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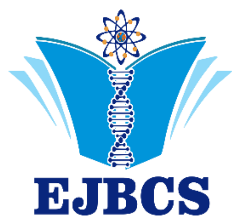

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Development of a dynamic multiple reaction monitoring LC-APCI-MS/MS method for quantification of ten nitrosamines in ranitidine API with simple extraction approach

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Abstract: Nitrosamines (NAs) are classified as probable or possible human carcinogens by the International Agency for Research on Cancer (IARC) and the presence of these impurities has resulted in numerous drug recalls from the market. In April 2020, ranitidine which is prescribed to reduce the amount of acid secreted by the stomach has been recalled owing to contamination with NAs. In this work, a simple and sensitive method for simultaneous determination of 10 NAs were developed, utilizing atmospheric pressure chemical ionisation source coupled liquid chromatography tandem mass spectrometer (LC-APCI-MS/MS). By performing dynamic multiple reaction monitoring (dMRM) mode, 10 NAs were separated on a Poroshell HPH C18 (4.6 x 150 mm, 2.7 µm) column with gradient elution implementing mobile phase A consisting of 0.2 % formic acid in water and mobile phase B consisting of methanol in 17 min. The proposed analytical method was successfully implemented in active pharmaceutical ingredient (API) of ranitidine with a water-based extraction procedure. Good linearity with a correlation coefficient (R^2) ≥ 0.994 was accomplished over the concentration in range of 0.5–50 ng/mL. The limits of detection (LODs) ranged in 0.06–0.17 ng/mL and limits of quantitation (LOQs) ranged in 0.21-0.58 ng/mL of the method met thresholds of US Food and Drug Administration (US-FDA) and European Medicines Agency (EMA) for testing of NAs. The accuracy of the developed method ranged from 83.1% to 111.9 % and the percent relative standard deviation (RSD %) was ≤ 8.9.

Keywords: N-nitrosamines, Ranitidine, Liquid chromatography, Mass spectrometry, Simultaneous determination

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1. Introduction

N-Nitrosamines, (NAs) which are commonly formed by the interaction of a nitrosating agent generating from either nitrite salts or nitrogen oxides with secondary, tertiary, or quaternary amines, belong to a larger group of potent carcinogens known as N-nitroso compounds (Bharate et al. 2021; Campillo et al. 2021). Therefore, the existence of NAs in pharmaceuticals is a cause of concern for patients as well as for regulatory authorities. Since July 2018, the United States Food and Drug Administration (US FDA) and the European Medicines Agency (EMA) recalled a great number of medicinal products owing to the detection of NAs above their daily acceptable limit (Wichitnithad et al. 2021). In the light of recent events in NA-contaminated medicinal products, the global concern has raised beyond angiotensin II receptor blockers (so called “sartans”) to comprise the ranitidine. Ranitidine medicines are used for decreasing the amount of acid produced by the stomach in patients with conditions such as heartburn and gastric ulcers. On April 1, 2020, the US FDA and EMA decided to withdraw all prescription and over the counter (OTC) ranitidine drugs from the market due to the presence of N-nitrosodimethylamine (NDMA) impurity (Tuesuwan et al. 2021). There is a considerable literature on investigation into the possible root causes of the NDMA formation in ranitidine (Bharate et al. 2021; Tuesuwan et al. 2021; King et al. 2020; Yokoo et al. 2021; Aldawsari et al. 2021; Shaik et al. 2020). Moreover, regulatory authorities and research groups have developed a number of analytical methods aimed at determining accurate concentration of NA-contaminants below the US FDA and EMA interim limits of the NAs in both active pharmaceutic ingredients (APIs) and final products (FPs) using gas chromatography-mass spectrometry (GC-MS) with different analysers (single quadrupole and triple quadrupole) equipped with either a headspace (HS) system or direct injection, supercritical

Table 1. Molecular structures, CAS numbers and abbreviations of NAs covered in the article.

Analyte	Abbreviation	CAS Number	Molecular Structure
N-nitrosodimethylamine	NDMA	62-75-9	
N-nitrosodiethylamine	NDEA	55-18-5	
N-nitrosodiisopropylamine	DIPNA	601-77-4	
N-nitrosoethylisopropylamine	EIPNA	16339-04-1	
N-nitrosomethylphenylamine	NMPhA	614-00-6	
N-nitrosodibutylamine	NDBA	924-16-3	
N-nitrosoethylmethylamine	NMEA	10595-95-6	
N-isopropylmethylnitrosamine	NMIPA	4747-21-1	
N-nitrosopiperidine	NPIP	100-75-4	
N-nitrosopyrrolidine	NPyR	930-55-2	

fluid chromatography, LC coupled with high resolution mass spectrometer, LC-MS/MS and HPLC-UV (U.S. Food & Drug Administration 2019; Health Canada 2019; U.S. Food & Drug Administration 2018; EDQM 2019; Schmidtsdorff et al. 2019; Parra et al. 2019; Masada et al. 2019; Ngongang et al. 2015; Ngongang et al. 2015; Lim et al. 2020; Ripollés et al. 2011; AlShehri et al. 2020; Giménez-Campillo et al. 2020; Liu et al. 2021).

The aim of this study was to develop and validate a simple, robust, and sensitive dMRM-based method using LC-APCI-MS/MS for simultaneous determination of ten (Table 1) nitrosamines in API of ranitidine in combination with water-based extraction.

2. Materials and methods

2.1. Chemicals, reagents, and pharmaceutical

NDMA, NDEA (N-nitrosodiethylamine), NMBA (N-nitroso-N-methyl-4-aminobutyric acid), DIPNA (N-nitrosodiisopropylamine), NDBA (N-nitrosodibutylamine), NMPhA (N-nitrosomethylphenylamine), EIPNA (N-nitrosoethylisopropylamine), NMEA (N-nitrosoethylmethylamine), NMIPA (N-isopropylmethylnitrosamine), NPIP (N-nitrosopiperidine), NPyR (N-nitrosopyrrolidine) and NDMA-D6 were supplied by Toronto Research Chemicals (Toronto, Canada). NDBA-D18, NDEA-D10 and NMPhA-D5 were purchased from by Cambridge Isotope Laboratories, Inc. (Massachusetts, USA). LC-MS grade methanol (MeOH), LC-MS grade acetonitrile (ACN) and formic acid (purity >98%) were purchased from Merck (Darmstadt, Germany). Deionized (DI) water was prepared at our laboratory using Elga Purelab (High Wycombe, United Kingdom) water purification system. The API of ranitidine (as ranitidine hydrochloride) was provided by local pharmaceutical company.

2.1.1. Preparation of working solutions, standard solutions, and quality controls

The primary stock solutions of 1000 µg/mL of the ten NAs (NDMA NDEA, DIPNA, NDBA, EIPNA, NMEA, NMIPA, NPIP, NPyR and NMPhA in MeOH) were used for the preparation of intermediary stock solutions. The intermediary stock solution of NAs mix (10 µg/mL) and stable isotope-labelled standard (IS) mix composed of NDMA-D6, NDBA-D18, NDEA-D5 and NMPhA-D5 (1 µg/mL) were prepared in MeOH and stored in a freezer at -20 ºC**.** The working mix solution of NAs was prepared by dilution with DI water at a concentration of 1 g/mL from the intermediary stock solution and stored at -20 ºC. Calibration standard solutions were made by fortifying appropriate volumes of NA working mix solution to obtain ultimate concentrations of 2.5, 10, 25, 50, and 250 ng/mL in DI water, which were then used in calibrator preparation. Furthermore, quality control samples (QCs) were prepared by spiking appropriate volumes of the working mix solution of NAs (10 g/mL) in not spiked ranitidine API to obtain QCs at concentrations of 1 and 10 ng/mL.

2.2. Instrumentation and chromatographic conditions

Agilent HPLC system (Agilent Technologies, Santa Clara, CA, USA) with flexible pump (G7104A), column compartment (G7116B), and autosampler (G7129C) coupled to Agilent Ultivo triple quadrupole LC/MS (6465B, Agilent Technologies, Santa Clara, CA, USA) equipped with APCI source was used for the experiments. Positive chemical ionisation in dMRM mode was used to detect NAs in MS/MS. In comparison to MRM, dMRM has a related delta retention time window (RTW) that can be turned on and off dynamically without affecting the total cycle time. It enables the instrument to only acquire MRM data during the retention time window, maximizing dwell time and improving sensitivity. The NAs were separated chromatographically using an Agilent InfinityLab Poroshell HPH C18, 4.6 x 150 mm 2.7 m (P/N 693975-702, Agilent Technologies, Santa Clara, CA, USA) analytical column maintained at 50 °C. DI water containing 0.2% formic acid (mobile phase A, pH 3) and MeOH were used to make the mobile phase (mobile phase B). The gradient elution was programmed as follows: linear elution at the flow rate of 0.4 mL/min starting at 2% mobile phase B increasing to 22% over 4 min, then converting to 66% mobile phase B at 0.6 mL/min, afterwards increasing linearly from 66% to 96% mobile phase B within 4 min at the flow rate of 0.6 mL/min, subsequently turning to 100% mobile phase B and maintaining for 3 min at 0.6 mL/min, finally equilibrating to the initial condition for 6 min. The analysis was performed in a total run time of 17 min. The injection volume was arranged as 20 µL. Following each sample injection, the needle was washed with a solvent mixture of MeOH and DI water (80:20; v/v). The autosampler's temperature was set to 8 °C. The method's mass spectrometer settings were as follows:; drying gas temperature 300 ºC, drying gas flow 6 L/min, nebulizer pressure 55 psi, vaporizer temperature 350 ºC, corona current 4 µA and capillary voltage 3000 V. Product ion transitions created by collision-induced dissociation (CID) of the corresponding precursor ion were used to detect NAs and ISs in MS/MS. NAs and IS dMRM transitions were studied at optimum fragmentation voltages (FV), which represented a common value for each precursor ion-product ion mass transition, and optimum collision energies (CE), which indicated a specific value for each product ion in terms of voltage (V) unit. Table 2 also shows the retention times (RTs) of NAs with a fixed RTW of 0.8 min for each analyte in dMRM mode. The analytes were quantified using calibration curves established on the calibrator concentrations, with matrix effect compensation based on the yields of the assigned ISs. Agilent MassHunter Acquisition (version 1.2), Agilent MassHunter Qualitative Analysis (version 10.0), and Agilent MassHunter Quantitative Analysis (version 10.1) software programs were used for data acquisition, qualification, and quantification, respectively.

Table 2. Mass transitions of analytes, ISs, RTs and MS/MS conditions

Compound Name	RT[1] (min.)	Precursor Ion (m/z)	Product[2] Ion(s) (m/z)	FV[3] (V)	CE[2,4] (V)	Polarity
NDBA	9.4	159	57/41	90	12/17	Positive
NDEA	7.7	103.1	75/47.1	80	8/15	Positive
DIPNA	8.3	131.1	89.1/43.1	60	4/10	Positive
NDMA	5.4	75	58/43	80	8/15	Positive
EIPNA	8.0	117	75/47	90	12/17	Positive
NMIPA	7.7	103.1	61/43	60	6/12	Positive
NMEA	7.0	89.1	61/43	70	9/9	Positive
NMPhA	8.3	137	107/65.8	80	10/21	Positive
NPIP	7.8	115.1	69/41	90	12/17	Positive
NPyR	7.1	101.1	55	90	15	Positive
NDMA-D6 (IS)	*5.4*	*81.1*	*64/46.1*	*80*	*12/17*	*Positive*
NDBA-D18 (IS)	*9.3*	*177.2*	*66.1*	*35*	*13*	*Positive*
NDEA-D10 (IS)	*7.7*	*113.1*	*81*	*45*	*10*	*Positive*
NMPhA-D5 (IS)	*8.3*	*142.1*	*71*	*45*	*22*	*Positive*

[1] Retention times acquired in dMRM mode
[2] Mass transitions as product ions are shown respectively with corresponding CE value
[3] FV: Fragmentor voltage
[4] CE: Collision energy

2.3. Sample preparation procedure

A 50 mg ranitidine API was weighted in a glass tube. Then, 250 µL of IS mixture was pipetted to the tube and vortex for 5 sec. Next, 2250 µL of DI water was added to the tube and agitated for 5 min. at room temperature. Following the extraction step, the solution was filtered through a 0.45 µm regenerated cellulose membrane prior to injection.

2.4. Quantification of NAs in prepared ranitidine API

In an HPLC vial, 100 µL of calibration standard solutions (prepared in section 2.1.1.) were transferred. Following that, 50 µL of IS mixture was added and swirled for 5 sec. Before injecting, 350 µL of DI water was added and vortexed for 5 seconds. After the injections were completed, a five-point calibration batch was established in the ranges of 0.5, 2, 5, 10, and 50 ng/mL for the quantification of NAs extracted from ranitidine API. The calibration curve was built using the peak area ratios of the standards to the assigned ISs. To achieve accurate quantification, great care was taken in assigning ISs that were not stable isotope forms of corresponding analytes. For API of ranitidine, *NDEA-D10* was selected as IS for NMEA, NMIPA, NPIP and NPyR; *NDBA-D18* was selected as IS for EIPNA. *NDMA-D6* was assigned as IS for DIPNA (Table 3).

Table 3. Assigned ISs for Nas.

Compound Name	API[1] of ranitidine
NDBA	*NDBA-D18*
NDEA	*NDEA-D10*
DIPNA	*NDMA-D6*
NDMA	*NDMA-D6*
EIPNA	*NDBA-D18*
NMIPA	*NDEA-D10*
NMEA	*NDEA-D10*
NMPhA	*NMPhA-D5*
NPIP	*NDEA-D10*
NPyR	*NDEA-D10*

[1] API: Active pharmaceutic ingredient

3. Method Validation

3.1. System Suitability

To evaluate the system suitability before validation experiments and impurity determination, the chromatographic system was checked in terms of reproducibility. For this reason, a solution containing a mixture of the lowest level of ten NAs was prepared. The system suitability solution was injected with six replicates. The system reproducibility was assessed by the variation of peak area (RSD % <9) and retention time (RSD % <1) to indicate the precision of injections.

3.2. Linearity and sensitivity

The linearity of the method was determined by analysing of ten NAs at 5 calibration points in the range of 0.5-50 ng/mL. The intercept, slope, and correlation coefficient (R^2) were evaluated by linear regression data analysis and fitting to a linear regression model with a weighting factor of 1/x. The correlation coefficient was ≥0.994 for all the calibration curves of NAs. For evaluation of the sensitivity of analytical method, limit of detection (LOD) and limit of quantitation (LOQ) were determined according to ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) validation of analytical procedures (CPMP/ICH/381/95), based on the standard deviations at low concentrations. Limit of detection for this method was determined from repeated analyses of neat solution at lowest concentration. The Lowest NAs concentration that could be measured with 99% confidence as 3 times the standard deviation was taken as the LOD. The experiments were repeated ten times (n= 10). The correlation coefficient, LOD and LOQ for each NAs are described in Table 4.

Table 4. Linearity, LOD and LOQ result of NAs.

Compound name	R^2	*LOD* (ng/mL)	*LOQ* (ng/mL)
NDBA	0.998	0.124	0.413
NDEA	0.998	0.065	0.210
DIPNA	0.994	0.076	0.254
NDMA	0.994	0.066	0.220
EIPNA	0.997	0.142	0.472
NMIPA	0.997	0.072	0.240
NMEA	0.998	0.175	0.582
NMPhA	0.995	0.134	0.445
NPIP	0.997	0.105	0.349
NPyR	0.998	0.092	0.308

3.3. Accuracy and precision

Considering the analytical range, two concentration levels of samples were used for accuracy and precision: low-level and high-level spiked samples (referred to as LLQC and HLQC). Accuracy was performed ten replicates of LLQC (1 ng/mL) and HLQC (10 ng/mL) samples. Accuracy was expressed as percentage (recovery %) of the nominal concentration. The results are summarized in Table 5. The method precision was evaluated by repeatability (intra-day) and intermediate precision (inter-day). The intra-day precision was measured by comparing the relative standard deviation (RSD %) of ten replicates of LLQC and HLQC during the same day. The inter-day precision was measured by comparing the percent relative standard deviation (RSD %) of six replicates of LLQC and HLQC on three consecutive days (Table 6).

Table 5. Accuracy values of NAs.

Compound name	**LLQC**[1] *n=10* **Recovery %**	**HLQC**[2] *n=10* **Recovery %**
NDBA	111.9	101.5
NDEA	108.9	107.7
DIPNA	83.1	90.7
NDMA	104.9	101.2
EIPNA	85.5	88.4
NMIPA	110.3	101.7
NMEA	88.8	92.8
NMPhA	105.9	105.7
NPIP	108.7	109.5
NPyR	101.0	101.7

[1]Low-level quality control (LLQC): 1 ng/mL
[2]High-level quality control (HLQC): 10 ng/mL

Table 6. Intra-day and inter-day precision of NAs.

Compound name	**Intra-day assay** *RSD % (n=10)*		**Inter-day assay** *RSD % (n=6x3)*	
	LLQC[1]	**HLQC**[2]	**LLQC**	**HLQC**
NDBA	2.44	4.27	5.94	3.96
NDEA	3.31	2.41	6.22	2.14
DIPNA	3.44	2.95	5.74	3.44
NDMA	4.83	1.96	5.85	1.76
EIPNA	6.15	5.89	8.91	5.80
NMIPA	2.64	2.72	4.21	2.43
NMEA	5.36	4.33	8.51	5.28
NMPhA	2.69	5.46	7.19	5.07
NPIP	3.97	2.67	4.56	2.34
NPyR	6.84	4.78	7.39	4.13

[1]Low-level quality control (LLQC): 1 ng/mL
[2]High-level quality control (HLQC): 10 ng/mL

3.4. Carry-over

The carry-over effect of the method was assessed by running the blank sample (MeOH) after the analysis of the highest concentration of the calibration curve. The response in the blank sample obtained after the highest concentration standard was not greater than 20% of the analyte response and not greater than 5% of the internal standard response.

3.5. Robustness

The robustness of the developed method was evaluated to investigate any influence on the peak retention and peak area by changing the volume of acidic additive for both of mobile phases (0.1 and 0.2 % formic acid). The results showed that the mobile phase conditions had only a slight effect on the peak retention time and all analytes were within acceptable RSD % (< 15%), indicating the robustness of the proposed method. With this in mind, ΔRTW values of NAs were adjusted 0.8 min. in dMRM mode.

4. Results and discussion

The APCI source, creating high signals for all NAs, was selected as the ionization source for the determination of NAs. Scan and MS/MS spectra were acquired from direct infusion of 10 µg/mL of each NAs and ISs (prepared in MeOH) at a flow rate of 0.3 mL/min, in positive mode with APCI. By using these solutions, MS conditions including MRM transition, drying gas temperature and flow, nebulizer pressure, vaporizer temperature, corona current and capillary voltage were individually optimized. To improve sensitivity, dMRM mode which is centred on individual retention time windows for each MRM transition was performed. The dMRM method is allowed the collection of satisfactory data points to provide an excellent quantitative accuracy with better precision. After optimizing MS/MS parameters, to identify the most suitable analytical column for separation of NAs, Poroshell HPH C18 (4.6 x 150 mm, 2.7 µm, Agilent, USA) and Poroshell HPH C18 (2.1 x 100 mm, 1.9 µm, Agilent, USA) were tested utilizing 100 ng/mL of NAs mixture prepared in MeOH. During the LC optimization process, different mobile phase combinations such as DI Water containing formic acid and buffers such as ammonium formate prepared at several concentrations (as mobile phase A), and ACN or MeOH (as mobile phase B) were experimented for best peak shape and peak area. Usage of an acidic content in mobile phase A (adjusted pH=3) proved to be fitted in terms of retention and separation of NAs. As the dMRM chromatogram shown in Fig. 1, Poroshell HPH C18 column ensured sufficient resolution of NAs in total run time of 17 min. Sample preparation procedure was optimized for ranitidine API sample as hydrochloride salt form. Extraction recoveries were evaluated by comparing the analyte responses observed in spiked sample (1 and 10 ng/mL). Peak shape, symmetry and baseline resolution were examined through the optimization of extraction procedures. In this context, ACN, MeOH and DI water with consisting of formic acid were tested as extraction solvents. Among these extraction solvents, DI water showed better resolution and recovery % by applying the developed method. NDMA was detected in not spiked API of ranitidine at a concentration of 7.9 ng/mL (Fig. 2). Besides, the analysis of low level-spiked (1 ng/mL) ranitidine API were demonstrated excellent detection responses for the rest of NAs (Fig. 3).

In the literature, liquid chromatography high resolution mass spectrometry (LC-HRMS) and LC-MS/MS-API (U.S. Food & Drug Administration 2019)-based methods were developed and validated for the detection and quantitation of NDMA in ranitidine drug substance and drug product. The U.S. FDA introduced these analytical procedures for ranitidine API (LOD 0.3 ng/mL). Besides that, Lima HH et al. (2020) reported a GC-MS/MS method for quantifying NDMA and NDEA in ranitidine (LOD 0.3 µg/kg and 0.07 µg/kg, respectively) with solid phase extraction (SPE). AlShehri YM et al. (2020) established a method for ranitidine based on headspace solid-phase microextraction (SPME) GC-MS technique for the determination of NDMA with LOD of 1.0 µg/L. Giménez-Campillo C et al. (2020) developed a method for the determination of nine volatile NAs in ranitidine pharmaceutical products using GC-MS in combination with dispersive liquid-liquid microextraction (DLLME) preconcentration technique. The LODs determined for all analytes in ranitidine ranged from 0.07 to 6.6 ng/g. In another literature, NDMA was measured by ESI-LC-MS/MS in ranitidine products with LOD 1.0 ng/mL. This study was demonstrated highly sensitive and selective method using a simple sample preparation procedure compared to the other methods (summarized in Table 7). U.S. FDA and EMA guidelines were taken into account to validate the performance of the developed LC-MS/MS method. The calibration curves for all NAs showed to be linearity in the analytical range 0.5-50 ng/mL with high correlation coefficients ($R^2 \geq 0.994$). LOD and LOQ levels of all NAs were calculated to range between 0.06–0.17 and 0.21–0.58 ng/mL, respectively. Intra-day and inter-day precision were tested at low- and high-level quality control samples. The method proved to be definite with RSD % values-based between 1.9-6.8 % (intra-day) and 1.7–8.9% (inter-day). The recovery of all NAs in API ranitidine was within 83.-111.9 % at LLQC and HLQC.

In the literature, various sample preparation methods such as liquid-liquid extraction (LLE) (Campillo et al. 2021), solid-phase extraction (SPE) (Lim et al. 2020) and solid phase microextraction (SPME) (AlShehri et al. 2020) methods have been developed for the extraction and preconcentration of nitrosamines. Therefore, the sample preparation procedures for the analysis of nitrosamines require excess solvent consumption and time costs. Contrary to above mentioned methods, the developed method in this article is based on “extract and shoot” approach that can detect low levels of the selected nitrosamines by LC-MS/MS with simple sample preparation providing less solvent consumption. In addition, the proposed method in this paper covers ten nitrosamines compared to the limited analyte list of literature (Liu et al. 2021; Campillo et al. 2021; Lim et al. 2020; AlShehri et al. 2020).

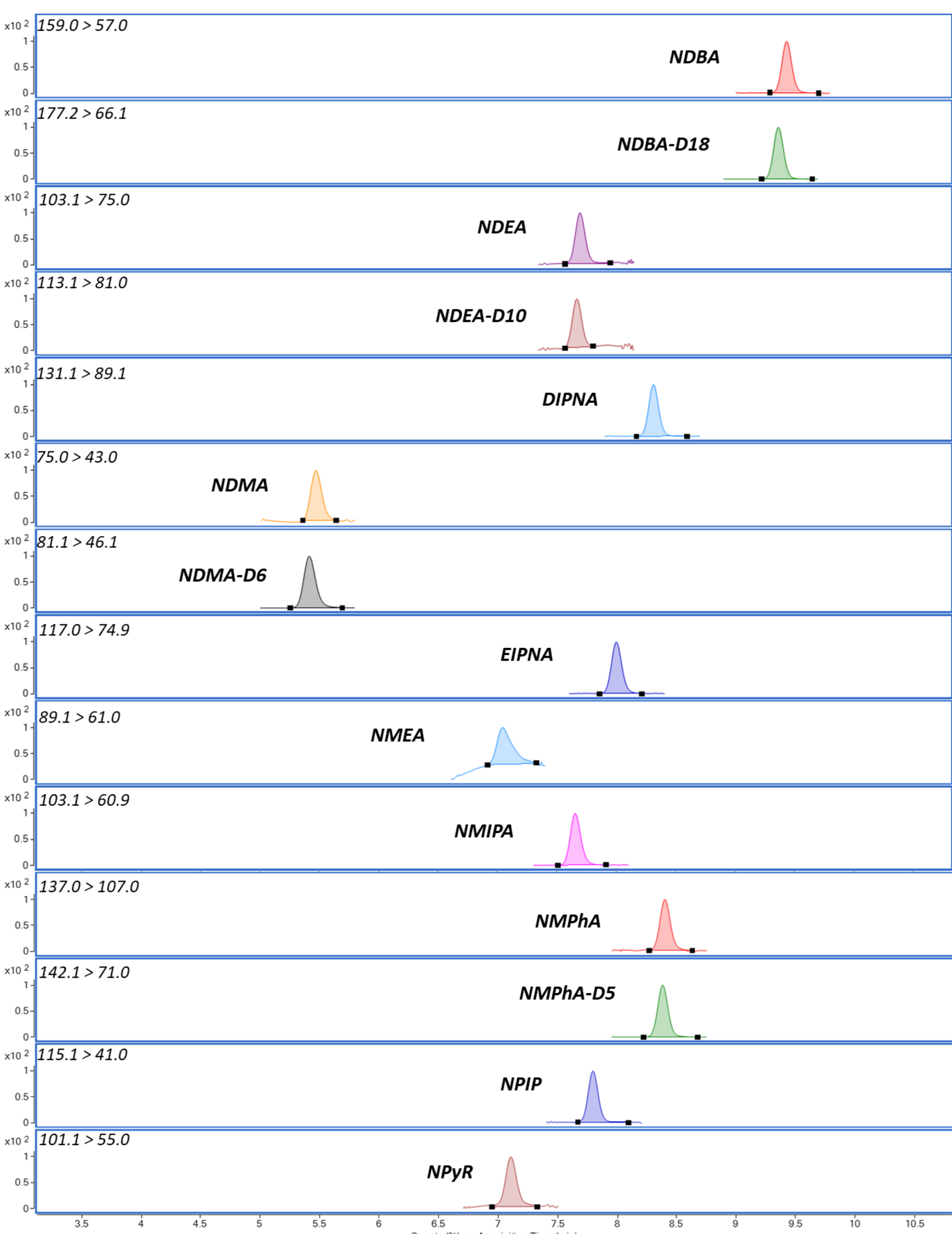

Fig. 1. Extracted dMRMs of NAs obtained from Poroshell HPH C18 column.

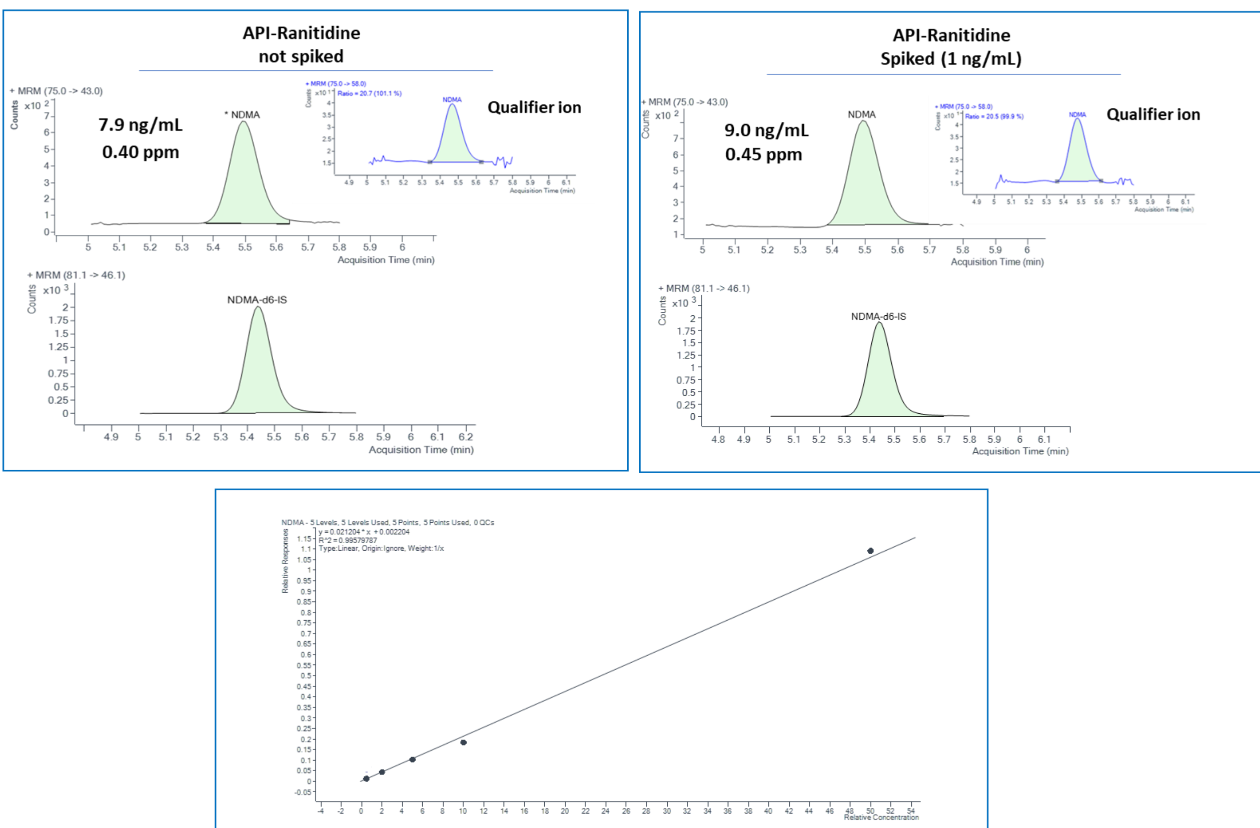

Fig. 2. The calibration curve for NDMA in not spiked and spiked (spiked with 1 ng/mL) API of ranitidine.

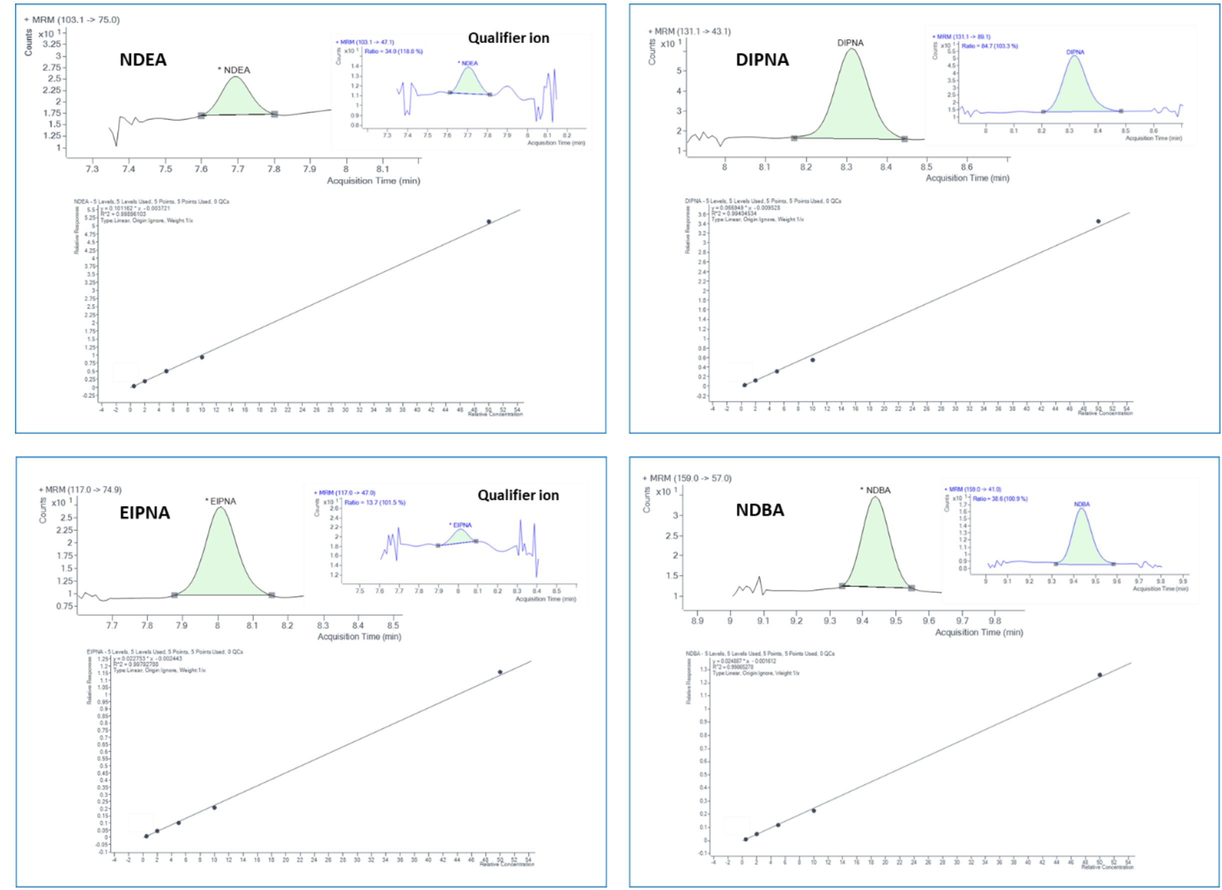

Fig. 3. The chromatograms and calibration curves of selected NAs (NDEA, DIPNA, EIPNA and NDBA) in 1 ng/mL spiked API of ranitidine.

Table 7. LOD values for nitrosamines to make comparison with other studies in literature.

Method	NDMA	NDEA	EIPNA	DIPNA	NDBA	NPIP	NPyR	NDPhA	NMEA	NMIPA	Ref.
LC-APCI-MS/MS[a]	0.066 ng/mL	0.065 ng/mL	0.142 ng/mL	0.076 ng/mL	0.124 ng/mL	0.105 ng/mL	0.092 ng/mL	0.134 ng/mL	0.175 ng/mL	0.072 ng/mL	This study
SPE-GC-MS/MS[b]	0.3 µg/kg	0.07 µg/kg	-	-	-	-	-	-	-	-	Lim 2020
LC-HRMS[c]	0.32 ng/mL	-	-	-	-	-	-	-	-	-	U.S. FDA 2019
LC-MS/MS[d]	0.3 ng/mL	-	-	-	-	-	-	-	-	-	U.S. FDA 2019
HS-SPME-GC-MS[e]	1.0 µg/L	-	-	-	-	-	-	-	-	-	AlShehri 2020
DLLME-GC-MS[f]	6.6 ng/g	0.29 ng/g	-	-	0.35 ng/g	0.47 ng/g	2.5 ng/g	0.07 ng/g	3.5 ng/g	-	Campillo 2021
LC-ESI-MS/MS[g]	1.0 ng/mL	-	-	-	-	-	-	-	-	-	Liu 2021

[a] LC-APCI-MS/MS: Liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry detection.
[b] SPE-GC-MS/MS: Solid phase extraction-gas chromatography–tandem mass spectrometry detection.
[c] LC-HRMS: Liquid chromatography-high resolution mass spectrometry detection.
[d] LC-MS/MS: Liquid chromatography- tandem mass spectrometry detection.
[e] HS-SPME-GC-MS: Headspace-solid-phase microextraction-gas chromatography mass spectrometry detection.
[f] DLLME-GC-MS: Dispersive liquid-liquid microextraction-gas chromatography mass spectrometry detection.
[g] LC-ESI-MS/MS: Liquid chromatography-electrospray ionization--tandem mass spectrometry detection.

5. Conclusion

In this work, an LC-APCI-MS/MS based method was developed by performing dMRM mode for the quantification of NDMA, NDEA, DIPNA, NDBA, NMPhA, EIPNA, NMEA, NMIPA, NPIP and NPyR in a single run for API of ranitidine. The dMRM significantly extended the dwell time, which provided much higher sensitivity and reproducibility than MRM. The developed method was validated with respect to the Q2(R1) ICH guidelines and ensured satisfying results of accuracy, precision, and other validation parameters. The LOQ and LOD of the proposed method were far below the US FDA and EMA interim limits of the corresponding NAs in ranitidine. This method could be modified for the simultaneous analysis of additional NAs in other type of drugs. However, the limitation is due to the fact that drugs not experienced in this study may cause unknown matrix effects over the chromatographic region which could not be compensated by the content of IS mixture. To minimize the risk of inaccuracy, future studies should address expanding the IS content placing corresponding stable isotope of target NAs.

Declaration of interests

☒ 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.

Conflict of Interest

- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

Contributions of the Authors to the Research

NUC, corresponding author, contributed to the research in the terms of methodology, software, investigation, writing draft, reviewing, and editing.
MEM contributed to the research in the terms of methodology, software, investigation, writing draft, reviewing, and editing.
GGG contributed to the research in the terms of methodology, software, investigation, writing draft reviewing, and editing.

References

Aldawsari FS, Alshehry YM, Alghamdi TS. 2021. N-nitrosodimethylamine (NDMA) Contamination of Ranitidine Products: A Review of Recent Findings. J Food Drug Anal. 29(1): 39–45.

AlShehri YM, Alghamdi TS, Aldawsari FS. 2020. HS-SPME-GC-MS as an Alternative Method for NDMA Analysis in Ranitidine Products. J Pharma Biomed Anal. 191:N113582.

Bharate SS. 2021. Critical Analysis of Drug Product Recalls due to Nitrosamine Impurities. J Med Chem. 64, 2923-2936.

Campillo CG, Belda MP, Campillo N, Hernandez-Cordoba M. 2021. Development of a new methodology for the determination of N-nitrosamines impurities in ranitidine pharmaceuticals using microextraction and gas chromatography-mass spectrometry. Talanta, 223: 121659.

Giménez-Campillo C, Pastor-Belda M, Campillo N, Hernández-Córdoba M, Viñas, P. 2020. Monitoring N-Nitrosamines impurities in ranitidine pharmaceuticals using microextraction and gas chromatography-mass spectrometry. Talanta. 121659.

Health Canada. 2019. Impurities found in certain angiotensin II receptor blocker (ARB) products, also known as Sartans. Information update. Available at: https:// www.canada.ca/en/health-canada/services/drugs-health-products/complianceenforcement/ information-health-product/drugs/angiotensin-receptor-blocker. html. Accessed January 7, 2021.

King FJ, Searle AD, Urquhart MW. 2020. Ranitidine-Investigations into the Root Cause for the Presence of

N-Nitroso-N,N-dimethylamine in Ranitidine Hydrochloride Drug Substances and Associated Drug Products. Org. Process Res. Dev. 24:2915–2926.

Lim HH, Oh YS, Shin HS. 2020. Determination of N-nitrosodimethylamine and N-nitrosomethylethylamine in drug substances and products of sartans, metformin and ranitidine by precipitation and solid phase extraction and gas chromatography–tandem mass spectrometry. J Pharma Biomed Anal. 189:113460.

Liu J, Zhao Z, Yang X., Jin Y, Liu X, Wang C, Zhang Z. 2021. Determination of N-nitrosodimethylamine in Ranitidine Dosag Forms by ESI-LC-MS/MS; Applications for Routine Laboratory Testing. Iran J Pharm Res. 20 (4): 255-264.

Masada S, Tsuji G, Arai R, Uchiyama N, Demizu Y, Tsutsumi T, Abe Y, Akiyama H, Hakamatsuka T, Izutsu K, Goda Y, Okuda H. 2019. Rapid and efficient high performance Liquid chromatography analysis of N-nitrosodimethylamine impurity in valsartan drug substance and its products. Sci Rep. 9:11852.

Ngongang AD, Duy SV, Sauve S. 2015. Analysis of nine N-nitrosamines using liquid chromatography-accurate mass high resolution mass spectrometry on a Q-Exactive instrument. Anal Methods. 7(14):5748–5759.

Parra MK, Josepha JF. 2019. NDMA impurity in valsartan and other pharmaceutical products:Analytical methods for the determination of N-nitrosamines J Pharma Biomed Anal. 164:536–549.

Ripollés C, Pitarch E, Sancho JV, López FJ, Hernández F. 2011. Determination of eight nitrosamines in water at the ng L1 levels by liquid chromatography coupled to atmospheric pressure chemical ionization tandem mass spectrometry. Anal Chim Acta. 702:62e71.

Schmidtsdorff S, Schmidt AH. 2019. Simultaneous detection of nitrosamines and other sartan-related impurities in active pharmaceutical ingredients by supercritical fluid chromatography. J Pharma Biomed Anal. 174:151–160.

Shaik KM, Sarmah B, Wadekar GS, Kumar P. 2020. Regulatory Updates and Analytical Methodologies for Nitrosamine Impurities Detection in Sartans, Ranitidine, Nizatidine, and Metformin along with Sample Preparation Techniques. Crit Rev Anal Chem. 52(1): 53-71.

The European Directorate for the Quality of Medicines & HealthCare (EDQM). 2019. LC-MS/MS method for LOSARTAN potassium. Test method. Available at: https://www.edqm.eu/sites/default/files/de_lgl_losartan_method_parameters_nm ba_lcms.pdf. Accessed Accessed January 7, 2021.

Tuesuwan B, Vongsutilers V. 2021. Nitrosamine Contamination in Pharmaceuticals: Threat, Impact, and Control. J Pharm Sci. 110:3118−3128.

U.S. Food & Drug Administration. 2018. Combined direct injection N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) impurity assay by GC/MS. Test method. Available at: https://www.fda.gov/media/117807/download. Accessed January 7, 2021.

U.S. Food & Drug Administration. 2019. Combined N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) impurity assay by GC/MS-headspace. Test method. Available at: https://www.fda.gov/media/117843/download. Accessed January 7, 2021.

U.S. Food & Drug Administration. 2019. GC/MS headspace method for detection of NDMA in valsartan drug substance and drug products. Test method. Available at: https://www.fda.gov/media/115965/download. Accessed January 7, 2021.

U.S. Food & Drug Administration. 2019. Liquid chromatography-high resolution mass spectrometry (LC-HRMS) method for the determination of NDMA in ranitidine drug substance and drug product. Test method. Available at: https://www.fda.gov/media/130801/download. Accessed December 1, 2020.

U.S. Food & Drug Administration. 2019. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of NDMA in ranitidine drug substance and solid dosage drug product. Test method. Available at: https://www.fda.gov/media/131868/download. Accessed December 1, 2020.

Wichitnithad W, Sudtanon O, Srisunak P, Cheewatanakornkool K, Nantaphol S, Rojsitthisak P. 2021. Development of a Sensitive Headspace Gas Chromatography−Mass Spectrometry Method for the Simultaneous Determination of Nitrosamines in Losartan Active Pharmaceutical Ingredients. ACS Omega. 6:11048−11058.

Yokoo H, Yamamoto E, Masada S, Uchiyama N, Tsuji G, Hakamatsuka T, Demizu Y, Izutsu K, Goda Y. 2021. N-Nitrosodimethylamine (NDMA) Formation from Ranitidine Impurities: Possible Root Causes of the Presence of NDMA in Ranitidine Hydrochloride. Chem Pharm Bulletin. 69: 872–876.