APPLICATION NOTE 000362

Reliable quantitation of 11 nitrosamine impurities in metformin drug products using Orbitrap Exploris 120 mass spectrometry

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Application benefits:

- Detection and quantification of 11 nitrosamines in a single liquid chromatography high-resolution accurate-mass mass spectrometry (LC-HRAM-MS) method
- Quantitation of nitrosamine impurities in metformin drug products below the daily acceptable intake level, that meets U.S. FDA regulatory guidelines
- Compliant data acquisition, processing and auditing built for a U.S. FDA 21 CFR part 11 compliant environment

Goal

Demonstrate highly sensitive and reliable quantitation of 11 nitrosamines in metformin drug products using an LC-HRAM-MS method on a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer

Introduction

On September 2020, the United States Food and Drug Administration (U.S. FDA) updated its regulatory guidelines for controlling nitrosamine impurities in human medicines after a series of voluntary recalls of drug products detected with elevated level of these contaminants. Considering the potent genotoxic and carcinogenic nature of these

nitrosamines, the updated guidelines also mandated that drug substance and product manufacturers must conduct risk assessments and implement control strategies to limit the formation of these impurities in all human drugs.[1] In addition, it also suggested expanding the screening panel to seven nitrosamine impurities, as well as establishing a new limit in which if more than one nitrosamine is detected in the drug products, the total nitrosamine contents cannot exceed 30 ppb, or more than 26.5 ng/day.[1] Similar regulatory requirements were published by the European Medicine Agency (EMA), with a distinction that the EMA committee for medicinal products for human use (CHMP) suggested assessing all biological medicines, in addition to synthetic chemical drugs, for possible presence of nitrosamine impurities.[2]

Given the complexity of the drug formulation matrix and high concentration of active pharmaceutical ingredient, reliable detection of trace levels of these impurities is often challenging. To address the above analytical challenges, we recently published an LC-HRAM-MS method using an Orbitrap-based mass spectrometer, which provides high selectivity using a mass resolution of 120,000 to differentiate minute chemical interferences.[3] The method has been used to evaluate and quantify nine nitrosamine impurities in commercially available ranitidine drug substances and products. As an extension to that work, we describe here a fit-for-purpose LC-HRAM-MS method using the same setup to detect and quantify 11 nitrosamines, the same nine nitrosamines plus *N*-Nitroso-*N*-methyl-4-aminobutyric acid (NMBA) and *N*-Nitrosomethylphenylamine (NMPA), in metformin drug products. The method is versatile as it can operate in either heated electrospray ionization (HESI) mode or atmospheric pressure chemical ionization (APCI) mode. Not only is the method highly sensitive, meeting all regulatory guidelines but is highly reproducible, well suited for routine screening of nitrosamine impurities in similar drug products.

Experimental

Reagents and consumables

- Water, UHPLC-MS Grade, Thermo Scientific™ (P/N W81)
- Methanol (MeOH), UHPLC-MS grade, Thermo Scientific™ (P/N A4581)
- Formic acid, Pierce™ LC/MS grade (P/N TS-28905)
- Nitrosamine reference standards (See Table 1)
- *N,N*-dimethylformamide (DMF), HPLC grade, Sigma-Aldrich (P/N 270547)
- Metformin hydrochloride extended-release tablets USP 500 mg

Sample preparation

Neat standards

Pooled neat standards ranging from 0.02 to 10 µg/mL were prepared by diluting the 1 mg/mL stock solutions with pure methanol. A working solution of pooled internal standards at 2 µg/mL was prepared by diluting the 1 mg/mL stock solution with pure methanol. Neat standards ranging from 0.2 to 100 ng/mL were prepared by mixing 10 µL of working standards with 10 µL of work internal standards, followed by adding 980 µL of pure methanol.

200 ppm DMF spiked neat standard (e.g., 20 ng/mL) was prepared by adding 0.5 µL of pure DMF to 1 mL of 20 ng/mL neat standard solution. 2 ppm and 20 ppm DMF spiked neat standards were prepared by first diluting pure DMF with pure methanol (1/10 and 1/100 dilution) prior to adding to the neat standard solution.

Metformin drug product preparation

Metformin drug tablets were ground into a fine powder and transferred to a 15 mL Falcon tube. Methanol containing 20 ng/mL of internal standards was added, bringing the final metformin concentration

Table 1. Nitrosamine reference standards

Standards	CAS	Vendor	P/N
Nitrosamine reference standards	See Table 1 in Application Note 738143		
***N*-Nitroso-*N*-methyl-4-aminobutyric acid (NMBA)**	61445-55-4	Cambridge Isotopes	ULM-10857-1.2
NMBA-$^{13}C_4$	61445-55-4	Cambridge Isotopes	CLM-10856-1.2
***N*-Nitrosomethylphenylamine (NMPA)**	614-00-6	Toronto Research Chemicals	N529925

to 100 mg/mL. Sample preparation was performed by continuous mixing for 30 minutes using a mechanical shaker, followed by cold centrifugation for 30 minutes at 4,200 rpm. The supernatant was transferred to a 100K molecular cut-off filter and cold centrifugation was performed for another 60 minutes at 4,200 rpm. Filtered extracts were transferred to an autosampler vial prior to LC-HRAM-MS analysis. For spiked samples, nitrosamine standards were added to the sample mixture prior to methanol extraction such that final spiked concentrations ranged from 0.2 to 100 ng/mL.

LC-MS method

A single targeted LC-HRAM-MS method was developed using a Thermo Scientific™ Vanquish™ Horizon™ UHPLC system coupled to an Orbitrap Exploris 120 mass spectrometer. 3 µL of samples were injected onto a Thermo Scientific™ Hypersil GOLD™ C18 column using the LC gradient and conditions outlined in Table 2. The method can operate in either heated electrospray ionization (HESI) or atmospheric pressure chemical ionization (APCI) mode with optimized and scan settings as outlined in Tables 3 and 4, respectively.

Table 1. LC and autosampler conditions

Parameter	Value		
HPLC column	Hypersil GOLD™ C18 HPLC column, 150 × 4.6 mm, 3 µm (P/N 25003-154630)		
Column temp.	20 °C		
Flow rate	0.5 mL/min		
Mobile phase A	Water + 0.1% formic acid		
Mobile phase B	Methanol + 0.1% formic acid		
Gradient	*Time) (min)*	*% Mobile phase A*	*% Mobile phase B*
	0.0	98	2
	5	98	2
	13	10	90
	21	10	90
	21.5	98	2
	30	98	2
Injection volume	3 µL		
Needle wash solution	80% Methanol with 0.1% formic acid		
Seal rinse solution	10% Methanol with 0.1% formic acid		
Autosampler temp.	6 °C		
Thermostatting mode	Still Air		
Gradient delay volume	None		
Needle wash option	Before and after injection		
Wash speed and time	30 µL/s for 10 s		
Divert valve	5.5–20 min		

Table 3. MS ion source parameters

Ionization	HESI	APCI
Spray voltage/current (Positive)	3,500 V	4 µA
Spray voltage/current (Negative)	4,000 V	40 µA
Sheath gas	40	
Auxiliary gas	10	
Sweep gas	0	
Ion transfer tube temp.	200 °C	
Vaporizer temp.	300 °C	

Table 4. Scan settings

Scan mode	tSIM	tMS²
Resolution	120,000	120,000
AGC target	1e5	1e5
Maximum IT	Auto	Auto
Isolation window	2.0 *m/z*	2.0 *m/z*

Software

Compliant-ready Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software version 7.2.10 was used for both data acquisition and analysis to meet modern regulatory requirements including United States Food and Drug Administration (U.S. FDA) 21 CFR Part 11 and European Commission (EU) Annex 11. Orbitrap Exploris 120 Tune 3.1 or higher software is required for data acquisition as it addresses the low mass ion transmission issue observed in an earlier version.

Results and discussion

Chromatographic separation of nitrosamine impurities in metformin products

Previously, we demonstrated that the LC-HRAM-MS method was capable of accurately quantifying below 17 ppb for nine nitrosamines spiked in excipient matrix.[3] However, due to co-elution of NMBA with ranitidine, accurate determination of NMBA could not be evaluated. With the Hypersil GOLD C18 column, this new method can baseline resolve metformin from the 11 nitrosamines. As shown in Figure 1, the retention time for metformin is around 4 minutes, whereas the retention time for NDMA, the first eluting nitrosamine, is around 9 minutes.

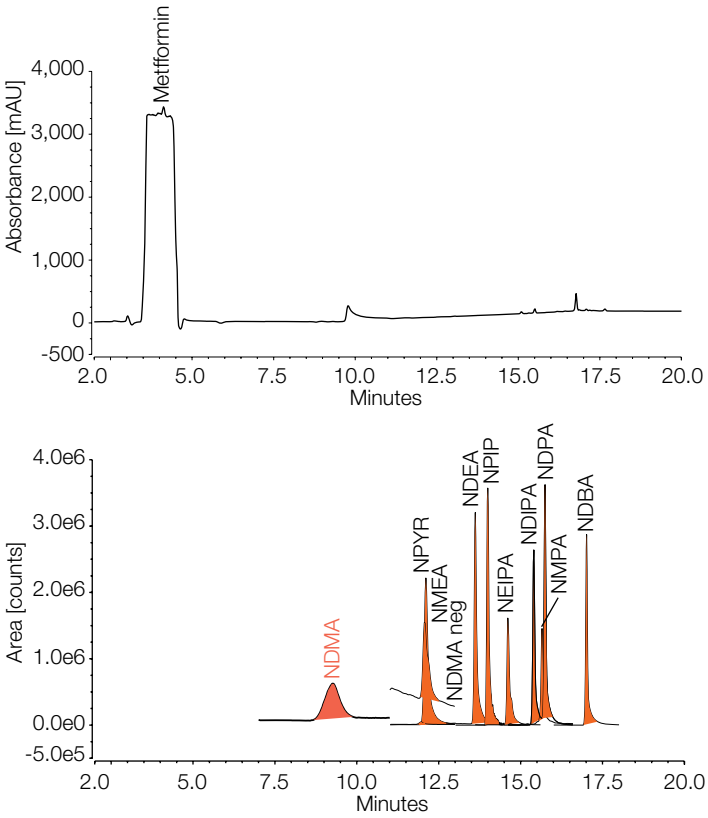

Figure 1. Chromatographic separation of metformin from nitrosamines (e.g., 20 ng/mL spiked sample) using the LC-HRAM-MS method. Example UV trace collected at 230 nm (top) and XIC of quantifier ions collected in APCI mode (bottom) are shown.

The use of a diverter valve could effectively avoid spraying a high concentration of metformin into the mass spectrometer and reduce source contamination. This method also provides adequate chromatographic resolution to separate DMF from NDMA. Although we could mass resolve the two molecules using 120,000 mass resolution on the Orbitrap Exploris 120 mass spectrometer, it is also important to chromatographically separate them to minimize any ion suppression effect as a result of high DMF concentration acceptable in drug products.[4] As illustrated in Figure 2, the baseline of NDMA quantifier ion takes a plunge as DMF elutes, and the duration of the sinking baseline increases as DMF concentration increases from 2 ppm to 200 ppm, potentially affecting the quantitation of NDMA if inadequate chromatographic resolution was achieved.

Achieving regulatory performance limits for the analysis nitrosamines in drugs

Like the previous method, this method was operated in targeted selective ion monitoring (tSIM) and targeted tandem MS (tMS^2) mode for optimal sensitivity and selectivity of target nitrosamines (Table 5). In addition to mixed targeted mode, there is polarity switching for the determination of NMBA in drug products.

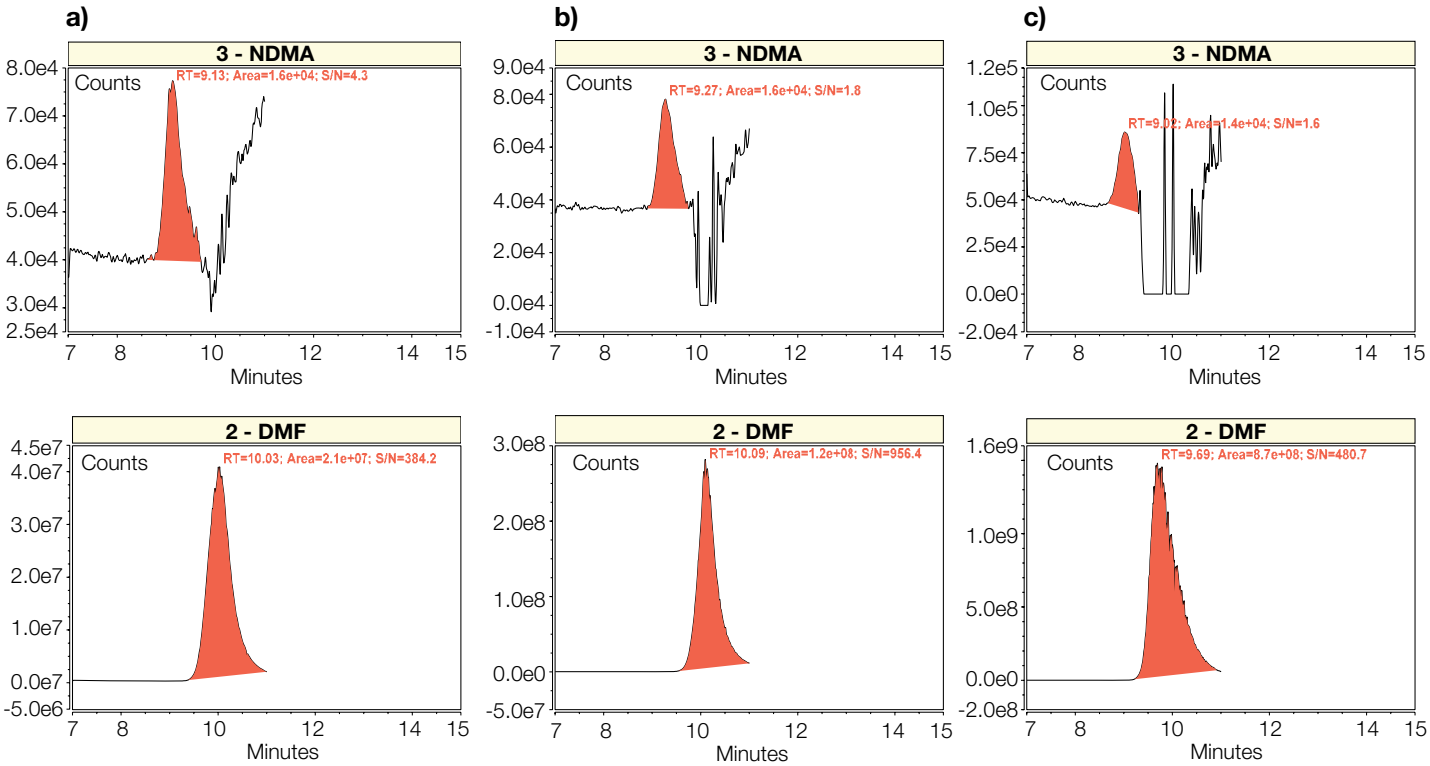

Figure 2. Chromatographic separation of various concentration of DMF from 2 ng/mL NDMA in neat solution. Example XICs of a) 2 ppm, b) 20 ppm, and c) 200 ppm DMF (*m/z* 74.0600). Only APCI data are shown.

Table 5. Optimized MS condition for target nitrosamines

	Polarity	Quantifier ion	Qualifier ion	HCD collision energy (%)	RF lens (%)
NDMA	Positive	75.0552	43.0290	60	100
NDMA-D6	Positive	81.0928	46.0479	60	100
NMEA	Positive	61.0397	89.0708	60	70
NMEA-D3	Positive	64.0585	92.0896	30	70
NPYR	Positive	101.0709	-	60	70
NPYR-D8	Positive	109.1212	-	60	70
NDEA	Positive	103.0866	-	15	70
NDEA-D10	Positive	113.1493	-	30	70
NPIP	Positive	115.0866	-	45	70
NPIP-D10	Positive	125.1494	-	45	70
NEIPA	Positive	75.0553	117.1022	15	70
NEIPA-D5	Positive	80.0866	122.1335	15	70
NDIPA	Positive	131.1179	-	15	70
NDIPA-D14	Positive	145.2058	-	15	70
NDPA	Positive	131.1179	-	15	70
NDPA-D14	Positive	145.2058	-	15	70
NDBA	Positive	159.1492	57.0699	15	70
NDBA-D18	Positive	177.2622	66.1264	15	70
NMPA	Positive	137.0709	-	15	70
NMBA	Negative	145.0619	-	15	70
NMBA-13C4	Negative	149.0753	-	15	70

Figure 3 shows examples of extracted ion chromatogram (XIC) of 2 ng/mL spiked metformin sample with a mass tolerance setting of 3 ppm. Other than NDMA and NDBA, no isobaric interferences were observed in blank metformin (data not shown). The determined amount of endogenous NDMA in blank metformin tablet samples was about 8 ppb or 0.8 ng/mL, well below the acceptable limit imposed by the regulatory guidelines. The amount of endogenous NDBA was estimated to be less than 2 ppb or 0.2 ng/mL, and the source of the endogenous NDBA was not from metformin product since same level of NDBA was found in pure methanol blank.

All target nitrosamines were quantified against their respective internal standards except NMPA as its deuterated counterpart was not available; therefore, for consistency, NDPA-D14 was used as a substitute. All calibration curves were constructed by plotting the peak area ratios of standard over internal standard against the concentrations of standard, with a 1/x weighting. Figure 4 shows the calibration curve for all compounds, and Table 6 lists instrument method limits of detection (LOD), limits of quantitation (LOQ), and linearity for all nitrosamines spiked in metformin. Table 7 shows a typical accuracy and precision obtained for 20 ppb or 2 ng/mL spiked metformin samples, well within 15% difference and %RSD. The method exhibits excellent linearity, up to 100 ng/mL, and superior LOQ, less than 20 ppb for all target nitrosamines regardless of which ionization mode was used. While the majority of the nitrosamines have identical LOD and LOQ in both ionization modes, APCI is preferred for the analysis of NDEA, NDIPA, and NDPA as it selectively improves the ionization for these compounds. On the contrary, HESI is preferred for the quantitation of NMBA as this fragile compound may undergo in-source fragmentation if a harder ionization is used, consistent with the previously reported findings on the analysis of the same set of nitrosamines in metformin drug products using the LC-MS/MS solution.[5]

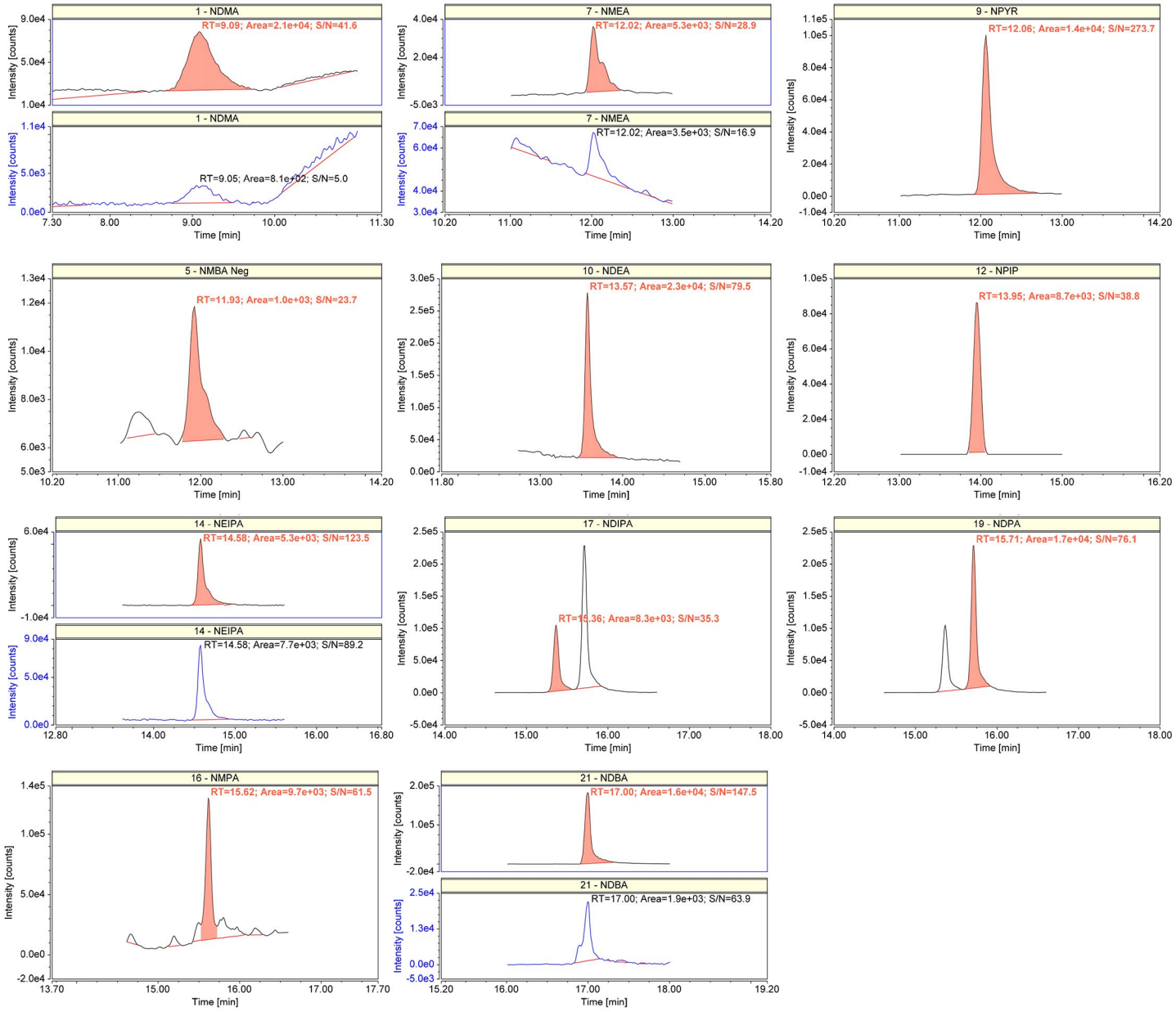

Figure 3. XIC of nitrosamine impurities in 2 ng/mL spiked sample. APCI data are shown.

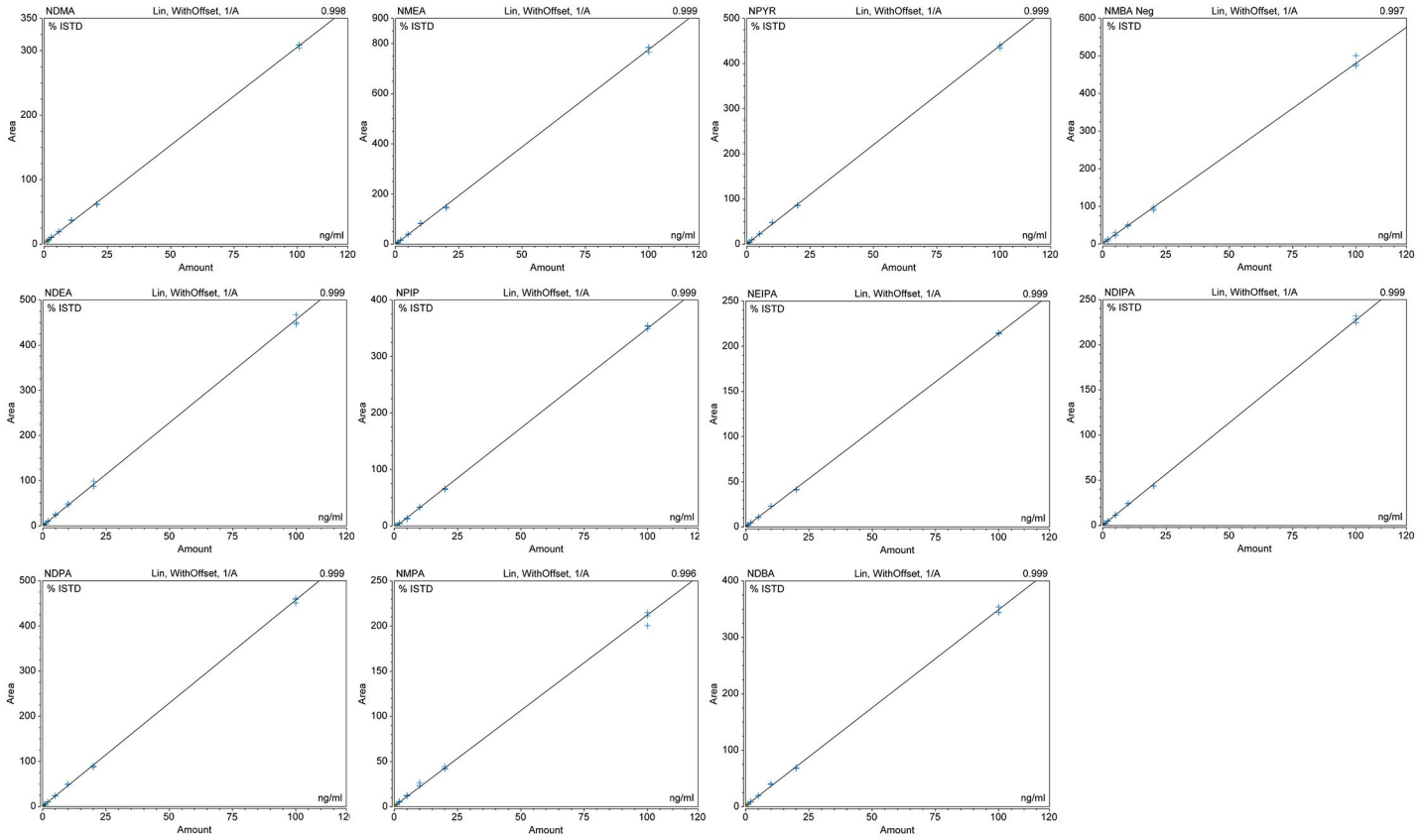

Figure 4. Calibration curves for all nitrosamines in metformin. APCI data are shown.

Table 6. Instrument LOD, LOQ, and linearity for all nitrosamines

	LOD*		LOQ**		Linearity
	ng/mL	PPB[3]	ng/mL	PPB†	
HESI mode					
NDMA	0.5	5	1.0	10	LLOQ – 100
NMEA	0.2	2	0.5	5	
NPYR	0.2	2	0.5	5	
NMBA	0.5	5	1.0	10	
NDEA	0.5	5	1.0	10	
NPIP	0.5	5	1.0	10	
NEIPA	0.2	2	0.5	5	
NDIPA	1.0	10	2.0	20	
NDPA	0.5	5	1.0	10	
NMPA	0.2	2	0.5	5	
NDBA	0.2	2	0.5	5	

	LOD*		LOQ**		Linearity
	ng/mL	PPB[3]	ng/mL	PPB†	
APCI mode					
NDMA	0.5	5	1.0	10	LLOQ – 100
NMEA	0.2	2	0.5	5	
NPYR	0.2	2	0.5	5	
NMBA	1.0	10	2.0	20	
NDEA	0.2	2	0.5	5	
NPIP	1.0	10	2.0	20	
NEIPA	0.2	2	0.5	5	
NDIPA	0.2	2	0.5	5	
NDPA	0.2	2	0.5	5	
NMPA	0.2	2	0.5	5	
NDBA	0.2	2	0.5	5	

*LOD defined as within 20% accuracy, and 15% RSD
**LOQ defined as within 15% accuracy, and 15% RSD
†PPB is calculated based on 100 mg/mL of metformin

Table 7. Accuracy and precision of 2 ng/mL spiked metformin (N=3). APCI data are shown.

	%Accuracy	%RSD
NDMA	98	3.2
NMEA	96	4.9
NPYR	95	1.0
NMBA	87	5.3
NDEA	94	1.8
NPIP	99	8.4
NEIPA	96	2.1
NDIPA	97	1.6
NDPA	96	0.5
NMPA	88	8.4
NDBA	97	2.5

Conclusion

A highly selective and sensitive method was developed using the Hypersil GOLD C18 HPLC column, Vanquish Horizon UHPLC, Orbitrap Exploris 120 mass spectrometer, and Chromeleon CDS software for detection and quantitation of 11 nitrosamines in metformin drug products. This fit-for-purpose method provides adequate chromatographic resolution and versatility for reliable quantitation of NDMA and other nitrosamines that can meet the new regulatory acceptance limits.

References

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