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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060NX

Determination of Nitrosamine Impurities and NDSRI in Anti-diabetic Drugs on Shimadzu LCMS-8060NX

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User Benefits

- Simple and sensitive LC-MS/MS method to quantify ten nitrosamine impurities with simple sample pre-treatment
- Achieve good linearity of $R^2 > 0.999$ with recovery within 70 – 120 % without matrix-matched calibration

■ Introduction

Detection of nitrosamines as impurities in drugs was first reported in June 2018 where N-nitrosodimethylamine (NDMA) was found in valsartan, an angiotensin II receptor blocker (ARB). Since then, nitrosamines have been found in other ARBs and drug classes. [1,2] Nitrosamines are a group of chemical compounds that can be found in several sources such as tobacco, cured meats, cosmetics and pharmaceutical products. They are formed when an amine reacts with nitrite or nitrosating agents e.g., nitrous acid. While nitrosamine drug substance related impurities (NDSRIs) are a class of nitrosamine impurities that share similar structure with the active pharmaceutical ingredients (APIs). Figure 1 shows an example of API sitagliptin and its NDSRI.

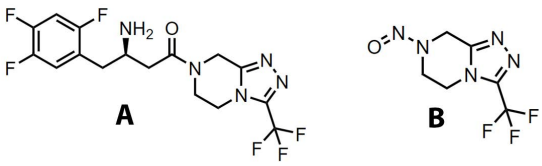

Figure 1. (A) Sitagliptin and (B) its NDSRI nitroso-sitagliptin (NTTP)

Nitrosamines have been a concern due to their potential health risks, as some studies have linked them to an increased risk of cancer. As a result, regulatory authorities have implemented strict limits on the presence of nitrosamines in pharmaceutical products. It is important for pharmaceutical manufacturers to be aware of the formation and presence of nitrosamines to ensure the safety and efficacy of their products and protect patient health.

The aim of this study is to present a simple and sensitive analytical method for ten nitrosamine impurities using APCI on Shimadzu LCMS-8060NX in MRM mode.

■ Measurement Conditions and Samples

The nitrosamine impurity standards were purchased from Toronto Research Chemicals and Restek. High concentration stock solutions were prepared in LCMS grade methanol from the purchased standards and later diluted serially for calibration levels.

Drug samples were prepared by first weighed and crushed into 50 mL centrifuge tube, then extracted with ultrapure water with an extraction ratio of API equivalent to 100 mg to 1 mL of extraction solvent. The samples were sonicated and centrifuged, and then filtered through 0.22 µm nylon filter before injecting into LCMS.

LCMS analytical conditions are described in Table 1, Table 2 and Table 3, respectively. Data were acquired with Shimadzu LabSolutions™ and analysed with LabSolutions Insight™ LCMS software.

Table 1. Analytical LC conditions for detection of nitrosamine impurities

Nexera™ XS LC-40	
Column	Shim-pack™ Scepter C18-120 (150 mm x 3.0 mm I.D., 1.9 µm) P/N: 227-31013-04
Mobile phase	A : 0.1 % formic acid in water B : 0.1 % formic acid in methanol
Gradient program	28 min elution gradient program
Flow rate	0.4 mL/min
Oven temperature	45 ˚C
Injection volume	10 µL
Switching valve	FCV-0206

Table 2. MS conditions for detection of nitrosamine impurities

LCMS-8060NX	
Interface	APCI
Acquisition Mode	MRM, positive mode
Heat block temperature	200 ˚C
DL temperature	200 ˚C
Interface temperature	400 ˚C
Nebulising gas	N_2, 4.4 L/min
Drying gas	N_2, 10 L/min

Table 3. MRM transitions for ten nitrosamine impurities

Compound	Mode	MRM Transitions	
		Quantifier	Reference
NDMA	+	75.05>43.05	75.05>58.10
NDEA	+	103.15>75.15	103.15>29.05
NMEA	+	89.10>61.10	89.10>43.10
NEIPA	+	117.05>75.10	117.05>47.10
NDIPA	+	131.05>89.10	131.05>47.10
NDPA	+	131.20>89.20	131.20>47.15
NDBA	+	159.25>57.20	159.25>41.10
NMBA	+	147.15>44.10	147.15>117.25
NPYR	+	101.10>55.15	101.10>41.10
NTTP	+	222.15>192.10	222.15>42.10

■ Result and Discussion

Calibration and Linearity

Calibration curves for ten nitrosamines were established with mixed standards ranging from 0.1 ng/mL to 10 ng/mL (the amount concentration was back calculated to be 1 ppb to 100 ppb in API). The limit of quantitation (LOQ) for each nitrosamine is less than 10% of the acceptable limit as per EMA guidelines to justify for omission of specification.

Figure 2 shows the calibration curve for four of the nitrosamine impurities. Excellent linearity was obtained with $R^2 > 0.999$ for all target nitrosamines throughout the calibration range as described in Table 4. Figure 3 shows the MRM chromatogram of the neat standard mixture at 1 ng/mL (10 ppb) while Figure 4 shows the chromatogram of each nitrosamine at their respective LOQs.

Accuracy and Precision

The percent accuracy results for the nitrosamines are in accordance with the ICH guideline of within ± 20 %.

Meanwhile, the average precision of <10 % was determined based on the investigation at the limit of quantitation (LOQ) with n = 6 also described in Table 4.

Spiked Sample Recovery

Recovery study was performed to examine the analytical performance of the method through the spiking of nitrosamine standard mixture at respective LLOQs on two sets of pharmaceutical drug samples, D1 and D2. Table 4 describes the respective recovery for the nitrosamine impurities.

Analysis of drug samples

The analytical method was employed on the anti-diabetic drug samples as indicated in Table 5. It was revealed that certain nitrosamine impurities were detected in the drug samples.

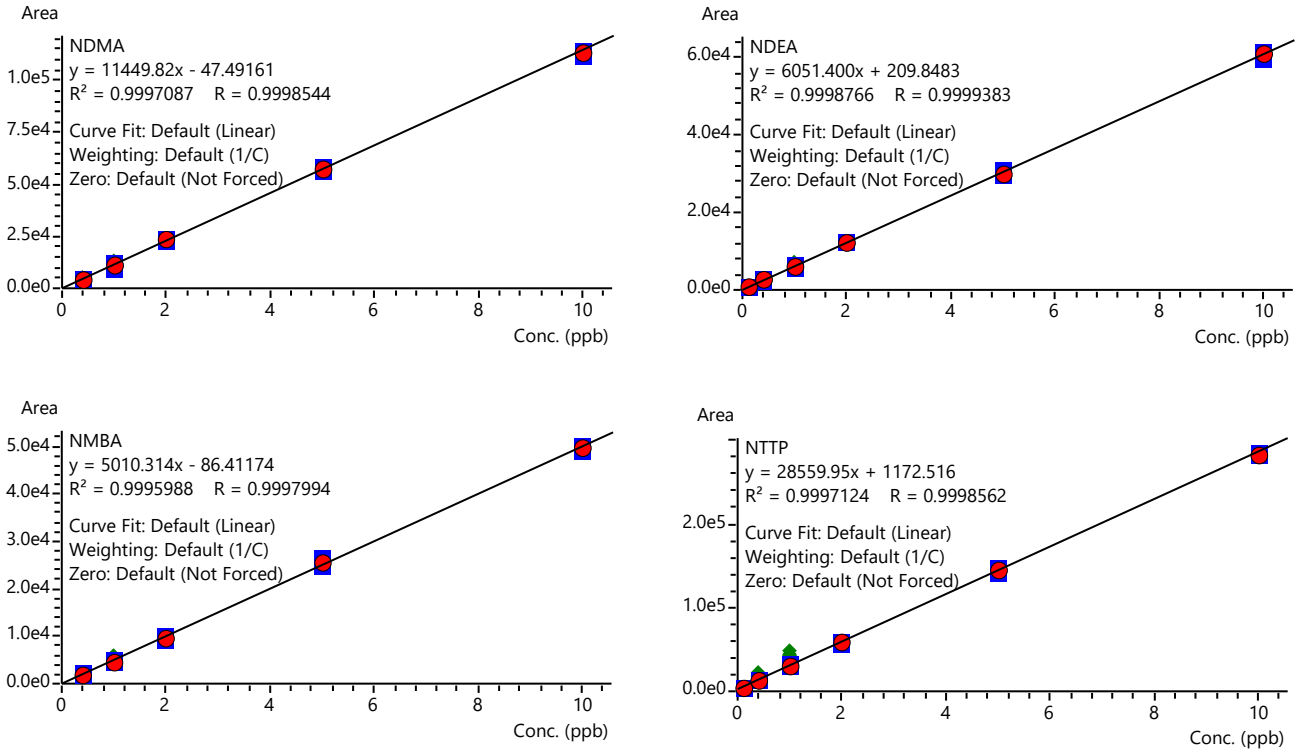

Figure 2. Calibration curves for NDMA, NDEA, NMBA and NTTP on Shimadzu LCMS-8060NX

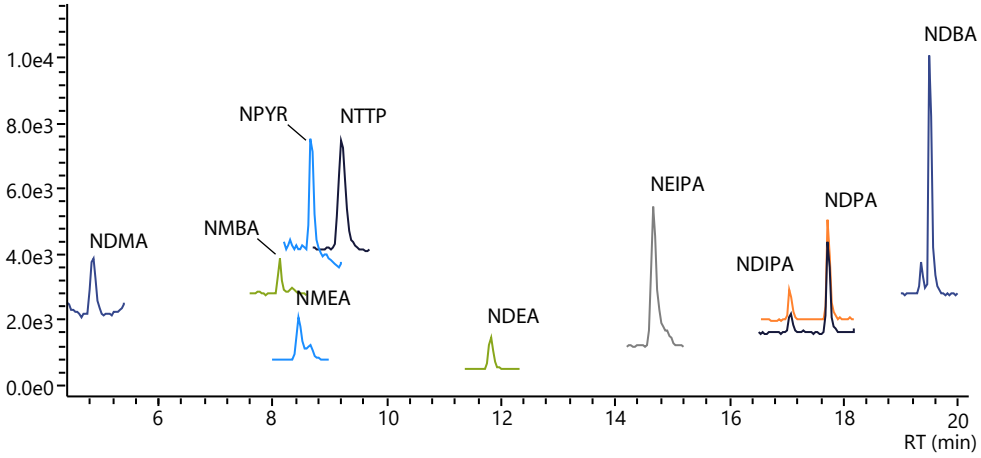

Figure 3. MRM chromatogram of ten mixed nitrosamine standards at 1 ng/mL (10 ppb)

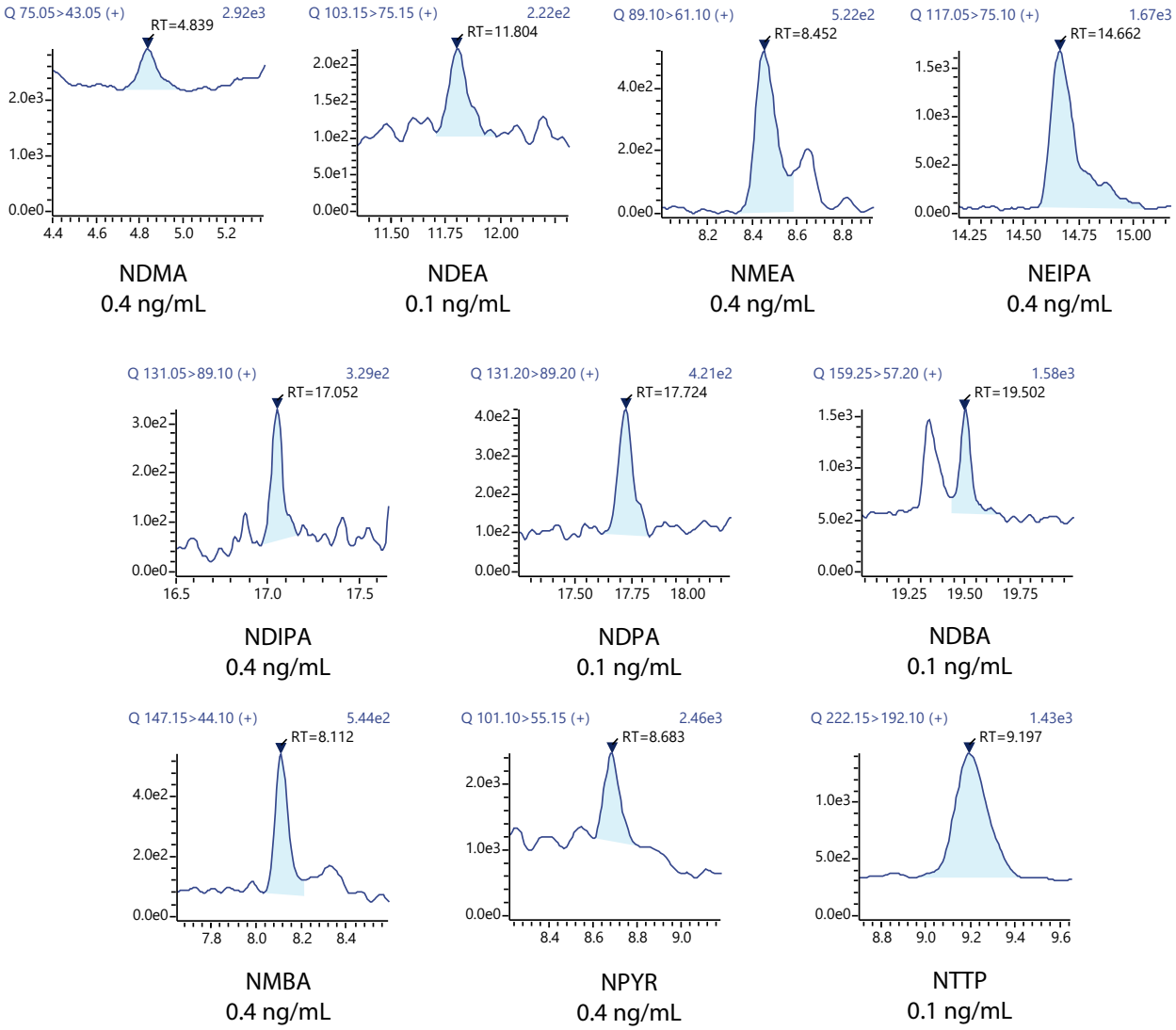

Figure 4. MRM chromatograms of each nitrosamine at respective LOQs

Table 4. Summary for nitrosamines with respective linearity, accuracy, % RSD and % recovery in spiked sample D1 and D2

Nitrosamine	R^2	Linearity range		Accuracy (%)	% RSD (Area)	% RSD (Conc.)	D1 Recovery# (%)	D2 Recovery# (%)
		(ng/mL)	(*ppb)					
NDMA	0.9997	0.4 – 10	4 – 100	97.2	6.20	6.13	110.4	74.5
NDEA	0.9999	0.1 – 10	1 – 100	97.4	5.90	8.00	80.3	85.9
NMEA	0.9996	0.4 – 10	4 – 100	100.3	7.74	8.33	120.0	102.2
NEIPA	0.9992	0.4 – 10	4 – 100	102.0	3.93	4.03	119.2	109.1
NDIPA	0.9995	0.4 – 10	4 – 100	104.4	4.22	4.35	98.6	98.8
NDPA	0.9999	0.1 – 10	1 – 100	97.0	6.78	7.78	101.9	81.2
NDBA	0.9999	0.1 – 10	1 – 100	96.3	4.69	6.76	87.1	94.2
NMBA	0.9996	0.4 – 10	4 – 100	103.7	6.84	6.56	113.9	108.5
NPYR	0.9998	0.4 – 10	4 – 100	100.5	5.77	6.19	115.6	83.5
NTTP	0.9997	0.1 – 10	1 – 100	92.3	6.25	9.03	81.7	119.4

* Concentration calculated with respect to target analytes in API

Recovery performed at respective LLOQs of nitrosamine impurities

Table 5. Results of drug samples tested for nitrosamine impurities (in ppb)

Nitrosamine	S1	S2	S3	S4	S5	S6	S7	S8	S9
NDMA	N.D.	N.D.	N.D.	N.D.	N.D.	< LOQ	N.D.	N.D.	N.D.
NDEA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
NMEA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
NEIPA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
NDIPA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
NDPA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
NDBA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
NMBA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
NPYR	19.4	N.D.	N.D.	N.D.	N.D.	13.33	4.10	< LOQ	< LOQ
NTTP	N.D.	N.D.	N.D.	158.77	170.05	53.36	49.91	59.21	88.85

* N.D. Not detected

■ Conclusion

A highly sensitive LC-MS/MS method using the Shimadzu LCMS-8060NX was developed to detect ten nitrosamine impurities. This straightforward yet effective method allows for the quantification of analytes at concentrations as low as 0.1 ng/mL.

With a small 10 µL injection volume and simple sample pre-treatment, the method demonstrated excellent recovery, accuracy and precision, making it well-suited for pharmaceutical safety testing. Additionally, by adjusting the chromatographic conditions based on the elution pattern of the specific drug product, this method can be adapted to quantify these impurities in a range of drug substances or products.

■ Reference

[1] *Nitrosamines as Impurities in Drugs – Health Risk Assessment and Mitigation Public Workshop*, Food and Drug Administration; 2021. https://www.fda.gov/media/150932/download.

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