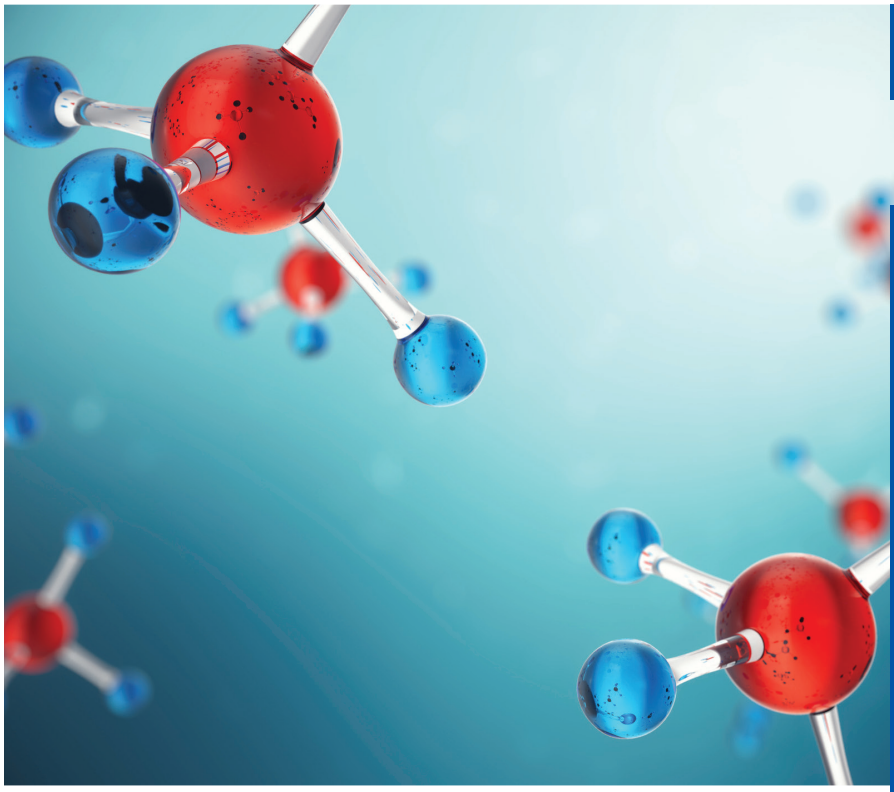

APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

Authors:

Dr. Narayan Kamble
Kailas Gavhane
Ravindra Mallavarapu
Dr. Umesh Talekar

PerkinElmer (India)
Pvt. Ltd. Mumbai

Estimation of N-nitroso Dimethyl Amine (NDMA) in Ranitidine Drug Substance by QSight® UHPLC-MS/MS

Introduction

N-nitrosodimethylamine (NDMA), a pharmaceutical Class 1 impurity, has been identified as a probable human carcinogen (a substance that could cause cancer) by a number of international regulatory bodies, including the United States Department of Health and Human Services (HHS) and the European Medicines Agency (EMA).[1-3] NDMA is also a known environmental contaminant that is found in water and foods, including meats, dairy products and vegetables. In 2018, EMA and the US Food and Drug Administration (FDA) began investigating NDMA, and similar nitrosamine compounds, when the impurities were found in a number of sartan blood pressure medicines (also known as angiotensin II receptor blockers).[4] The investigation resulted in a number of recalls to protect human health.

The source of nitrosamine impurities in active pharmaceutical ingredients (API) is varied, and the impurity can be introduced under a variety of stages in the API manufacturing process. For example, the use of sodium nitrite ($NaNO_2$), or other nitrites, in the presence of secondary or tertiary amines poses the potential for nitrosamine impurity formation. Another possible source of nitrosamine impurity formation occurs in raw materials, starting materials, and intermediates which contain nitrite and amines. The use of recycled solvents, reagents and catalysts, may also pose a risk for nitrosamine formation.

PerkinElmer®
For the Better

The presence of NDMA impurities has also been observed in the drug ranitidine, an H2 (histamine-2) blocker, commonly used to relieve and prevent heartburn.[4,5] EMA and FDA have recently set temporary interim acceptable daily intake limits for NDMA in ranitidine at 96 ng per day (0.32 ppm).[5,6] To limit NDMA impurities in products, it is imperative that pharmaceutical manufacturers identify and limit the possible sources of nitrosamine formation in their manufacturing process. To achieve proper control and reduce the possibility of formation of these carcinogenic impurities, it is essential to have a sensitive, specific, precise and accurate analytical method. The method described herein, utilizing a QSight® 120 UHPLC/MS/MS, offers a comprehensive solution to quantify NDMA impurities in ranitidine, at or below the FDA limit of 96 ng/day[5-7].

Experimental

Hardware/Software

Chromatographic separation was achieved utilizing a PerkinElmer LX-50 UHPLC, with subsequent analyte determination achieved using a PerkinElmer QSight 120 triple quadrupole mass spectrometer, equipped with both ESI and APCI ionization sources. All instrument control, data acquisition and data processing were performed using single-window Simplicity 3Q™ compliance software.

A QSight system with photo diode array (PDA) detector and diverter valve was used to determine the elution time of the API. This information is necessary to determine the valve divert time for the API to waste and avoid contamination of mass spectrometer. This helps to keep the mass spectrometer cleaner for an extended period of time, minimizing down time for this analysis.

Materials and Methods

Solvents and Standards

LC/MS grade solvents and reagents were used in the preparation of solutions. The NDMA impurity standard was purchased from Sigma-Aldrich Inc.

Stock Standard Solutions and Calibration Standard

The stock solutions and calibration standards were prepared as follows:

- **NDMA standard stock solution (100 ppm):** 10 mg of NDMA standard was weighed and transferred in a 100 mL volumetric flask, dissolved, and diluted up to the mark using methanol as the diluent.
- **Standard stock solution-1 (1 ppm):** The 1.0 mL of 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 standard stock solution-1 (1ppm) was diluted to 10 mL in a volumetric flask using acidified water as the diluent.
- **Working level 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 make calibration standards ranging from 0.3 to 100 ppb

Sample Preparation

The 30,000 ppm concentration ranitidine API sample was prepared in diluent (0.1 % v/v formic acid in water). The sample solution was filtered through 0.2 µm nylon filter paper and transferred to an LC vial for UHPLC-MS/MS analysis.

UHPLC-MS/MS Parameters

UHPLC-MS/MS parameters, including column and mobile phase gradient program, are presented in Table 1.

Table 1. LC Parameters.

LC Parameters				
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	95	5
	2	3.0	95	5
	3	5.0	5	95
	4	5.10	95	5
	5	7.0	95	5
Column Oven Temperature	40 °C			
Auto Sampler Temperature	10 °C			
Injection Volume	40 µL			
Flow	1.8 mL/min			
Run Time	7.0 min			

Table 2. QSight LC-MS/MS Parameters.

QSight LC-MS/MS Parameters	
APCI Corona Discharge (Positive)	3.0 µA
Drying Gas	300
Nebulizer Gas	350
Source Temperature	450 °C
HSID Temperature	320 °C

Table 3. MRM parameters and retention time of NDMA.

Sr. No	Compound Name	Polarity	Q1 Mass	Q2 Mass	RT (min)	CE
1	NDMA-1	Positive	75.1	43.1	0.68	-21
2	NDMA-2	Positive	75.1	58.1	0.68	-16

Results and Discussion

Using a QSight 120 UHPLC-MS/MS, the limit of detection (LOD) was determined to be 0.3 ppb, with a S/N value greater than 3, and a limit of quantitation (LOQ) of 1 ppb, with a S/N value greater than 10. Figures 1 and 2 present MRM chromatograms for NDMA at 0.3 ppb (LOD) and 1 ppb (LOQ). Figure 3 shows the MRM chromatograms for the NDMA standard at 10 ppb, allowing for better visualization of the analyte signal for the 75 > 43 transition.

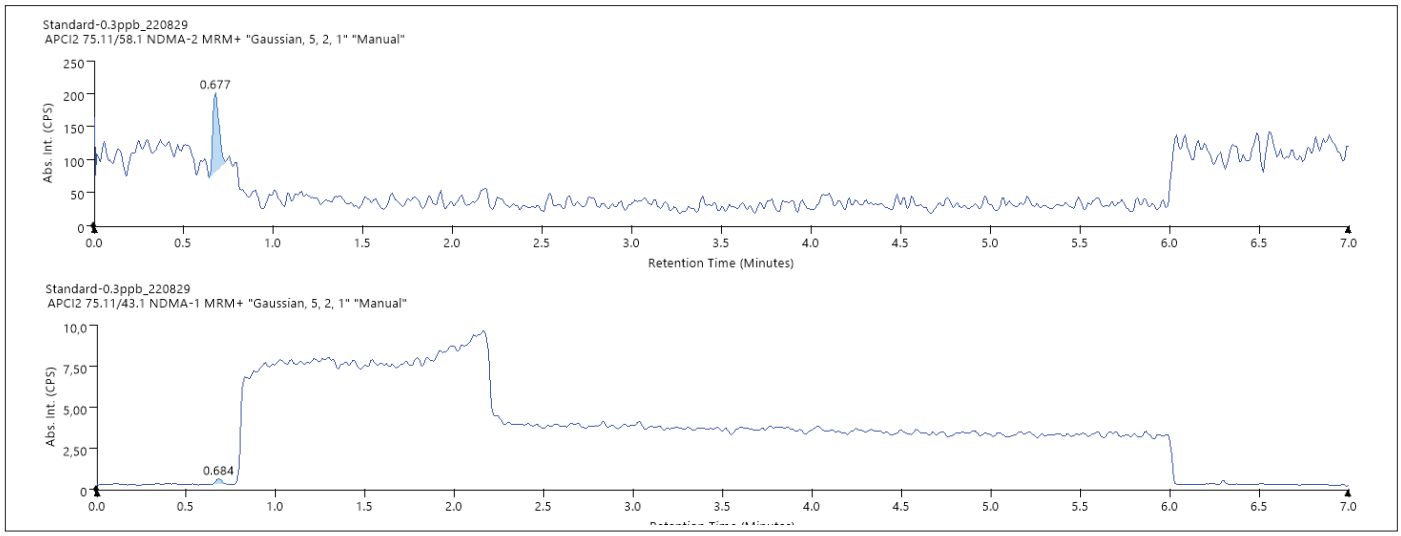

Figure 1. MRM chromatograms for the 0.3 ppb (LOD) NDMA standard.

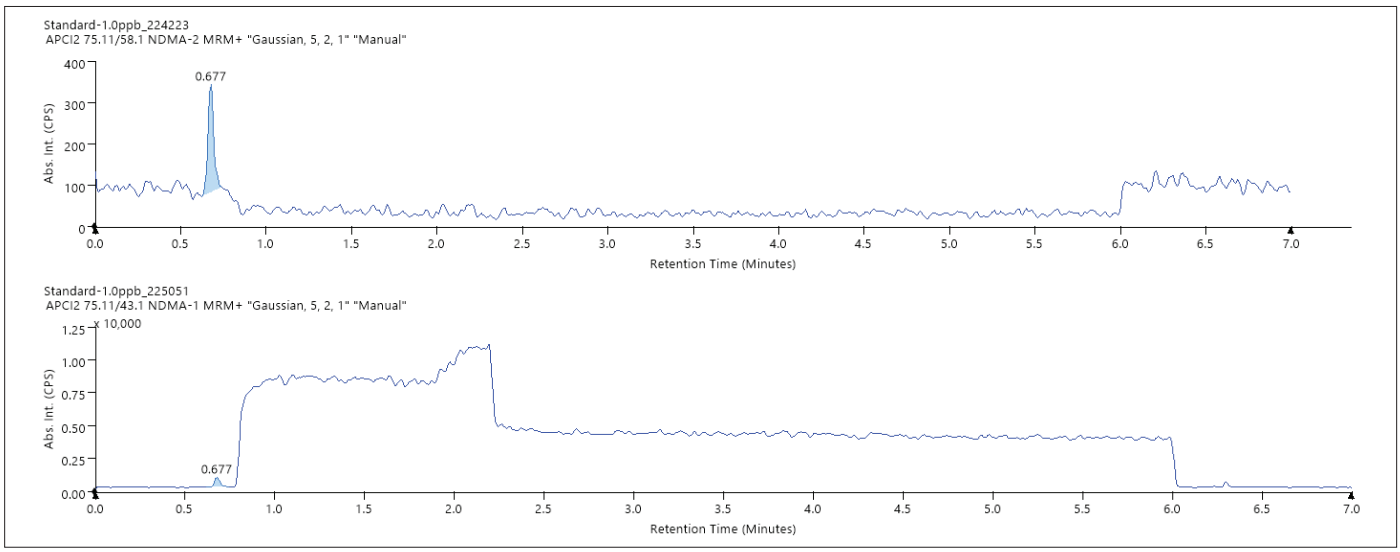

Figure 2. MRM chromatograms for the 1 ppb (LOQ) NDMA standard.

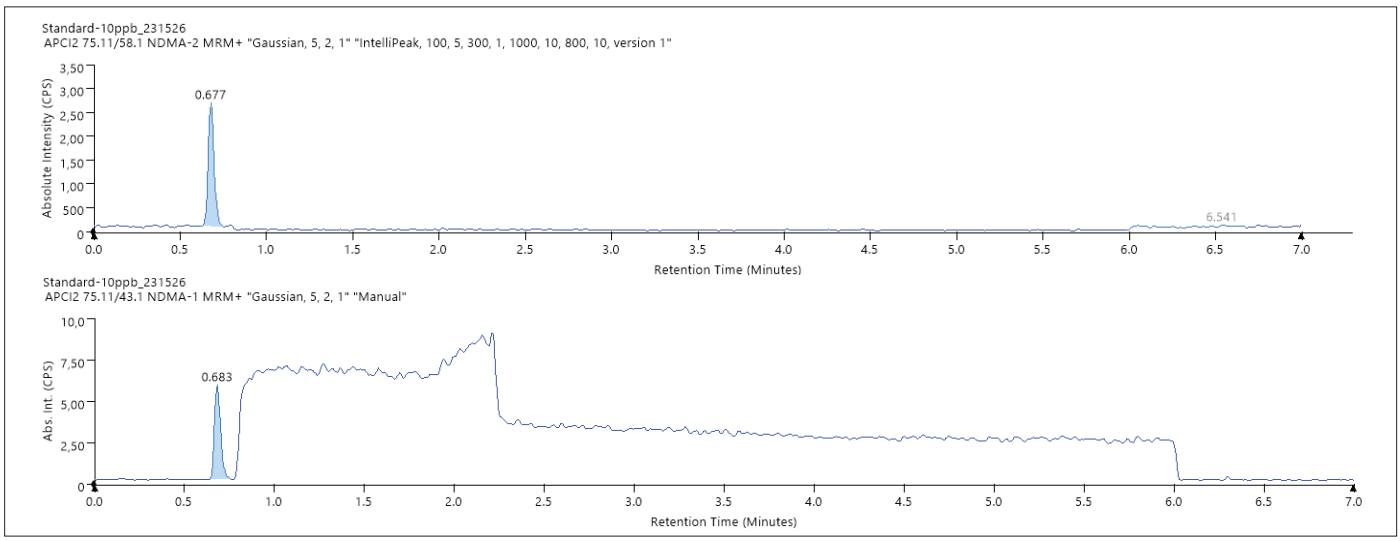

Figure 3. MRM chromatograms for the 10 ppb (working level) NDMA standard.

The calibration curves for the NDMA standard solution showed good linearity over the concentration range of 0.3 to 100 ppb, with a correlation coefficient (R^2) more than 0.99. Figure 4 shows the calibration curve for NDMA.

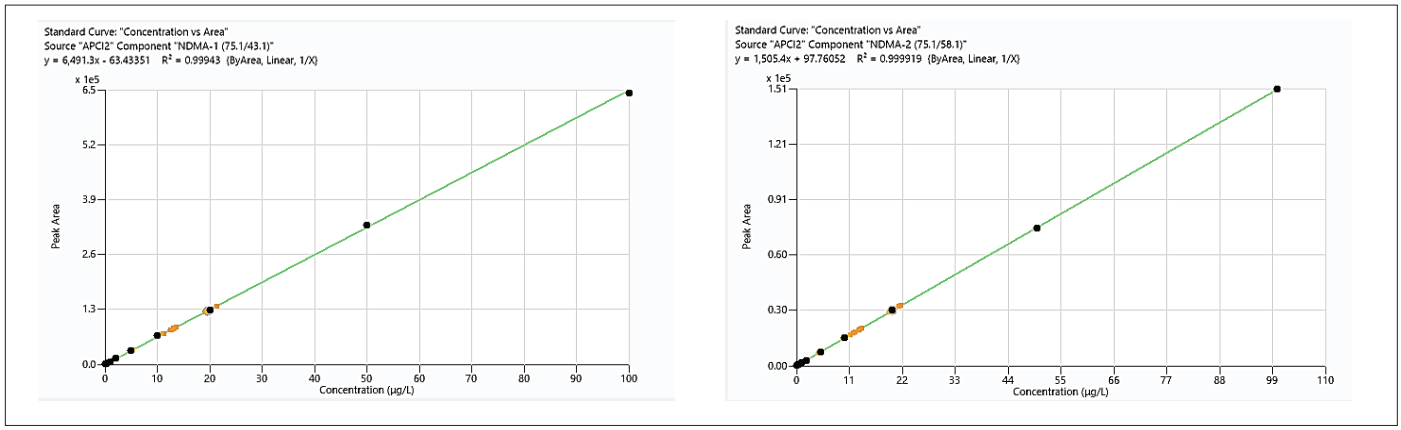

Figure 4. Calibration curve for NDMA over concentration range of 0.3 to 100 ppb.

Figures 5 and 6 show MRM chromatograms for the 1 ppb and 10 ppb spike, respectively, of NDMA in Ranitidine API. As shown in the figures, NDMA gives a good signal response, allowing for robust peak detection and quantitation.

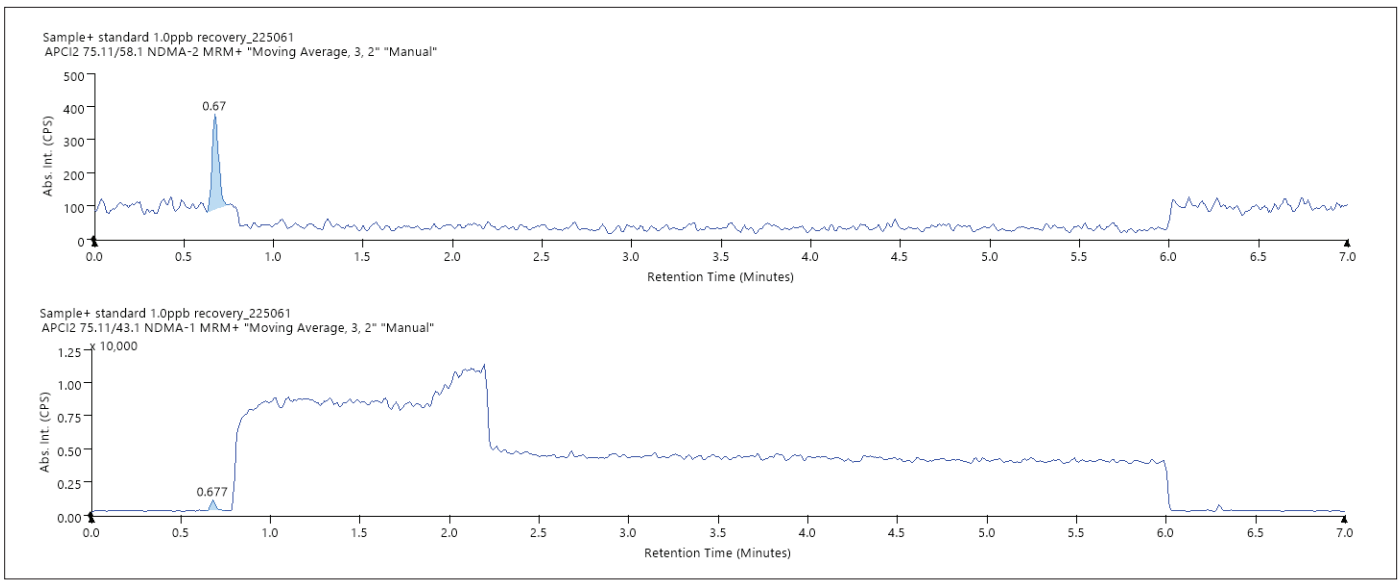

Figure 5. MRM chromatogram for the 1 ppb (LOQ) NDMA spike in Ranitidine API sample.

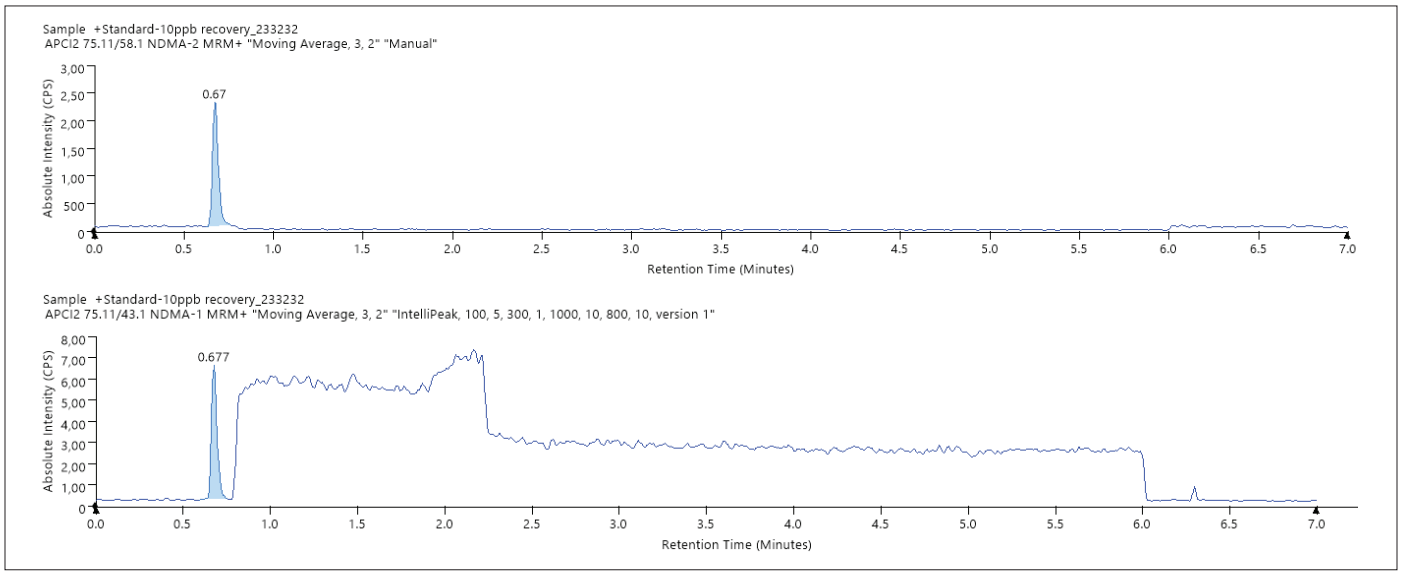

Figure 6. MRM chromatogram for the 10 ppb NDMA spike in Ranitidine API sample.

Table 4 displays the results for the LOD, LOQ, and linearity for NDMA neat in solvent, as well as with respect to the sample concentration. As can be seen, this method meets the regulatory requirement.

Table 4. Summary of LOD, LOQ and Linearity for NDMA achieved for Ranitidine API sample.

	NDMA Actual Standard (ppb)	NDMA with Respect to Sample Concentration (ppb)
LOD	0.3	10
LOQ	1.0	33.3
Linearity	0.3 to 100	33 to 3330

Method precision and accuracy were assessed based on six replicate analyses of the spiked standard at 1 ppb and 10 ppb in the sample matrix. The average recovery values were observed between 80% to 120%. The peak area RSDs for six replicate injections were observed at less than 10%. Table 5 shows the spike recovery analysis data.

Table 5. Spike recovery at 1 ppb and 10 ppb in sample matrix (6 replicates).

	% Recovery	
Preparation	1 ppb spike	10 ppb spike
1	112.3	84.1
2	117.3	95.7
3	114.6	81.7
4	111.9	80.6
5	110.8	85.4
6	111.6	88.8
Average	113.1	86.1
%RSD	2.15	6.44

The samples were analyzed continuously for 3 days. After every batch analysis of samples, a 5 ppb bracketing standard was injected. The area count for the 5-ppb standard was found to be comparable and within ±10% of the limit of average value of six consecutive standard injections. Figure 7 shows an image of the sample cone before and after sample analysis for three days, with negligible deposition. The QSight's StayClean™ source showed excellent long term signal stability and reproducibility, without the need for any cleaning. This demonstrates the reduction in maintenance downtime for analyzing an NDMA impurity in samples, improving laboratory productivity.

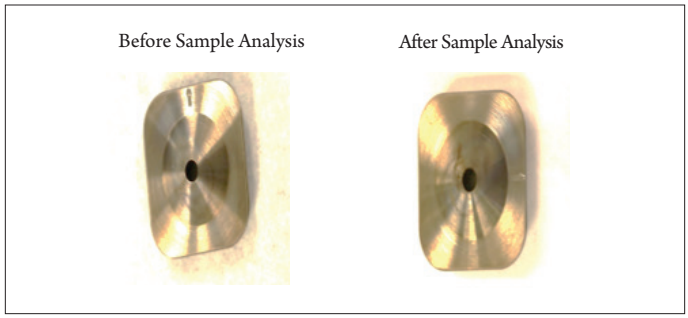

Figure 7: The image of sample cone before and after sample analysis over three days.

The results show excellent accuracy and repeatability. The identity of the NDMA impurity was confirmed by ensuring the quantifier/qualifier ion ratio in the sample containing 30,000 ppm of API ranitidine was within 30% of the ion ratio obtained with the NDMA standard.

Conclusions

The method described herein, using a PerkinElmer QSight UHPLC-MS/MS, offers a fast (7 min), selective, sensitive and robust analysis for the detection and quantification of the nitrosamine impurity NDMA, in ranitidine API. The results demonstrate that the developed method is suitable for the analysis of NDMA in ranitidine at the daily acceptable intake limit of NDMA. The method parameters can be extended to the determination of NDMA in other drug substances for quality control, with some optimization to the chromatography to achieve separation of the API from NDMA.

References

1. National Toxicology program US Department of Health and Human Services, Report on Carcinogens, 14th Edition, https://ntp.niehs.nih.gov
2. ICH M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals To Limit Potential Carcinogenic Risk, Step 4 version, 31st March 2017, https://www.ich.org
3. International Agency for Research on Cancer, https://www.iarc.
4. European Medicines Agency, EMA/217823/2019, EMA/189634/2019, https://www.ema.europa.eu.
5. European Medicines Agency, EMA/351053/2019 rev 1 https://www.ema.europa.eu
6. U.S. FDA Statement, 1 Nov 2019, https://www.fda.gov
7. U.S. Food and Drug Administration, Doc.c 17 Oct 2019, https://www.fda.gov.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

276940 (208683) PKI