Inspect the ions obtained for the peak in full scan mode and use at least one of the ions in SIM to obtain a better scan rate.

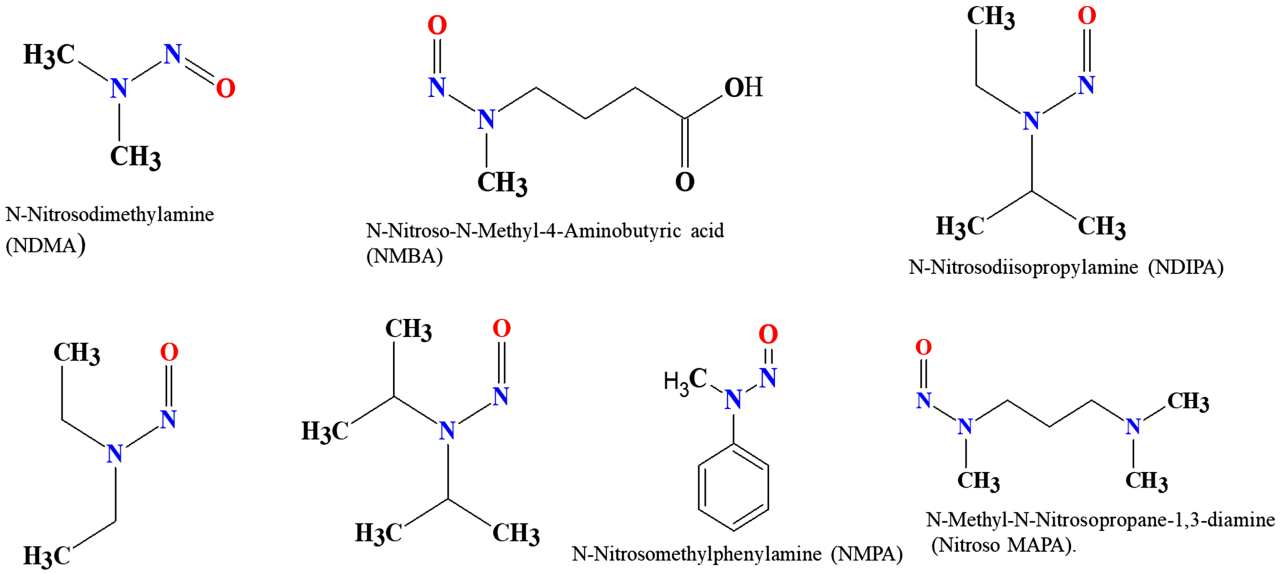


Figure 2. Nitrosamines impurities.

2. Experimental

2.1. Material

Cinacalcet API sample, N-Nitrosodimethylamine (NDMA), N-Nitroso-N-Methyl-4-Aminobutyric acid (NMBA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosomethylphenylamine (NMPA), N-Methyl-N-Nitrosopropane-1,3-diamine (Nitroso MAPA) Impurities were obtained from the Validation Cell Department, Megafine Pharma (P) ltd. Nashik.

2.2. Chemicals and Reagents

Methanol, Formic Acid, Milli-Q Water are used for the solution preparation (all solvents/chemicals used are LCMS grade).

2.3. Instrumentation

Liquid Chromatographic with triple Quadrupole Mass Spectrometer Model-ALTIS, Make: Thermo Fischer is equipped with a binary gradient pump, degasser, automated sampler, and column oven. This LC system is hyphened with a triple quadrupole mass spectrometer along with Chromeleon software, the Analytical balance is made by Sartorius.

2.4. Chromatographic Conditions

LC column used for the analysis was Kinetex 2.6 µ Phenyl-Hexyl, 150 mm length 4.6 mm internal diameter. Mobile Phase A and B used for elution were 0.1 % Formic Acid in water and 0.1 % Formic acid Methanol respectively. The injection volume was optimized to 5.0 µL. Elution was obtained at a flow rate 0.6 mL/min in a gradient mode: 50% Solvents (0 - 8 min.), Column oven temperature 40˚C with autosampler temperature 25˚C.

The mass spectrometer was equipped with Electron Spray Ionization (ESI) source with SRM Mode Dwell time 100, CID gas 1.5 m torr and Chromatographic peak width 6. The details of MS parameters are shown in Table 1.

Table 1. Method parameters.

Sr. No.	Name of Impurity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)	Start Time	End Tine	Q1 Resolution (FWHM)	Q1 Resolution (FWHM)
1	NDMA	75.06	58.05	13	33	0	5	0.7	1.2
2	NMBA	147.1	116.9	6	30	0	5	0.7	1.2
3	NDEA	103.1	75.0	7	30	0	5	0.7	1.2
4	NEIPA	117.2	75.0	10	30	0	8	0.7	1.2
5	NDIPA	131.1	89.0	9	30	0	8	0.7	1.2
6	NMPA	137.1	107.0	12	40	0	8	0.7	1.2
7	MAPA	118.1	71.1	11	30	0	5	0.7	1.2

2.5. Preparation of Solutions

All preparations were made using Milli-Q water as a diluent. After weighing and transferring 500 mg of the test sample into a 10 mL volumetric flask, 3 mL of pure water was added, the mixture was vortexed, filtered through a 0.45-µ nylon syringe, and the clear filtrate was collected in an HPLC vial (Conc. 50 mg/mL). For each of the nitrosamine impurities NDMA, NMBA, NDEA, NEIPA, NMPA, and MAPA a standard solution was made at a concentration of 0.030 µg/mL relative to the sample.

3. Results and Discussion

3.1. Method Validation

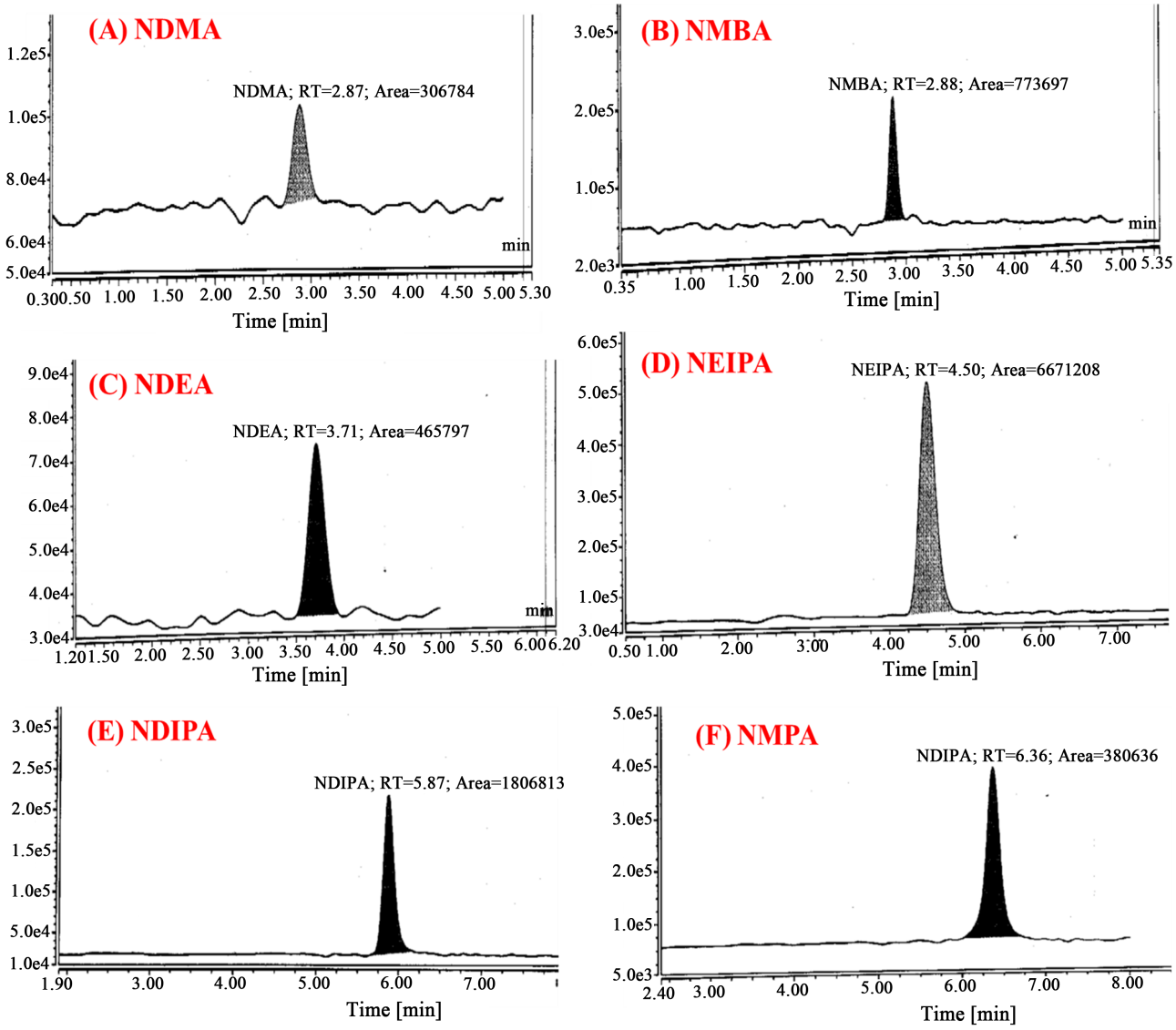
The developed analytical method met the requirements of system suitability criteria during the Method validation and batch analysis, indicating a more reliable method according to the ICH (International Conference on Harmonization) guidelines. The analytical method validation was carried out with Specificity, Precision, Limit of detection, Limit of quantitation, Linearity and Accuracy [2]-[6].

3.1.1. Specificity

The Specificity to definitively evaluate the analyte in the presence of potentially predicted components is known as specificity [2]. The specificity of the approach was established by confirming that the m/z value of each individual impurity should not interfere with the m/z value of the others and that no blank interference peak should be found at the retention time of NDMA, NMBA, NDEA, NEIPA, NMPA, and MAPA. The list the retention periods for NDMA, NMBA, NDEA, NEIPA, NMPA, and MAPA are mentioned in **Table 2**, Therefore, the specificity of the approach has been proven in **Figure 3**.

Table 2. Retention times (RT).

Details	NDMA	NMBA	NDEA	NEIPA	NDIPA	NMPA	MAPA
RT in (min.)	2.87	2.88	3.72	4.50	5.85	6.35	2.34



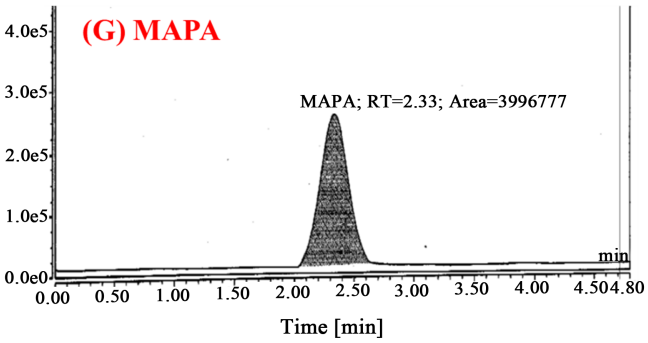


Figure 3. (A) Specificity of NDMA, (B) Specificity of NMBA, (C) Specificity of NDEA, (D) Specificity of NEIPA, (E) Specificity of NDIPA, (F) Specificity of NMPA and (G) Specificity of MAPA.

3.1.2. Precision

The degree of agreement (or scatter) between a set of measurements made by repeatedly sampling the same homogeneous sample under specified conditions is expressed as the precision of an analytical method [8]. System Precision for seven nitrosamine impurities was demonstrated by analysing six separates the obtained percent relative standard deviation (%RSD) for NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, and MAPA is found to be less than 15.0%. The result are reported in **Table 3**.

Table 3. %RSD for system precision data.

Name of Nitrosamine Impurity	% RSD System Precision (n = 6)
NDMA	10.52
NMBA	2.83
NDEA	11.19
NEIPA	1.72
NDIPA	2.46
NMPA	1.84
MAPA	2.64

In order to establish method precision, six injections of standard solution were used to spike each impurity at 0.030 ppm. The obtained results of each nitrosamine impurities NDMA, NMBA, NDEA, NEIPA, NMPA, and MAPA the percent relative standard deviation (%RSD) for is found to be 2.16% - 11.38%, the results are reported in **Table 4** below.

3.1.3. Limit of Detection and Limit of Quantitation

The lowest amount of analyte in a sample that can be identified but not always quantified as an exact value is known as the detection limit of a particular analytical process [8]. The lowest concentration of analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the quan-

titation limit of a particular analytical process [8]. The predefined limits of quantitation (LOQ) and detection (LOD) were 0.009 ppm and 0.003 ppm, respectively.

Table 4. %RSD for method precision data.

Sample Replicate	NDMA ppm	NMBA ppm	NDEA ppm	NEIPA ppm	NDIPA ppm	NMPA ppm	MAPA ppm
Sample-1	0.031	0.031	0.028	0.036	0.031	0.027	0.033
Sample-2	0.025	0.029	0.030	0.036	0.030	0.022	0.033
Sample-3	0.030	0.030	0.028	0.036	0.030	0.023	0.032
Sample-4	0.030	0.030	0.028	0.037	0.031	0.027	0.034
Sample-5	0.025	0.031	0.027	0.036	0.030	0.022	0.033
Sample-6	0.033	0.031	0.031	0.038	0.032	0.027	0.036
Mean	0.029	0.030	0.029	0.037	0.031	0.025	0.034
Std. Dev.	0.0033	0.0008	0.0015	0.0008	0.0008	0.0026	0.0014
%RSD	11.38	2.67	5.17	2.16	2.58	10.40	4.12

3.1.4. Linearity

The linearity of an analytical procedure is defined as its ability (within a specified range) to produce test results that are directly proportional to the concentration (amount) of analyte in the sample [8].

The correlation coefficient (r), after the regression analysis of the results should not be less than 0.99 [8]. Plotting the peak area of NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, and MAPA impurities against their corresponding concentration of linearity solution (**Figure 4**) yielded linearity curves. A series of standard solutions of NDMA, NMBA, NDEA, NDEA, NEIPA, NDIPA, NMPA, and MAPA impurities were prepared in a concentration ranging from LOQ to 250% (10%, 80%, 100%, 150% and 250%) of the target concentration (0.03 µg/mL w.r.t. sample). Plotting the peak area of NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, and MAPA impurities against its corresponding concentration of linearity solution yielded linearity curves, with an observed correlation coefficient of each nitrosamine impurities are reported in **Table 5** and the Linear regression data for all seven nitrosamine impurities are represented in **Figure 4**.

3.1.5. Accuracy

The degree of agreement between the value found and the value that is recognized as either a conventional true value or an acceptable reference value is a measure of an analytical procedure's accuracy [8]. By spiking known concentrations of NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, and MAPA in Cinacalcet Hydrochloride API at levels LOQ, 100%, and 150%,The obtained % recovery should be in the range of 70% to 130%.The accuracy of the procedure was assessed in

terms of recovery (Table 6). According to the approach, three preparations of each level were made and examined; the mean and percentage recovery were determined. The recovery of NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, and MAPA in Cinacalcet Hydrochloride API at all levels was accomplished with great accuracy using this approach.

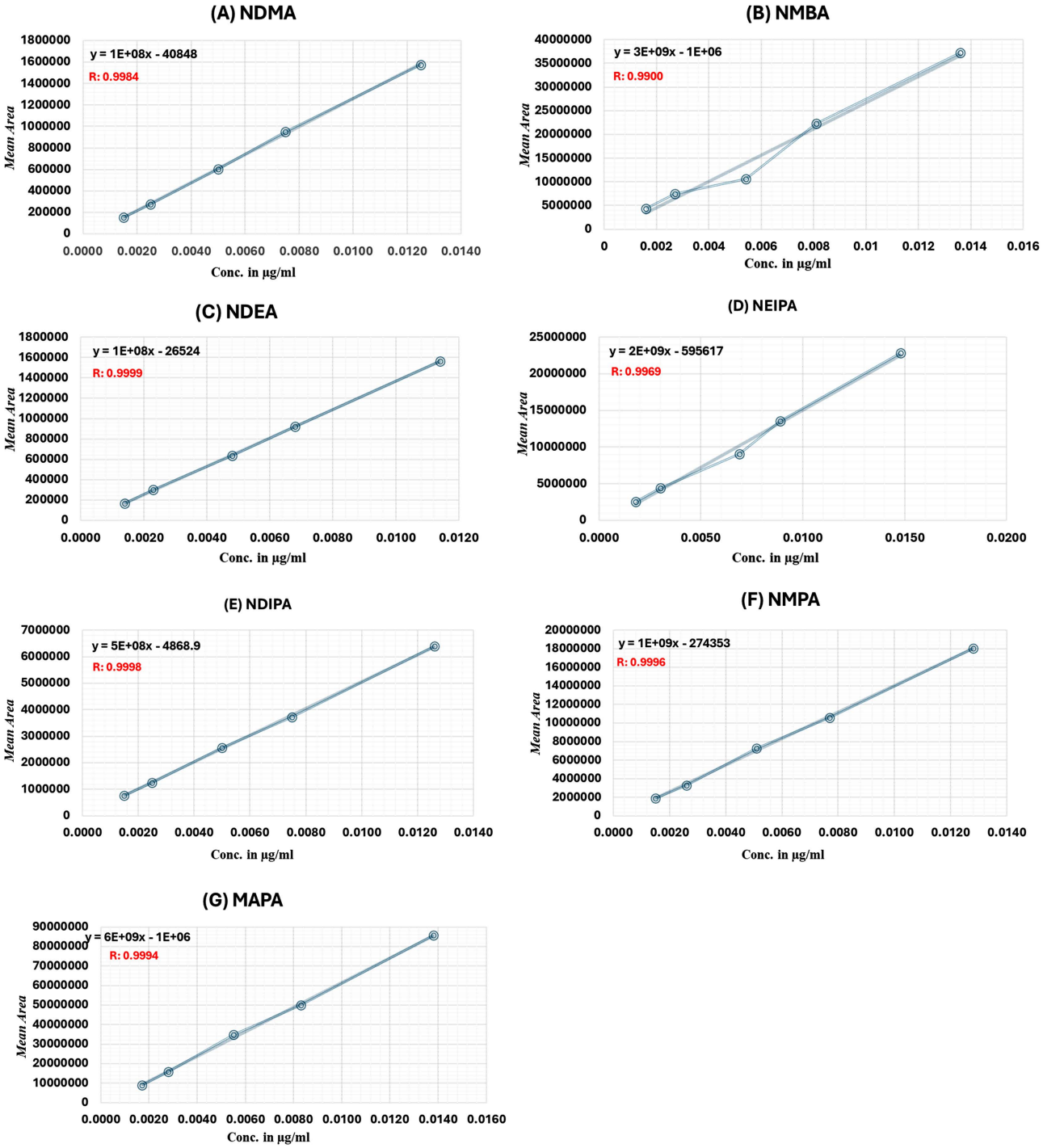


Figure 4. Linearity of NDMA (A), Linearity of NMBA (B), Linearity of NDEA (C), Linearity of NEIPA (D), Linearity of NDIPA (E), Linearity of NMPA (F) and Linearity of MAPA (G).

Table 5. Correlation coefficient data.

Name of Nitrosamine impurity	Slope	Intercept	Correlation Coefficient
NDMA	129932849.19	−40847.86	0.99984
NMBA	2773990210.63	−1031028.39	0.99008
NDEA	139441777.34	−26524.29	0.99995
NEIPA	1564229367.19	−595616.59	0.99690
NDIPA	506495562.41	−4868.91	0.99986
NMPA	1431507625.98	−274353.23	0.99962
MAPA	6306173354.35	−1476060.93	0.99949

Table 6. Accuracy of NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, MAPA.

Recovery Concentration (µg/mL)	% Recovery						
	NDMA ppm	NMBA ppm	NDEA ppm	NEIPA ppm	NDIPA ppm	NMPA ppm	MAPA ppm
0.009 µg/mL	77.78	80.00	125.00	100.00	111.11	77.78	100.00
0.009 µg/mL	100.00	90.00	127.50	109.09	111.11	100.00	111.11
0.009 µg/mL	88.89	90.00	112.50	109.09	111.11	100.00	111.11
0.03 µg/mL	103.33	90.63	103.70	100.00	103.33	89.66	100.00
0.03 µg/mL	83.33	84.38	111.11	100.00	100.00	72.41	100.00
0.03 µg/mL	100.00	87.50	103.70	100.00	100.00	75.86	96.88
0.045µg/mL	108.89	89.58	112.20	105.66	108.89	95.35	108.51
0.045µg/mL	108.89	87.50	107.32	105.66	104.44	81.40	106.38
0.045µg/mL	117.78	97.92	112.20	107.55	108.89	95.35	112.77

3.1.6. Application of Method

The examination of the drug substance showed that the method is more accurate and highly specific for identifying the seven nitrosamine contaminants in cinacalcet hydrochloride. The sample batch data is represented in Table 7.

Table 7. Sample batch data

Sample Replicate	NDMA ppm	NMBA ppm	NDEA ppm	NEIPA ppm	NDIPA ppm	NMPA ppm	MAPA ppm
Batch No-1	ND	ND	ND	ND	ND	ND	ND
Batch No-2	ND	ND	ND	ND	ND	ND	ND
Batch No-3	ND	ND	ND	ND	ND	ND	ND

4. Conclusion

The present study introduces a sensitive, specific, linear, accurate, and efficient liquid chromatography with mass spectrometer method that separates and effectively detects the seven nitrosamine impurities NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, and MAPA in Cinacalcet Hydrochloride API. This method has been validated as being more precise, linear, and accurate. Based on the results of all the data produced, we can conclude that the present method can be useful for identifying and quantifying the nitrosamine impurities in Cinacalcet Hydrochloride API. As a result, the method is deemed suitable for routine analysis of NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA and MAPA impurities.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Recommended Acceptable Intake Limits for Nitrosamine Drug Substance Related Impurities (NDSRIs) Guidance for Industry, S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) August 2023 Pharmacology/Toxicology Nitrosamine Guidance at 9.

International Agency for Research on Cancer (IARC) Monographs on the Identification of Carcinogenic Hazards to Humans web page at https://monographs.iarc.who.int/list-of-classifications. See, e.g., NDMA, N-Nitroso Piperidine (NPIP), 4-(Methylnitrosoamino)-1-(3-Pyridinyl)-1-Butanone (NNK), N-Nitroso Pyrrolidine (NPYR), and N-Nitroso Morpholine (NMOR).

ICH M7(R2), available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

Nitrosamine Guidance at 10 and Appendix B (FDA Determination of Acceptable Intake Limits).

An AI limit can be converted into a parts per million (ppm) control limit for a manufacturer or applicant. The conversion varies by product and is calculated based on a drug's maximum daily dose (MDD) as reflected in the drug labeling (ppm = AI (nanograms (ng))/MDD (milligrams)). Nitrosamine Guidance at 10.

FDA Statement on FDA's Ongoing Investigation Into Valsartan Impurities and Recalls and an Update on FDA's Current Findings, available at https://www.fda.gov/news-events/press-announcements/fda-statement-fdasongoing-investigation-valsartan-impurities-and-recalls-and-update-fdas-current.

ICH M7(R2) at 9 to 11 and Note 4 on calculating a compound-specific AI limit.

Lhasa Carcinogenicity Database (LCDB) is available at https://carcdb.lhasalimited.org. As indicated in the Field Descriptions from LCDB, LCDB includes data from Carcinogenic Potency Database (CPDB). CPDB is available at https://files.toxplanet.com/cpdb/index.html.

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