APPLICATION NOTE 000239

Improved quantitation of eight nitrosamine impurities in metformin drug products using a TSQ Fortis Plus mass spectrometer

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Keywords: Nitrosamines, NDMA, DMF, HESI, APCI, selected reaction monitoring, liquid chromatography, TSQ Fortis Plus, mass spectrometry, triple quadrupole, chromatography data system, compliance-ready, genotoxic impurities, metformin

Application benefits

- Detection and quantification of eight nitrosamines in a single liquid chromatography-selected reaction monitoring-mass spectrometry (LC-SRM-MS) method
- Quantitation of nitrosamine impurities in metformin drug products below the daily acceptable intake level, that meets FDA regulatory guidelines
- Compliant data acquisition, processing, and auditing built for regulatory compliant environment

Goal

Demonstrate highly selective and sensitive quantitation of eight nitrosamines in metformin drug products using a single LC-SRM-MS method on a Thermo Scientific™ Vanquish™ Core system coupled to a Thermo Scientific™ TSQ Fortis™ Plus mass spectrometer

Introduction

Nitrosamines have become an emerging group of contaminants in recent drug product recalls.[1] Given the potent genotoxic and carcinogenic nature of certain nitrosamines, regulatory agencies around the world have updated their guidelines to control and limit the formation of these impurities in human medicines.[2,3] To assist analytical testing, the United States Food and Drug Administration (U.S. FDA) has published several validated liquid chromatographic mass spectrometric (LC-MS) methods for the determination of nitrosamine impurities in drug products.[4–7] While a high-resolution mass spectrometric method provides high accuracy and confidence in the measurement and is the preferred primary screening method, it requires higher ownership costs and is less widespread than its triple quadrupole counterparts for targeted quantitative analysis.

Previously, we reported a fit-for-purpose LC-MS/MS method for the quantitation of nitrosamine impurities in metformin drug products.[8] This specific drug product did not contain any detectable DMF, an interferent that has overlapping SRM transitions with NDMA. However, for drug products that may contain significant DMF concentration, it is critical to chromatographically separate the two compounds to avoid overestimation of NDMA. In response to the chromatographic challenge, we developed an alternative LC method using the Vanquish Core HPLC and Thermo Scientific™ Hypersil GOLD C18 HPLC column; this setup was coupled to a TSQ Fortis Plus mass spectrometer for highly selective and sensitive quantitation of eight nitrosamines in the same drug products. Not only can the method separate NDMA from DMF, this method can provide detection and quantitation limits comparable to high-resolution methods (data not shown).

The new TSQ Fortis Plus mass spectrometer comes with advanced active ion management plus (AIM+) technology, a design that incorporates segmented quadrupoles and enhanced RF electronics, enabling ultra-fast polarity switching speed and improved Q2 active collision cell. These improvements provide the ultimate robust quantitative performance that is required to meet the emerging analytical challenges associated with nitrosamine impurity analysis.

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, Thermo Scientific™ 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

Reference standards: Reference standards ranging from 0.2 to 100 ng/mL were prepared using the same procedure as outlined in a previous application note.[4]

200 ppm DMF spiked standard (e.g., 20 ng/mL) was prepared by adding 0.2 µL of pure DMF to 1 mL of 20 ng/mL neat standard solution. 20 ppm DMF spiked standards were prepared by diluting pure DMF with pure methanol 1/10 (v/v) prior to adding to the standard solution.

Metformin drug product preparation: Metformin drug tablets and spiked samples were prepared using the same procedure as outlined in a previous application note.[8]

Table 1. Nitrosamine reference standards

Standards	CAS	Vendor	P/N
N-Nitrosodimethylamine (NDMA)	62-75-9	Restek	31898
N-Nitrosodiethylamine (NDEA)	55-18-5		
N-Nitrosodi-n-butylamine (NDBA)	924-16-3		
N-Nitroso-di-n-propylamine (NDPA)	621-64-7		
N-Ethyl-N-nitroso-2-propanamine (NEIPA)	16339-04-1	Enamine	EN300-1296534
N-Nitroso-di-isopropylamine (NDIPA)	601-77-4	Enamine	EN300-7456222
N-Nitroso-n-methyl-4-aminobutyric acid (NMBA)	61445-55-4	Cambridge Isotopes	ULM-10857-1.2
N-Nitrosomethylphenylamine (NMPA)	614-00-6	Toronto Research Chemicals	N529925
NDMA-D_6	62-75-9	Restek	33910
NDEA-D_{10}	55-18-5	Cambridge Isotopes	DLM-7982-S
NDBA-D_{18}	924-16-3	CDN Isotopes	D-6711-0.05g
NDPA-D_{14}	621-64-7	Cambridge Isotopes	DLM-2131-S
NEIPA-D_5	16339-04-1	Toronto Research Chemicals	E932796
NDIPA-D_{14}	601-77-4	Toronto Research Chemicals	N525602
NMBA-$^{13}C_4$	61445-55-4	Cambridge Isotopes	CLM-10856-1.2

LC-MS method

A single targeted LC-SRM-MS method was developed using a Vanquish Core system coupled to a TSQ Fortis Plus mass spectrometer.

The Vanquish Core system consists of the following modules:

- Thermo Scientific™ System Base Vanquish™ Core (P/N VC-S01-A-02)
- Thermo Scientific™ Vanquish™ Binary Pump C (P/N VC-P10-A-01)
- Thermo Scientific™ Vanquish™ Split Sampler CT (P/N VC-A10-A-02)
- Thermo Scientific™ Vanquish™ Column Compartment C (P/N VC-C10-A-03)
- Thermo Scientific™ Vanquish™ Diode Array Detector (P/N VF-D11-A-01)

Unless otherwise stated, 6 µL of undiluted sample extract were injected onto a 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 scan settings as outlined in Tables 3 and 4, respectively.

Software

Compliant-ready Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.2.10 software was used for both data acquisition and analysis to meet regulatory requirements including U.S. FDA 21 CFR Part 11 and European Commission (EU) Annex 11. TSQ Fortis Plus tune version 3.4 was used for instrument control.

Table 2. LC and autosampler conditions

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		
Solvent A	Water + 0.1% formic acid		
Solvent B	Methanol + 0.1% formic acid		
Gradient	Time (min)	% Solvent A	% Solvent B
	0.0	98	2
	5	98	2
	13	10	90
	21	10	90
	21.5	98	2
	30	98	2
Injection volume	6 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		
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	3,500 V	—
Spray current	—	4 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	SRM
Q1 FWHM (Da)	1.2
Q3 FWHM (Da)	1.2
Chromatographic peak width (s)	30
Points across peak	15
CID gas (mTorr)	1.5

Results and discussion

Chromatographic separation of nitrosamine impurities

Due to the presence of high concentration of drug substance and complex formulation, it is imperative to chromatographically separate interfering species for accurate detection and quantification of nitrosamine impurities using the LC-SRM-MS method. As shown in Figure 1, this gradient using the Hypersil GOLD C18 HPLC column could effectively separate metformin from the rest of nitrosamines. With the 30-minute cycle time, all nitrosamines, except for NDPA and NMPA, were baseline resolved. In addition, this method allows adequate separation of NDMA from DMF, providing high selectivity for accurate and reliable quantification of NDMA. As illustrated in Figure 2, DMF elutes after NDMA; an isobaric interfering peak was detected as a result of contribution from low abundant ^{15}N and ^{13}C-DMF isotopes. It appears that the level of interference is significantly less for the qualifier ion (*m/z* 58.05) compared to the quantifier ion (*m/z* 43.03), making it a possible alternative for quantification of NDMA in presence of high DMF concentration (e.g., 200 ppm).

Achieving regulatory performance limits

Like the previously reported LC-SRM-MS method, this method can operate in either HESI or APCI mode for all target nitrosamines (Table 5). Figure 3 shows a comparison of the extracted ion chromatogram (XIC) of each nitrosamine impurity in blank metformin with a 2 ng/mL spiked sample. Although small amounts of endogenous NDMA and NDBA were detected in blank metformin, the levels were below the instrument method quantitation limit, and the estimated total nitrosamine content in the tested drug product was below the acceptable limit imposed by the regulatory guidelines. Unlike NDMA, the source of the endogenous NDBA was not from metformin product since the same level of NDBA was found in pure methanol blank. These results were also verified by a high-resolution accurate mass method using a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer.[5]

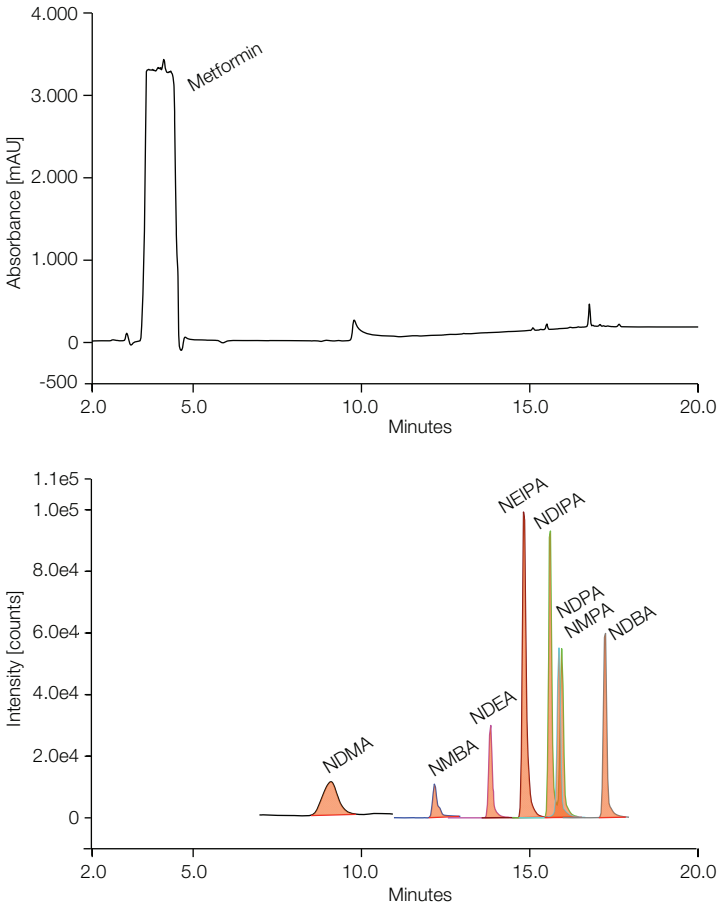

Figure 1. Chromatographic separation of metformin from nitrosamines (e.g., 100 ng/mL spiked sample) using the LC-SRM-MS method. Example UV trace collected at 230 nm (top), and XIC of quantifier ions collected in APCI mode (bottom) are shown.

The instrument method limits of detection (LOD), limits of quantitation (LOQ), and linearity were evaluated for target nitrosamines in both HESI and APCI mode. All reference nitrosamine standards 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. As shown in Table 6, this method can quantify these impurities below 20 ppb and 10 ppb using the HESI and APCI source, respectively, with linearity up to 1,000 ppb relative to metformin concentration. Figure 4 shows sample calibration curves with 1/x weighting, all of which had a linear regression coefficient above 0.997. Table 7 shows typical accuracy and precision obtained for 20 ppb spiked metformin samples, well within 15% difference and %RSD.

Table 5. Optimized MS parameter settings for target nitrosamines

	Precursor ion (*m/z*)	Quantifier ion (*m/z*)	Qualifier ion (*m/z*)	CE (V) Quan, Qual	Tube lens (V)	Source fragmentation (V)
NDMA	75.055	43.03	58.054	15.57, 12.75	90	30
NDMA-D6	81.093	46.113	—	17.93	53	14.7
NMBA	147.076	117.125	44.018	7.32, 14.22	90	15
NMBA-13C4	151.09	121.125	—	6.4	99	5
NDEA	103.087	75.113	29.208	11.19, 14.65	90	25
NDEA-D10	113.149	81.196	—	12.67	61	9.8
NEIPA	117.102	75.054	47	10.48, 16.67	90	20
NEIPA-D5	122.134	80.125	—	10.69	61	6.5
NDIPA	131.118	89.125	47	9.34, 14.56	90	25
NDIPA-D14	145.206	97.155	—	10.31	56	6.5
NMPA	137.1	66.1	107.1	19, 12	90	30
NDBA	159.149	103.054	41.054	11.36, 17.72	90	15
NDBA-D18	177.262	113.137	—	12.79	64	8.2

Enhanced detection of nitrosamines with better confidence

With the improved design of the active collision cell, the dissociation of energetically stable nitrosamines increases and results in better confidence and determination of nitrosamines using the TSQ Fortis Plus mass spectrometer. To demonstrate this enhancement, the same method was executed on a TSQ Fortis mass spectrometer and peak areas of selected nitrosamines were compared with the results obtained on TSQ Fortis Plus system. While the majority of nitrosamines had a slight gain in integrated peak area (e.g., between 1.1 and 1.5), and the amount varied between the quantifier and qualifier ion, a few had up to 4x improvement in integrated peak area. For instance, as shown in Figure 5, the NDMA quantifier ion had a 2x improvement in peak area but only about a 15% increase for the qualifier ion; on the contrary, the NDEA qualifier ion had a 4x improvement in peak area but only a 25% increase for the quantifier ion. These measurements were taken at 200 ppb where the %RSD for five replicate injections were below 3%; therefore, the observed gains are statistically significant and are likely not attributed to sample injection and spray variations. These improvements could provide higher confidence in the determination of LOD and LOQ and enable flexibility to switch between quantifier and qualifier ion for better selectivity as in the case of NDMA when closely eluting interferences are present.

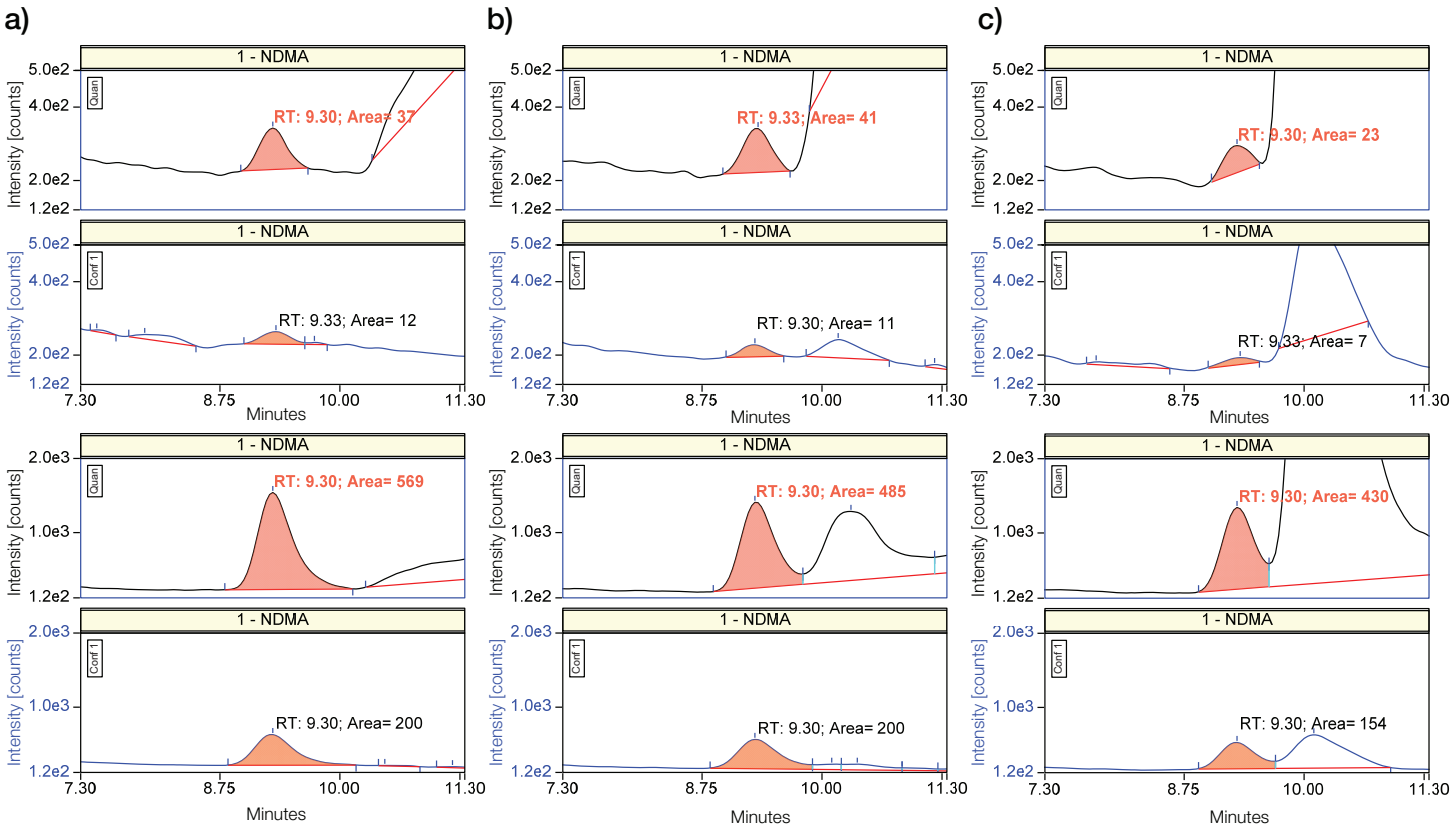

Figure 2. Chromatographic separation of DMF from NDMA. Example XIC of 20 ppb (top) and 200 ppb (bottom) NDMA a) in neat, b) with 20 ppm DMF, and c) with 200 ppm DMF; APCI data are shown and the injection volume is 3 µL.

a) Blank metformin

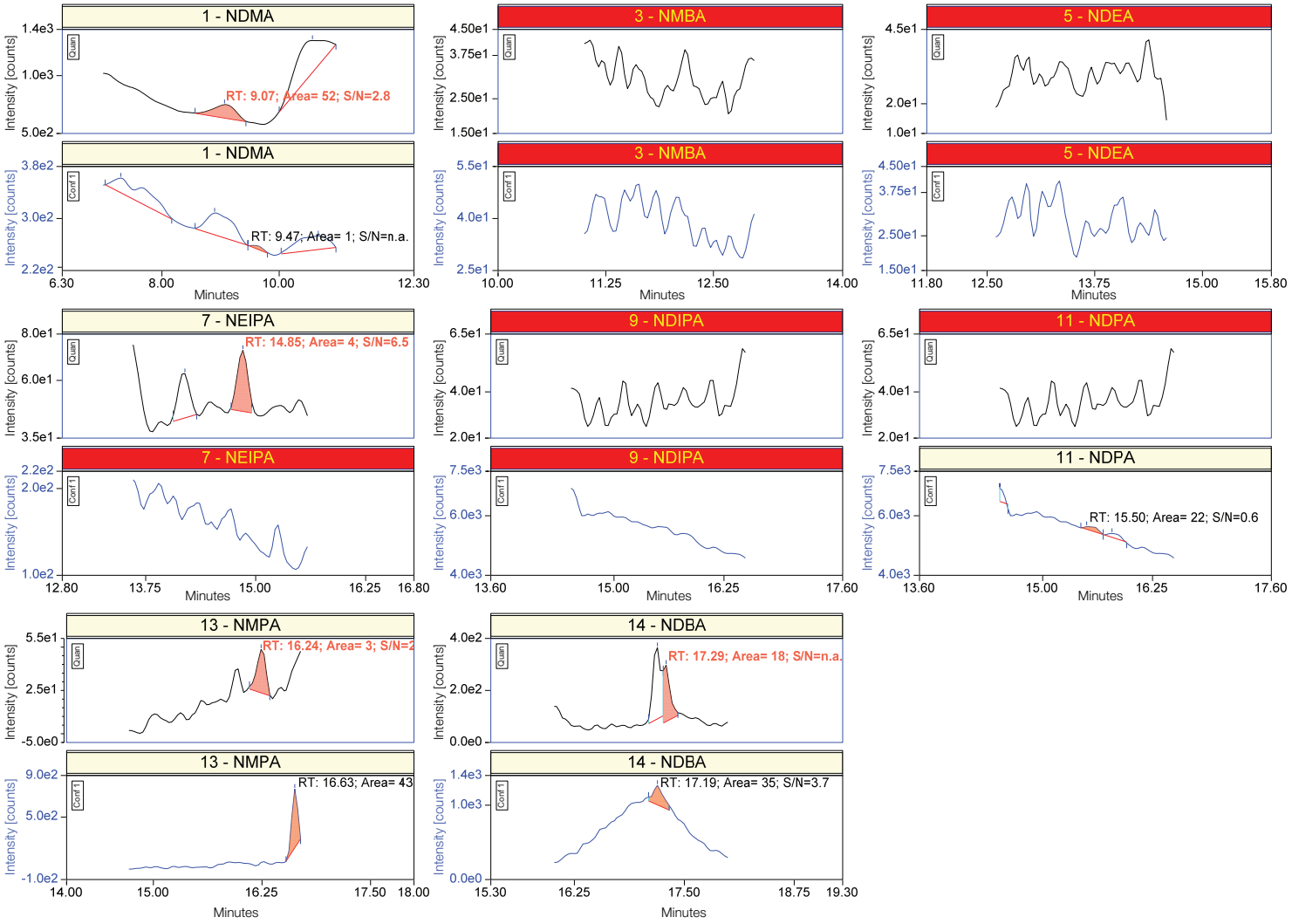

Figure 3a. XIC of nitrosamine impurities in blank metformin. APCI data are shown.

b) 2 ng/mL spiked metformin

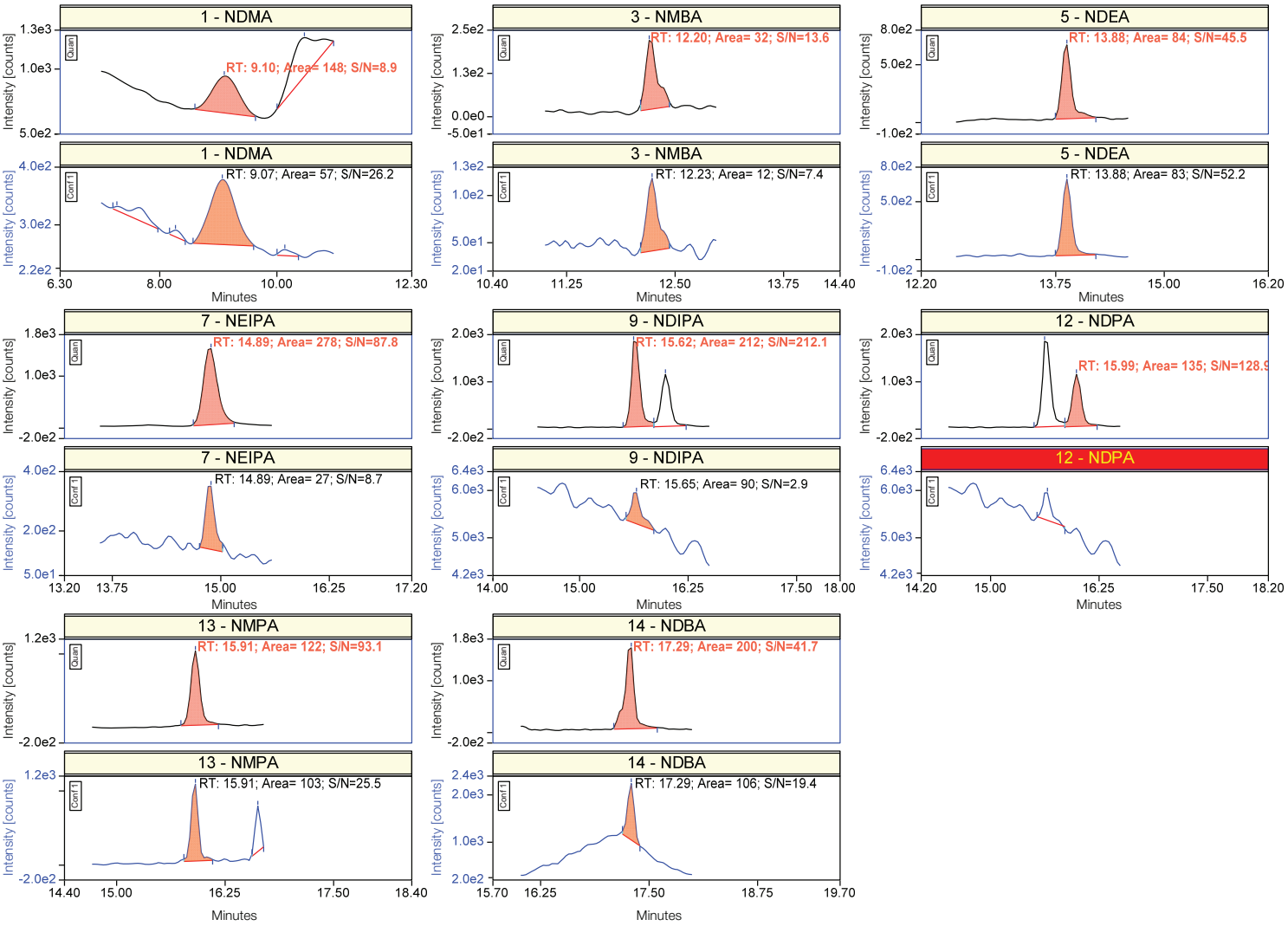

Figure 3b. XIC of nitrosamine impurities in 2 ng/mL spiked sample. APCI data are shown.

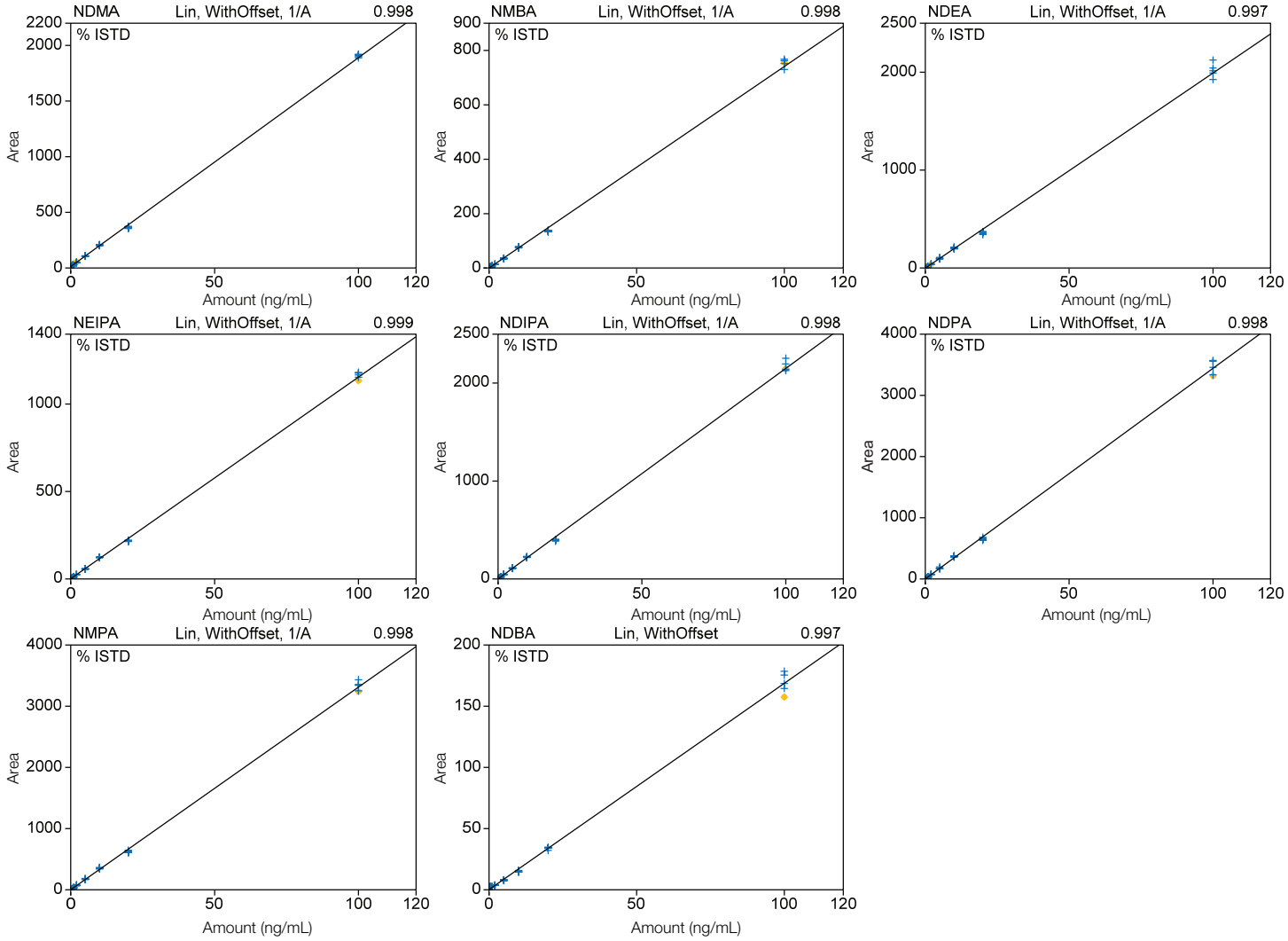

Figure 4. Calibration curves for all nitrosamines in metformin. APCI data are shown.

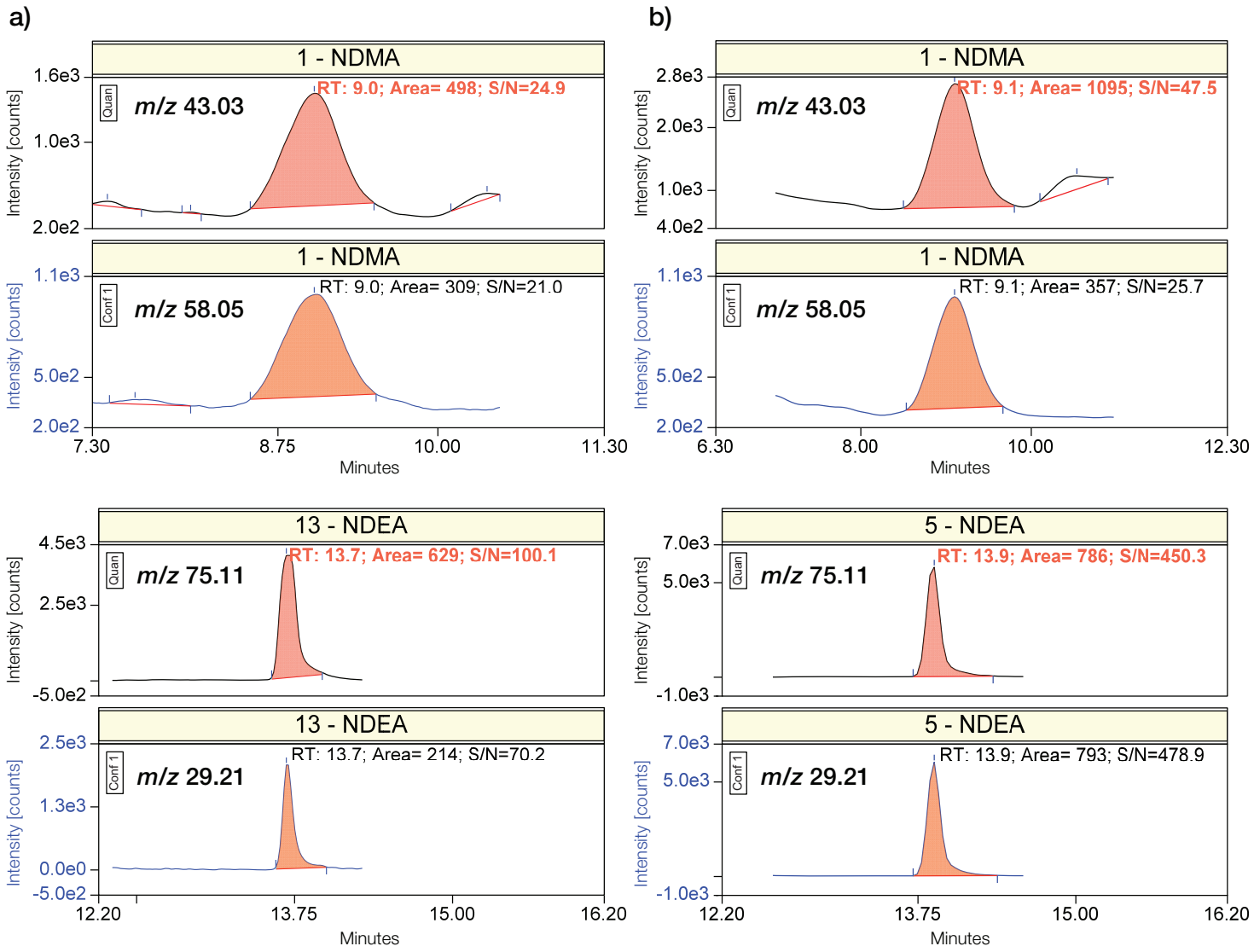

Figure 5. Peak area comparison for NDMA and NDEA collected on a) TSQ Fortis mass spectrometer and b) TSQ Fortis Plus mass spectrometer. 200 ppb spiked metformin, APCI data are shown.

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

a) HESI mode

Standards	LOD		LOQ		Linearity
	ng/mL	PPB	ng/mL	PPB	
NDMA	0.5	5	1.0	10	LLOQ – 100
NMBA	0.2	2	0.5	5	
NDEA	0.2	2	0.5	5	
NEIPA	0.2	2	0.5	5	
NDIPA	0.5	5	1.0	10	
NDPA	1.0	10	2.0	20	
NMPA	0.5	5	1.0	10	
NDBA	0.2	2	0.5	5	

b) APCI mode

Standards	LOD		LOQ		Linearity
	ng/mL	PPB	ng/mL	PPB	
NDMA	0.5	5	1.0	10	LLOQ – 100
NMBA	0.5	5	1.0	10	
NDEA	0.2	2	0.5	5	
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	

1. LOD defined as within 20% accuracy, and 15% RSD
2. LOQ defined as within 15% accuracy, and 15% RSD
3. PPB is calculated based on 100 mg/mL of metformin

Table 7. Accuracy and precision of 2 ng/mL spiked metformin (n=5), APCI data are shown.

Standards	% Accuracy	% RSD
NDMA	102	8.3
NMBA	95	2.4
NDEA	104	3.4
NEIPA	105	1.7
NDIPA	105	2.5
NDPA	106	2.1
NMPA	107	6.1
NDBA	106	2.8

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Conclusion

A highly selective and sensitive method was developed using the Hypersil GOLD C18 HPLC column, Vanquish Core HPLC, TSQ Fortis Plus mass spectrometer, and Chromeleon software for detection and quantitation of eight 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 will meet the new regulatory acceptance limits.

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