APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Determination of Nitrosamine Impurities in Active Pharmaceutical Ingredient (API) Using QSight 220 LC-MS/MS

Introduction

Nitrosamines are a well-known group of highly potent, mutagenic impurities formed by the reaction of secondary amines with nitrite under acidic conditions[1-4]. In pharmaceutical drug substances, the formation of nitrosamines is possible in the presence of secondary, tertiary, or quaternary amines and nitrite salts under acidic reaction conditions. Under these conditions, nitrite salts may form nitrous acid, which can react with an amine to form a nitrosamine. Additional sources of nitrosamines in pharmaceutical drug substances includes the use of recovered solvents or equipment contaminated with N-nitrosamines formed outside of the declared synthetic process[2,3]. Evidence of carcinogenicity associated with nitrosamines was briefly summarized by the National Toxicology Program in the 14th report on carcinogens[1]. The most probable nitrosamine impurities are N-Nitroso-di-methyl amine (NDMA, CAS No. 62-75-9), N-Nitroso-di-ethyl amine (NDEA, CAS No. 55-18-5), N-nitroso-N-methyl-4-aminobutyric acid (NMBA, CAS No. 61445-55-4), N-nitroso-di-butylamine (NDBA, CAS No. 924-16-3), N-nitroso-di-isopropyl amine (NDIPA, CAS No. 601-77-4), and N-ethyl-n-nitroso-2-propanamine (NEIPA, CAS No. 16339-04-1).

In mid-2018, nitrosamine impurities were observed for the first time in Valsartan drugs, which are used to treat high blood pressure and congestive heart failure[2]. The US Food

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and Drug Administration (US FDA) and European Medicines Agency (EMA) made an announcement in July 2018 regarding the presence of NDMA and NDEA in generic drug substance and drug products, especially in angiotensin II receptor blockers (ARBs) also known as sartan family drugs[2,4]. The ICH guideline, ICH M7 (R1) classified these compounds in Class 1, which is known to be mutagenic and carcinogenic to the human body with high risk[5]. Based on available toxicity data, acceptable daily intake for these impurities, which has been adopted by most of the regulatory bodies, is 96 ng/day for NDMA and NMBA, and 26.5 ng/day for NDEA, NDBA, NDIPA and NEIPA[4].

For safety assessment and control of the most probable impurities in pharmaceutical drug substances and products, it is critical to have a specific, sensitive, and reliable method[5-7]. This application note describes an efficient, sensitive and reproducible method developed using the PerkinElmer QSight 220® LC/MS/MS for the detection and quantification of NDMA, NDEA, NMBA, NDBA, NDIPA and NEIPA impurities in sartan and metformin active pharmaceutical ingredients (APIs). This work demonstrates that all six nitrosamine impurities can be determined at the lowest concentration limits set by US and European regulatory agencies.

Experimental

Hardware/Software

Chromatographic separation was carried out by a PerkinElmer LX50 UHPLC system, with subsequent detection achieved using a PerkinElmer QSight 220 triple quadrupole mass spectrometer equipped with an APCI ionization source. All instrument control, data acquisition, data processing and result reporting were performed using Simplicity 3Q™ software. The QSight system with photodiode array (PDA) detector and diverter valve was used to determine the elution time of the APIs and nitrosamine impurities during the development stage to avoid blocking of the APCI source needle. The PDA detector may be removed once the time window is set in the MS method parameter for diverting the main API to waste through the diverter valve. This is necessary to keep the mass spectrometer clean for extended periods of time, thus minimizing down time.

Method Parameters

LC parameters, including column and mobile phase gradient program, are given in Table 1. The MS/MS parameters are given in Table 2. The MRM transition parameters for all targeted analytes are listed in Table 3.

Table 1. LC Parameters.

LC Conditions				
LC Column	C18, 50 x 4.6 mm, 3 µm			
Mobile Phase A	0.1% Formic acid in water			
Mobile Phase B	0.1% Formic acid in Methanol			
Mobile Phase Gradient	Sr. No	Time	%A	%B
	1	0.00	90	10
	2	0.87	90	10
	3	4.04	45	55
	4	9.81	45	55
	5	9.87	10	90
	6	12.12	10	90
	7	12.87	90	10
	8	14.00	90	10
Column Oven Temperature	45 °C			
Auto sampler Temperature	10 °C			
Injection Volume	40 µL			
Flow	1 mL/ min			
Run Time	14 min			

Table 2. MS/MS Source Parameters

Parameter	Setting Value
APCI Corona Discharge Current (Positive)	3 µA
Drying Gas	120
Nebulizer Gas	350
Source Temperature	450 °C
HSID Temperature	250 °C

Table 3. MRM transition parameters for the six nitrosamine analytes.

Sr. No	Name	Q1 Mass	Q2 Mass	Entrance Voltage	Collision Cell Lens 2 Voltage	Collision Energy
1	NDMA-1	75.1	43.1	10	-21	-22
2	NDMA-2	75.1	58.1	18	-25	-16
3	NDEA-1	103	75.1	9	-25	-15
4	NDEA-2	103	47	18	-25	-24
5	NDBA-1	159.1	103.1	15	-37	-15
6	NDBA-2	159.1	57.2	8	-37	-19
7	NDBA-3	159.1	41.2	12	-33	-31
8	NDIPA-1	131.1	89.1	15	-29	-12
9	NDIPA-2	131.1	43.1	12	-29	-24
10	NDIPA-3	131.1	47.1	4	-21	-22
11	NEIPA-1	117.1	75.1	12	-29	-13
12	NEIPA-2	117.1	43	10	-25	-22
13	NEIPA-3	117.1	29.1	13	-29	-23
14	NMBA-1	147.1	44.1	9	-28	-11
15	NMBA-2	147.1	87.2	9	-29	-11
16	NMBA-3	147.1	117.2	8	-68	-3

Materials and Methods

Solvents and Standards

All the nitrosamine impurity standards (NDMA, NDEA, NDBA, NDIPA, NEIPA and NMBA) used in this study were obtained from Cleanchem laboratories. LC/MS grade solvents and reagents were used in the preparation of solutions.

Stock Solutions and Calibration Standards

The stock solutions and calibration standards were prepared as follows:

- Individual standard stock solution of NDMA, NDEA, NDBA, NDIPA NEIPA (100 ppm) and NMBA (500 ppm): 10 mg of each individual impurity (NDMA, NDEA, NDBA, NDIPA and NEIPA) standard was weighed and transferred into a 100 mL volumetric flask. The impurity standard was dissolved and diluted up to the mark using methanol as the diluent. Similarly, the NMBA individual standard stock solution of 500 ppm concentration was prepared.
- Mix standard stock solution-1 (1000 ppb): The 1.0 mL of individual standard stock solution (100 ppm) was further diluted to 100 mL in a volumetric flask using acidified water as the diluent.
- Standard stock solution-2 (100 ppb): The 1.0 mL of mix standard stock solution-1 (1000 ppb) was diluted to 10 mL in a volumetric flask using acidified water as the diluent.
- Working Level standard solution (10 ppb): The 1.0 mL of standard stock solution-2 (100 ppb) was diluted to 10 mL in a volumetric flask using acidified water as the diluent.
- Calibration standard solutions: The stock solution was serially diluted with LC/MS grade water to prepare calibration standards ranging from 0.1 ppb to 100.0 ppb for all five nitrosamine target analytes, except for NMBA. The calibration standard concentrations for NMBA were five times higher in comparison to the other five nitrosamine target analytes.

Sample Preparation

The study was conducted using Irbesartan and Metformin APIs. The API sample of 30,000 ppm concentration was prepared in water diluent. The sample solution was filtered through a 0.2 µm nylon filter, and transferred to an amber colored LC vial for LC/MS/MS analysis. Recovery for all target analytes at different concentration levels was measured using an API solution at 30,000 ppm concentration.

Results and Discussion

A single LC/MS/MS method was developed for six nitrosamine impurities. Specificity is the critical factor in this analysis, which decides the ruggedness of the method developed. The specificity parameter, as defined by separation between API and the closely eluting nitrosamine target analyte, was optimized using a C18 column and gradient program. The method performance was established based on the limit of detection (LOD), limit of quantitation (LOQ), linearity, reproducibility and recovery. The developed method covers a linearity range from 0.1 ppb to 100 ppb (0.1 ppb, 0.3 ppb, 0.5 ppb, 1.0 ppb, 5.0 ppb, 10 ppb, 20 ppb, 50 ppb and 100 ppb). All six nitrosamine impurities show linear response over the concentration range from 0.1 to 100 ppb, with correlation coefficient (R^2) values greater than 0.99 for all analytes. The results of the LOD, LOQ, and linearity are summarized in Table 4. The representative calibration curves for all six nitrosamine target analytes are shown in Figure 1.

The area response reproducibility data for all six nitrosamine impurities are given in Table 5. The method shows excellent repeatability results at 1 ppb concentration level with peak area RSDs less than 10% for six injections.

The spike recovery study was performed at six different concentrations: level-1 (0.5 ppb), level-2 (1.0 ppb), level-3 (2.0 ppb), level-4 (6.0 ppb), level-5 (10 ppb), and level-6 (15 ppb). The spike recovery was tested with six replicates for all of 6 nitrosamines. The average recovery results for analytes were in the range of 70-120%, with peak area RSDs less than 15%, as shown in Tables 6 and 7 for Irbesartan and Metformin API, respectively.

The API sample concentration of 30,000 ppm of Irbesartan and Metformin was used to comply, as per set regulatory limits. PerkinElmer QSight 220 LC/MS/MS is capable of low limits of detection, with respect to sample concentration based on the daily dose. Low limits of detection can be achieved by increasing the sample size, concentration and injection volume, provided specificity parameters could be passed.

Table 4. Result summary for LOD, LOQ, coefficient of regression, and calibration curve range.

Compound	Neat Standard Concentration			With respect to sample Concentration			Correlation Coeff. (R^2)
	LOD (ppb)	LOQ (ppb)	Linearity (ppb)	LOD (ppb)	LOQ (ppb)	Linearity (ppb)	
NDMA	0.1	0.2	0.1-100	4.0	8.0	4.0-3000	0.998
NDEA	0.1	0.3	0.1-100	3.0	12.0	3.0-3000	0.999
NDBA	0.1	0.3	0.1-100	4.0	10.0	4.0-3000	0.997
NDIPA	0.1	0.3	0.1-100	4.0	9.0	4.0-3000	0.999
NEIPA	0.1	0.3	0.1-100	3.0	10.0	3.0-3000	0.997
NMBA	0.3	0.5	0.3-500	9.0	18.0	9.0-16000	0.992

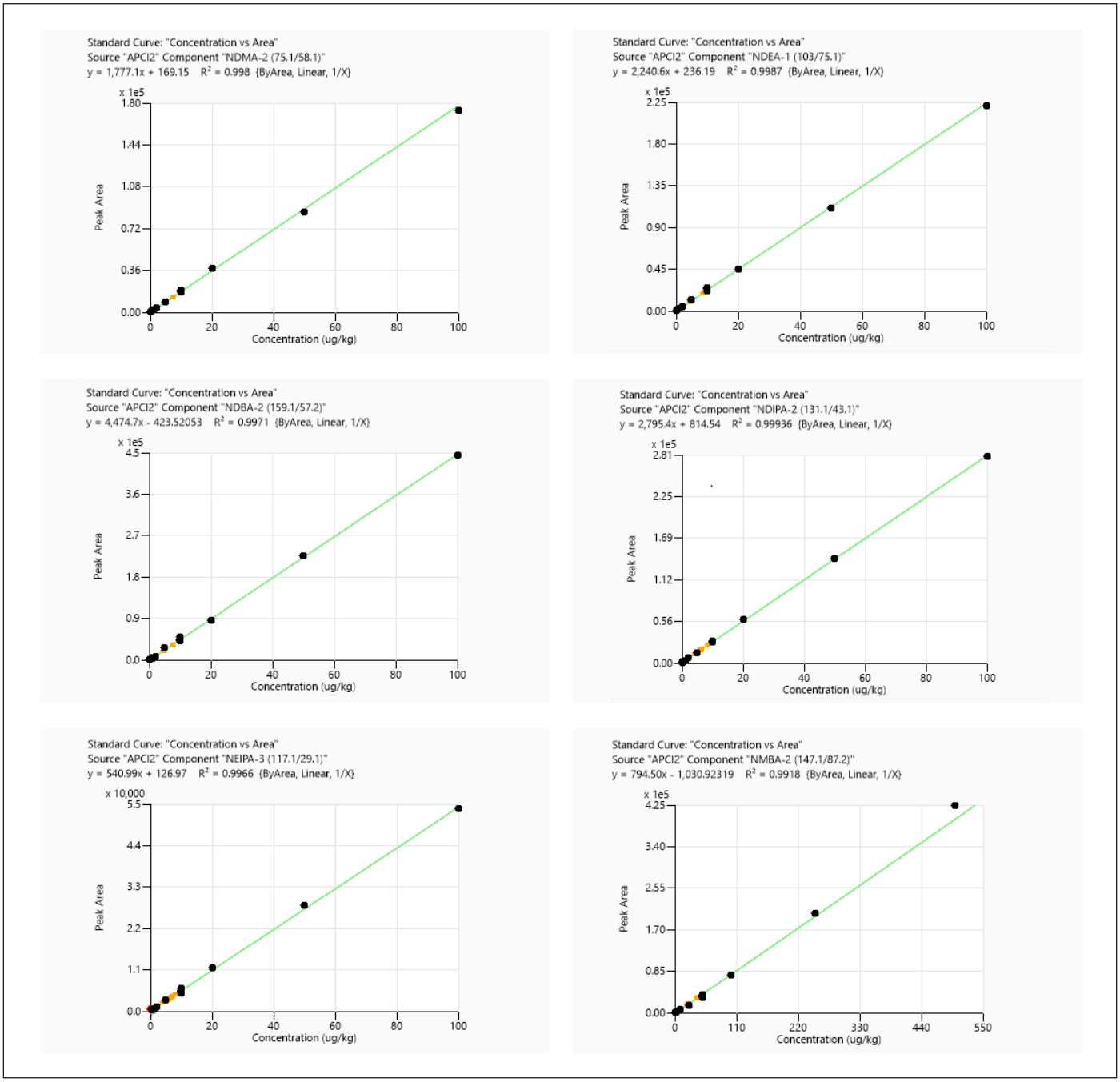

Figure 1. Calibration curves for all 6 Nitrosamine target analytes.

Table 5. Area reproducibility of all nitrosamine impurities was tested at 1 ppb.

Compound Name	NDMA	NDEA	NDBA	NDIPA	NEIPA	NMBA
Injection 1	1859	2311	4126	2822	6032	3072
Injection 2	2074	2346	3698	2379	4814	3143
Injection 3	1850	2311	3309	2766	4942	2963
Injection 4	2153	2609	3284	2332	4984	2562
Injection 5	2145	2663	3512	2802	5313	2614
Injection 6	2160	2570	4020	2477	5876	2750
Average	2040.17	2468.33	3658.17	2596.33	5326.83	2850.67
Std DEV	147.11	162.78	356.28	225.10	515.30	243.53
% RSD	7.21	6.59	9.74	8.67	9.67	8.54

Table 6. Spike recovery results of the six impurity's neat standards spiked in an Irbesartan API at 30,000 ppm concentration.

Name	Level-1 (0.5 ppb)	Level-2 (1 ppb)	Level-3 (2 ppb)	Level-4 (6 ppb)	Level-5 (10 ppb)	Level-6 (15 ppb)
NDMA	101.83	95.13	84.67	83.22	72.14	74.47
NDEA	96.07	88.77	88.23	77.46	70.47	71.57
NDBA	103.43	95.88	77.94	78.24	71.62	73.80
NDIPA	94.20	91.08	91.17	77.04	72.13	70.98
NEIPA	97.97	77.77	83.78	74.26	70.58	71.24
NMBA	91.17	81.53	75.35	78.52	74.37	74.27

Table 7. Spike recovery results of six impurity's neat standards spiked in a Metformin API at 30,000 ppm concentration.

Name	Level-1 (0.5 ppb)	Level-2 (1 ppb)	Level-3 (2 ppb)	Level-4 (6 ppb)	Level-5 (10 ppb)	Level-6 (15 ppb)
NDMA	88.50	78.90	80.63	120.23	100.44	86.25
NDEA	90.40	95.57	102.13	116.65	92.51	91.11
NDBA	88.50	86.40	88.90	102.67	95.35	93.61
NDIPA	87.80	95.33	96.33	110.74	99.89	90.79
NEIPA	116.70	111.57	95.45	108.27	95.38	89.86
NMBA	104.10	119.33	107.04	107.21	91.96	87.40

Figure 2 (A and B) represents the total ion chromatogram (TIC) for Irbesartan and Metformin. Figure 3 shows MRM chromatograms for all six analytes in Irbesartan, using a QSight 220 LC/MS/MS. All six impurities spiked into the Irbesartan API at a concentration of 6 ppb were baseline separated from each other and Irbesartan in 9 the min runtime.

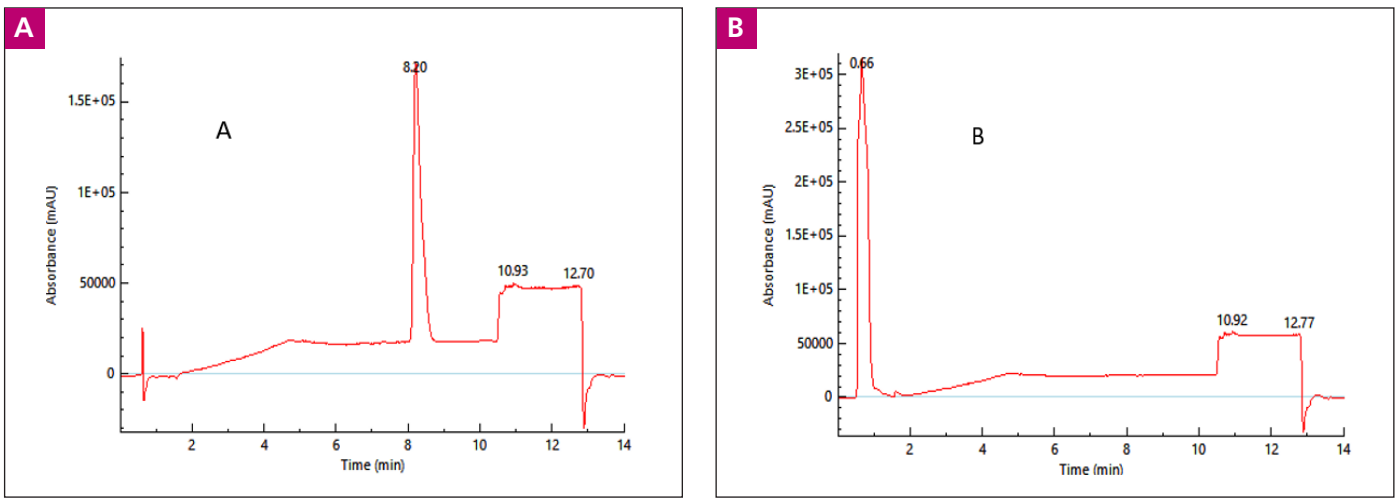

Figure 2. Total ion chromatograms (TICs) of (A) Irbesartan sample, API shown eluting at 8.20 min and (B) Metformin sample, API shown eluting at 0.66 min.

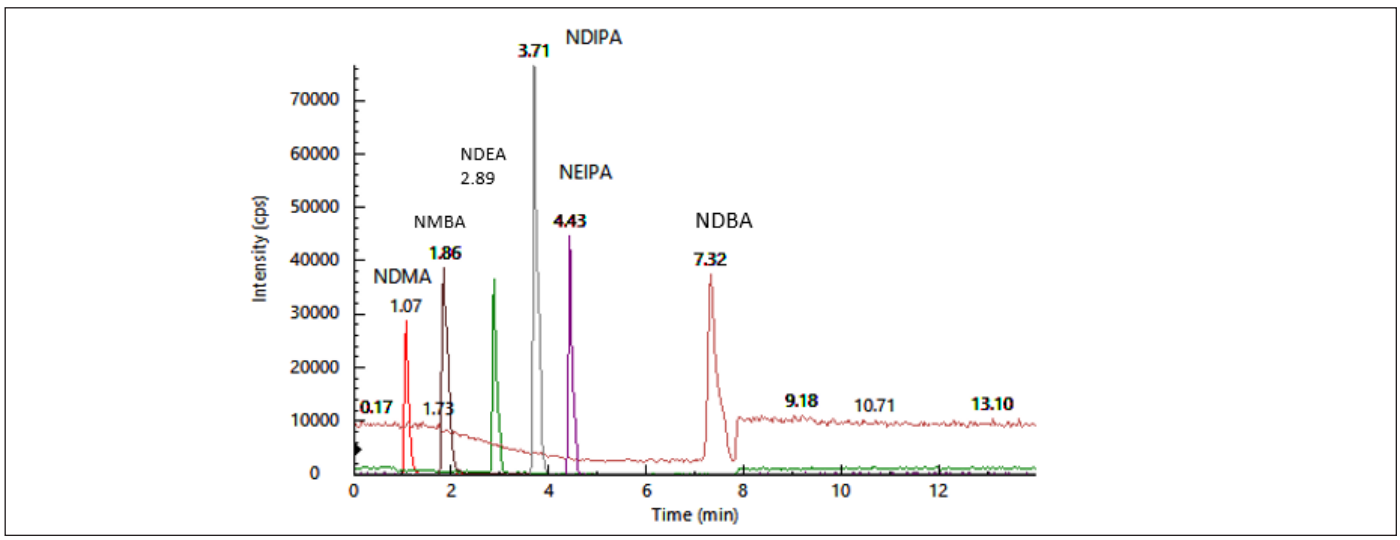

Figure 3. MRM chromatogram of 6 nitrosamine impurities well separated in less than 9 minutes.

Conclusion

The PerkinElmer QSight 220 LC/MS/MS can be used to determine nitrosamine impurities at the lowest levels set by regulatory agencies. This application note describes a fast, selective, sensitive and robust method for the detection and quantification of nitrosamine impurities in Metformin and Irbesartan. This method can be extended to the analysis of these impurities in other drug products with some modifications to the chromatography to achieve separation of the API from these impurities.

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