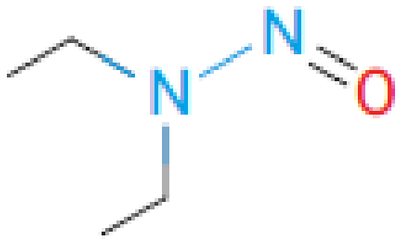
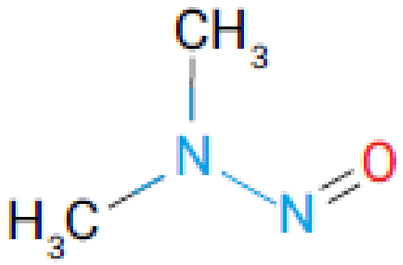
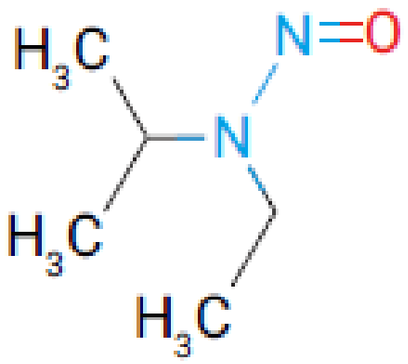
Nitrosamine Impurities Application Guide

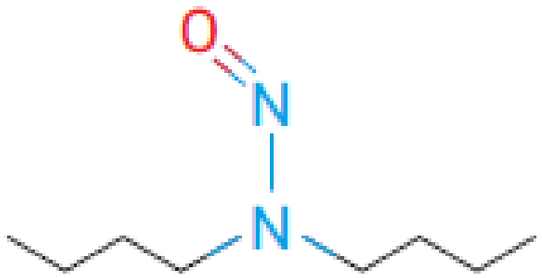
Confidently Detect and Quantify Mutagenic Impurities in APIs and Drug Products

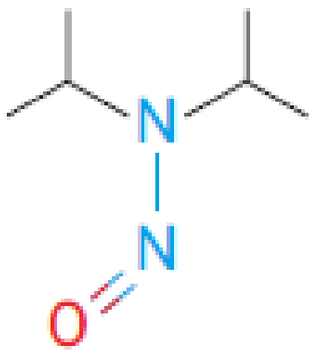
Introduction

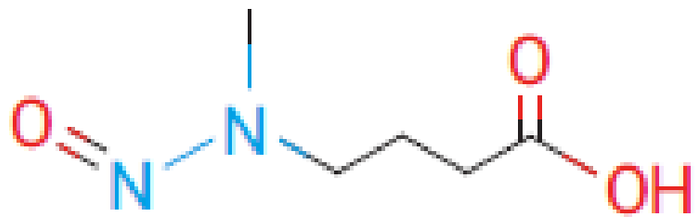












Mutagenic impurities in APIs and drug products pose a significant risk to health and safety—even in small quantities—and thus are a major concern for drug makers. Mutagenic impurities can damage DNA, leading to mutations and potentially cancer.

Nitrosamines are formed by chemical reactions that occur during API manufacturing whether from starting materials, intermediates, reactants, reuse of solvents and by-products; they can form through degradation products generated during formulation or storage or from environmental contaminants. Recently, nitrosamines have been found in sartan drugs, a class of medications used to treat high blood pressure and heart failure, prompting recalls of angiotensin receptor blockers (ARBs)—valsartan, losartan, and irbesartan—which were contaminated with N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA), two carcinogenic impurities. Since then, several other N-nitrosamines have also been identified and are being investigated by regulators: N-Nitrosodiispropylamine (NDIPA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodibutylamine (NDBA), and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA). Nitrosamines have now also been identified in ranitidine medications (which are used to treat heartburn and acid reflux) and metformin, an oral diabetes medication.

For detailed info, refer to: FDA Press Releases; EMA Press Releases

Diverse Pathways for Nitrosamine Formation

Regulatory agencies advise companies on steps to take to avoid nitrosamines in medicines.

Risky preparations must be tested for nitrosamine contamination via validated and appropriately sensitive analytical methods. Manufacturers must inform authorities of nitrosamine detection, irrespective of the amount detected.

Nitrosamines in Recent News!

Efforts to address and control the presence of trace levels of mutagenic impurities is of special concern to global regulators. As a result, US FDA and other regulatory agencies have taken steps to address the issue of mutagenic impurities in pharmaceuticals.

July 2018

- FDA recalled valsartan DP due to NDMA contaminant

Oct 2018

- FDA expanded recall to other sartans, due to NDMA & NDEA

Feb 2019

- FDA expanded recall as new contaminant, NDMA identified

April 2019

- FDA identified additional contaminants, EIPNA & DIPNA

April 2020

- FDA recalled ranitidine-based drugs

May 2020

- FDA recalled metformin-based drugs

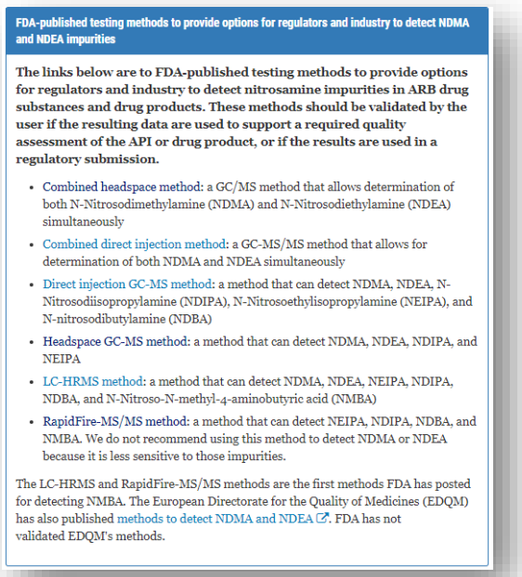
Detection and quantification of these trace nitrosamines in APIs and drug products can be challenging and necessitates the use of advanced and sensitive tools to meet regulatory requirements. Agilent offers reliable systems and solutions that not only fulfill FDA directives, but also meet and exceed FDA's established regulatory requirements to help pharmaceutical customers identify and quantify trace (ppb) nitrosamine and other mutagenic impurities confidently.

Sartan-Based
Valsartan
Losartan
Telmisartan
Candesartan
Irbesartan
Olmesartan
Pregabalin
Ranitidine
Metformin

Sartan-Based Drugs

- Valsartan, losartan, irbesartan and other “-sartan” drugs are a class of medicines known as angiotensin II receptor blocker (ARBs) used to treat high blood pressure and heart failure
- Regulatory agencies reported that some generic versions of the angiotensin II receptor blocker (ARB) medicines contain nitrosamine impurities that don’t meet the agency’s safety standards
- Regulatory agencies (for e.g. including US Food and Drug administration (US FDA)) published guidance on the detection and quantification of nitrosamine impurities in sartan-based drugs

US FDA



FDA-published testing methods to provide options for regulators and industry to detect NDMA and NDEA impurities

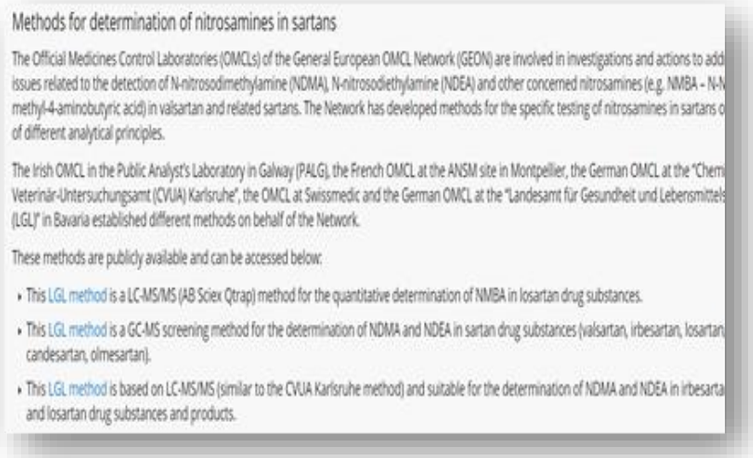
The links below are to FDA-published testing methods to provide options for regulators and industry to detect nitrosamine impurities in ARB drug substances and drug products. These methods should be validated by the user if the resulting data are used to support a required quality assessment of the API or drug product, or if the results are used in a regulatory submission.

- Combined headspace method: a GC/MS method that allows determination of both N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) simultaneously
- Combined direct injection method: a GC-MS/MS method that allows for determination of both NDMA and NDEA simultaneously
- Direct injection GC-MS method: a method that can detect NDMA, NDEA, N-Nitrosodiisopropylamine (NDIPA), N-Nitrosoethylisopropylamine (NEIPA), and N-nitrosodibutylamine (NDBA)
- Headspace GC-MS method: a method that can detect NDMA, NDEA, NDIPA, and NEIPA
- LC-HRMS method: a method that can detect NDMA, NDEA, NEIPA, NDIPA, NDBA, and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA)
- RapidFire-MS/MS method: a method that can detect NEIPA, NDIPA, NDBA, and NMBA. We do not recommend using this method to detect NDMA or NDEA because it is less sensitive to those impurities.

The LC-HRMS and RapidFire-MS/MS methods are the first methods FDA has posted for detecting NMBA. The European Directorate for the Quality of Medicines (EDQM) has also published methods to detect NDMA and NDEA. FDA has not validated EDQM's methods.

https://www.fda.gov/media/131868/download

Council of Europe



Methods for determination of nitrosamines in sartans

The Official Medicines Control Laboratories (OMCLs) of the General European OMCL Network (GEON) are involved in investigations and actions to addi issues related to the detection of N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA) and other concerned nitrosamines (e.g. NMBA - N-N methyl-4-aminobutyric acid) in valsartan and related sartans. The Network has developed methods for the specific testing of nitrosamines in sartans o of different analytical principles.

The Irish OMCL in the Public Analyst's Laboratory in Galway (PALG), the French OMCL at the ANSM site in Montpellier, the German OMCL at the "Chem Veterinär-Untersuchungsamt (CVUA) Karlsruhe", the OMCL at Swissmedic and the German OMCL at the "Landesamt für Gesundheit und Lebensmittels (LGL)" in Bavaria established different methods on behalf of the Network.

These methods are publicly available and can be accessed below:

- This LGL method is a LC-MS/MS (AB Sciex Qtrap) method for the quantitative determination of NMBA in losartan drug substances.
- This LGL method is a GC-MS screening method for the determination of NDMA and NDEA in sartan drug substances (valsartan, irbesartan, losartan candesartan, olmesartan).
- This LGL method is based on LC-MS/MS (similar to the CVUA Karlsruhe method) and suitable for the determination of NDMA and NDEA in irbesarta and losartan drug substances and products.

https://www.edqm.eu/en/news/omcls-release-three-methods-determination-ndma-sartans

Health Canada

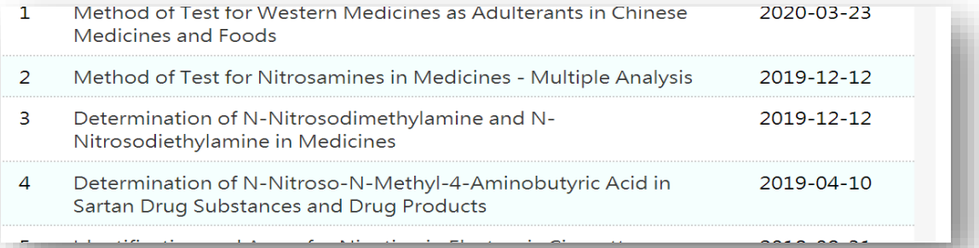
MENU

Canada.ca > Departments and agencies > Health Canada > Drugs and health products > Compliance and enforcement: Drug and health products > Information by Health Product > Drugs

Impurities found in certain angiotensin II receptor blocker (ARB) products, also known as sartans

https://healthycanadians.gc.ca/recall-alert-rappel-avis/hc-sc/2020/72963a-eng.php

Taiwan FDA



1	Method of Test for Western Medicines as Adulterants in Chinese Medicines and Foods	2020-03-23
2	Method of Test for Nitrosamines in Medicines - Multiple Analysis	2019-12-12
3	Determination of N-Nitrosodimethylamine and N-Nitrosodiethylamine in Medicines	2019-12-12
4	Determination of N-Nitroso-N-Methyl-4-Aminobutyric Acid in Sartan Drug Substances and Drug Products	2019-04-10

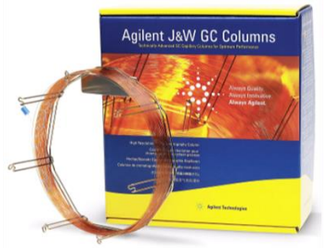
https://www.fda.gov.tw/ENG/siteList.aspx?sid=10360

Sartan-Based Drugs

Mutagenic Impurity Analysis GC/MS Workflow Solution

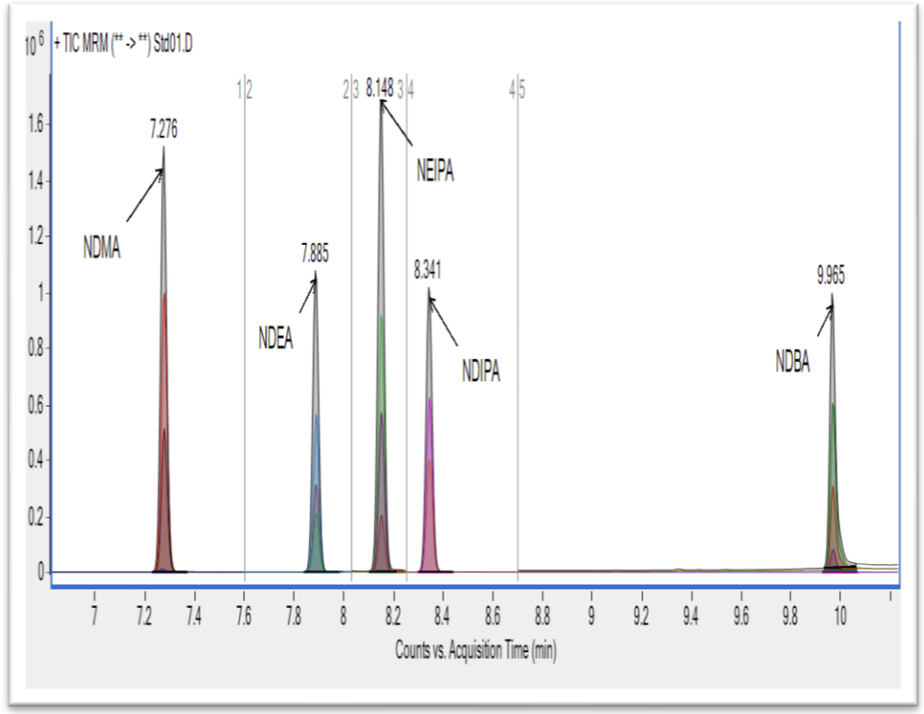
Separate	Acquire	Analyze

[8890 GC/7697A HSS/ 5977B GC/MSD]



[WAX GC Columns]

[8890 GC/7693 LS/ 7010B GC/TQ]



[MassHunter Software]

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Sartan-Based Drugs

Agilent GC/MS Solution for Analysis of Nitrosamines

Typical Configuration

GC	**Add 8890 GC and ALS with one of the MS Options**		
	G3540A	Agilent 8890 GC System	112, 201, 313 (for TQ only)
	G4513A	7693A Autoinjector	NO OPT
	G4514A	7693A Tray, 150 Vial	NO OPT
TQ	**MS Option 1**		
	G7012BA	7010B Quadrupole MS/MS EI Bundle	#010 (optional),245
HSS –GC with SQ	**MS Option 2**		
	G7079BA	5977B High Efficiency Source (HES) EI GC/MSD	#010 (optional),245
	G4557A	7697A Headspace Sampler, 111 Vial capacity	NO OPT
	G3449A	8890/8860 Transfer Line Interface Accessory	NO OPT
	MS Option 3		
	G7077BA	5977B InertPlus EI GC/MSD	#010 (optional),245
	G4557A	7697A Headspace Sampler, 111 Vial capacity	NO OPT
	G3449A	8890/8860 Transfer Line Interface Accessory	NO OPT

8890 GC/7697A HSS/ 5977B GC/MSD

8890 GC/7693 LS/ 7010B GC/TQ

Application Area

Analytes	NDMA, NDEA, NEIPA, NDIPA, NDBA
Matrices	Sartan drug substances and drug products
Customers	Pharmaceuticals and contract labs

Columns and supplies

Columns J&W VF-WAXms GC Column, 30 m, 0.25 mm, 1.00 µm, 7 inch cage (CP9206)

GC Vials and Caps: Screw top MS analyzed vial kit (5190-2277)

Syringe Filter Paper: Nylon, 0.45 µm (5190-5091)

GC Inlet Liner: Ultra Inert, splitless, single taper, glass wool (5190-2293)

Highlights – GC/MS/MS approaches

- ❑ Cost effective, easy to use
- ❑ Quick implementation in labs
- ❑ Optimized methods and RTL based MRMs
- ❑ More API can be used (100 mg/mL or more) for sample prep.
- ❑ Most APIs are insoluble in Dichloromethane, so it doesn't overload column
- ❑ Easy sample preparation
- ❑ All sartans can be analyzed by a single GC/MS/MS method. No method modification because of API or formulation. Lower detection limits can be achieved

Sartan-Based Drugs

Mutagenic Impurity Analysis LC/MS Workflow Solution

Separate

InfinityLab Poroshell Column

1290 Infinity II LC System

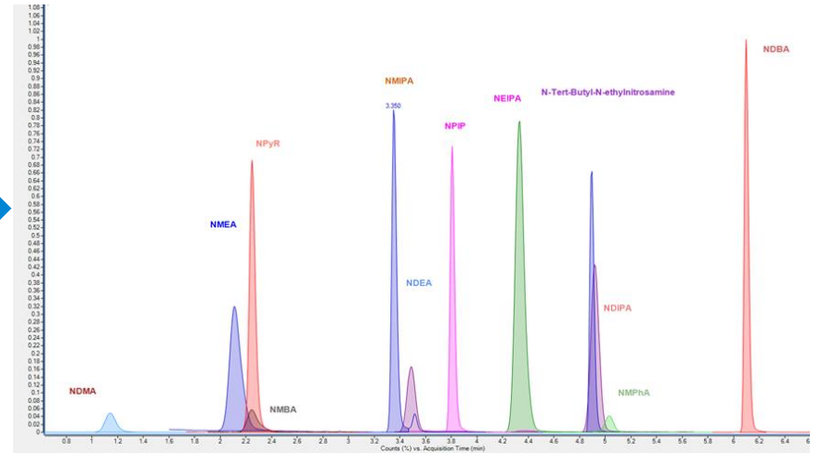
Acquire

6470 LC/TQ

Ultivo LC/TQ

6546 LC/Q-TOF

Analyze



MassHunter Software

Confirmatory analysis of nitrosamine impurities in sartan-based drug substances and drug products are presented per the US FDA published method here

Back to Introduction

Sartan-Based Drugs

Agilent LC/MS Solution for Nitrosamines Analyses

Typical LC Configuration
Agilent 1290 Infinity II High-Speed Pump (G7120A)
Agilent 1290 Infinity II Multisampler (G7167B)
Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
Agilent 1290 Infinity II Variable Wavelength Detector (G7114B)

HPLC

1290 Infinity II LC System

TQ

6470LC/TQ

Ultivo LC/TQ

Application Area	
Analytes	NDMA, NDEA, NEIPA, NDIPA, NDBA and NMBA
Matrices	Sartan drug substances
Customers	Pharmaceuticals and contract labs

Columns and supplies

Columns: Varies for each sartan drug

HPLC Vials and Caps: Vial, screw 2mL Amber p/n 5182-0716 and Cap p/n 5183-2077

Syringe Filter Paper: 5190-5261 (PVDF, 13mm 0.2 µm)

Highlights – LC/MS/MS approaches

- ❑ Easy to operate
- ❑ Quick implementation in labs
- ❑ Optimized methods
- ❑ Sample size used as per US FDA recommendations
- ❑ Easy sample preparation
- ❑ Valsartan API elutes after all nitrosamines, so diverter valve programmed accordingly

GC/MS Method for Analysis

Instrument Method

ALS	GC	MS
Injection Volume: 2µL	Carrier Gas: He 1mL/min	EI Mode

Parameter	Value
MMI injection mode	Pulsed splitless: 12.285 psi until 0.5 min
Inlet temperature	250 °C
Oven temperature program	40 °C (0.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)
Total run time	12.33 min
MS transfer line temperature	250 °C

Parameter	Value	
Source temperature	250 °C	
Quadrupole temperature	Q1 and Q2 = 150 °C	
MS1 and MS2 resolution	All compounds Unit	
Collision gas flow	Nitrogen at 1.5 mL/min,	
Quenching gas flow	Helium at 4 mL/min	
Quant./qual. transitions (FDA method)	Start time: 6.5 min NDMA	74 → 44, CE 15, dwell 150 ms 74 → 42, CE 20, dwell 50 ms NDMA:C13-d_6 82 →48, CE 20, dwell 100 ms
	Start time: 7.60 min NDEA	102 →85, CE 10 V, dwell 150 ms 102 →56, CE 18 V, dwell 150 ms
	Start time: 8.03 min NEIPA	116 →99, CE 10 V, dwell 150 ms 71 →56, CE 10 V, dwell 150 ms
	Start time: 8.25 min NDIPA	130 →88, CE 10 V, dwell 150 ms 130 →42, CE 10 V, dwell 150 ms
	Start time: 8.70 min NDBA	158 →99, CE 10 V, dwell 150 ms 84 →56, CE 22 V, dwell 150 ms

Sample Preparation

For API

- 500 mg of Drug substance
- Add 5 mL NDMA-C13-D6 internal standard prepared in Dichloromethane (50 ng/mL
- Vortex for 1 min followed by centrifugation for 2 min at 4000 rpm
- Filter 1 mL supernatant through a 0.45 µm filter paper in a GC vial

For Drug Product

- Weight tablet amount equivalent to 500 mg of API
- Add 5 mL NDMA-C13-D6 internal standard prepared in Dichloromethane (50 ng/mL
- Vortex for 1 min followed by centrifugation for 2 min at 4000 rpm
- Filter 0.5 mL supernatant through a 0.45 µm filter paper in a GC vial

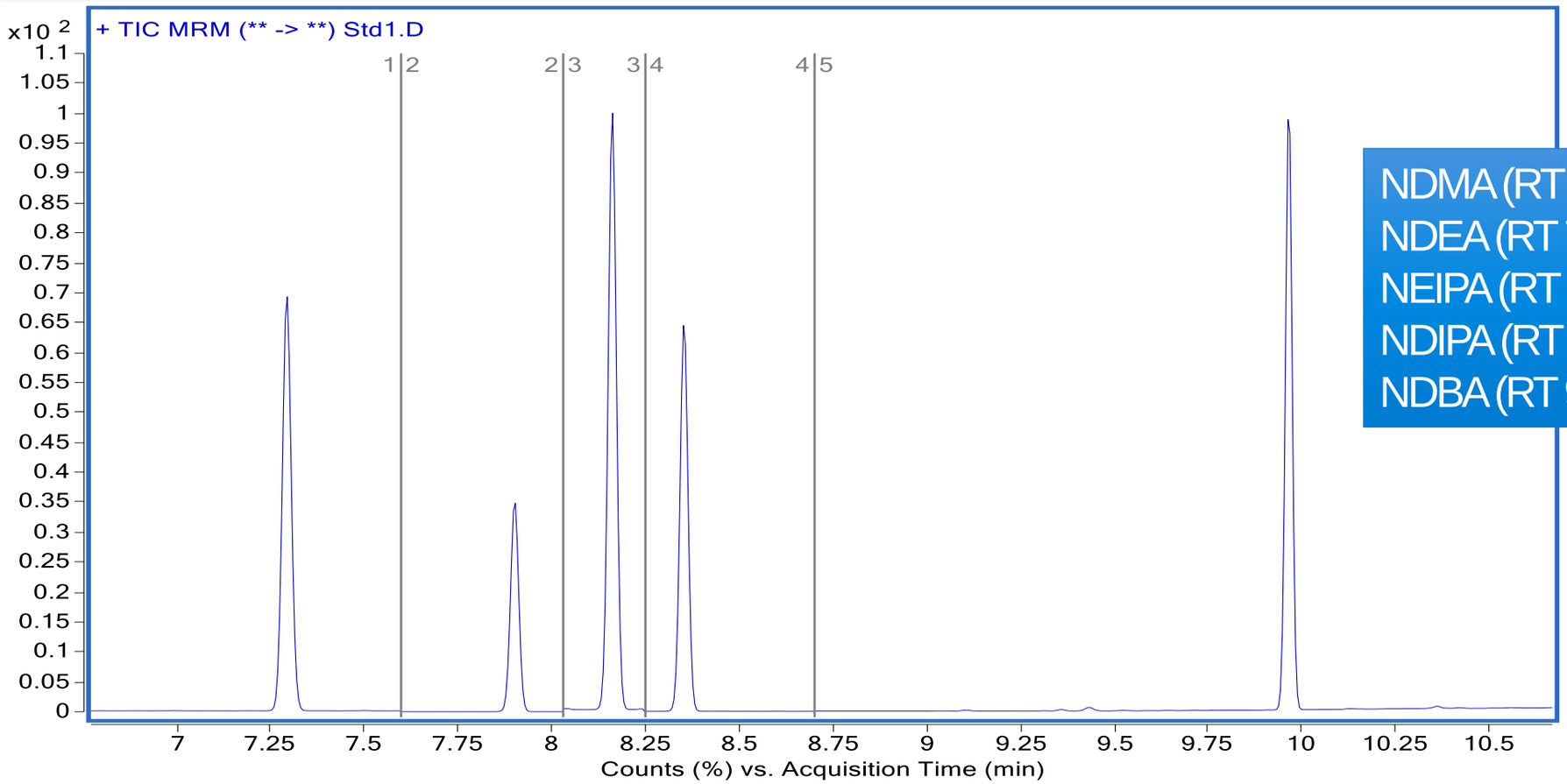
Calibrations

2.5 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 40 ng/ml, 80 ng/ml and 100 ng/ml each prepared in Dichloromethane containing 50 ng/mL of NDMA –C13-D6

System Suitability

The coefficient of determination (R2) of the linear calibration curve should be ≥ 0.998.
The S/N ratio of the 5 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 40 ng/mL standard should be ≤ 5

Results for 5 nitrosamine impurities at 100 ng/mL in Valsartan API

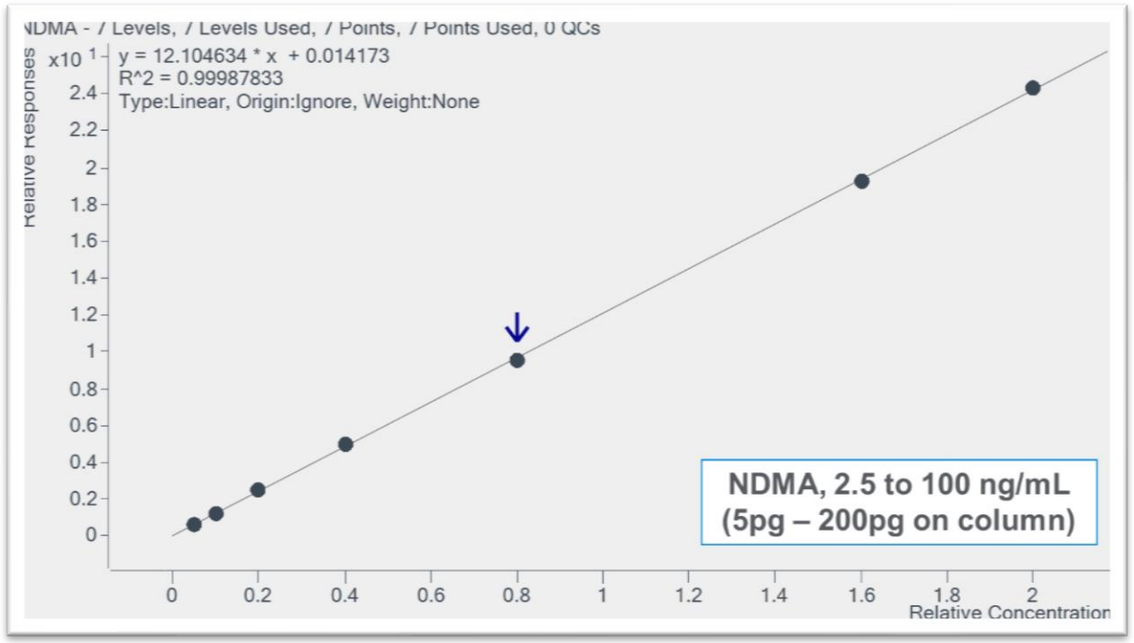


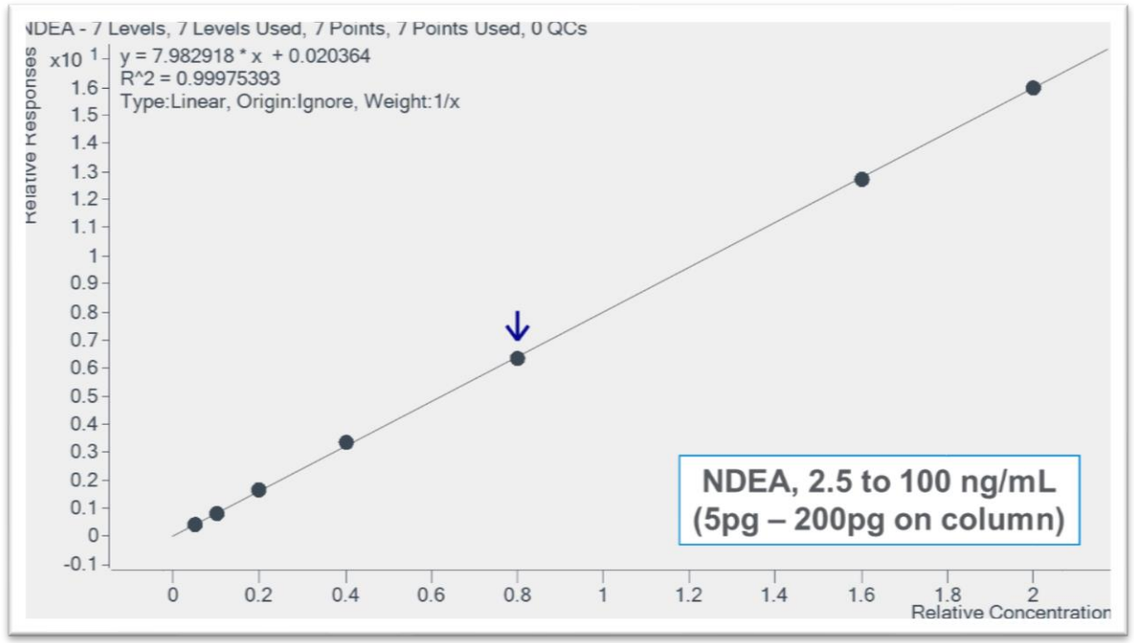
NDMA (RT 7.294 min)
NDEA (RT 7.903 min)
NEIPA (RT 8.157 min)
NDIPA (RT 8.346 min)
NDBA (RT 9.777 min)

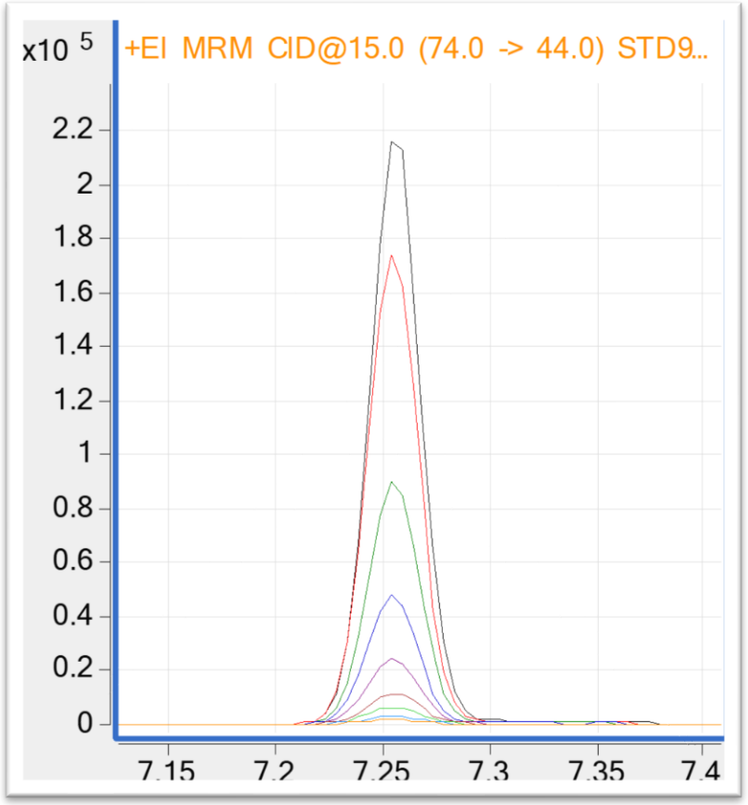
Benefits Agilent GC/TQ	
Optimized methods	• Optimized method for both API and Formulation • Compatible with stringent FDA regulations
Scalable application	• Best precision = best ion ratios = best quant results Rugged ion source design • Retention Time Locking for reproducible methods over time and between labs
Sample prep	• Sample preparation as per FDA guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument. • Efficient Quant review with MassHunter • Data Integrity

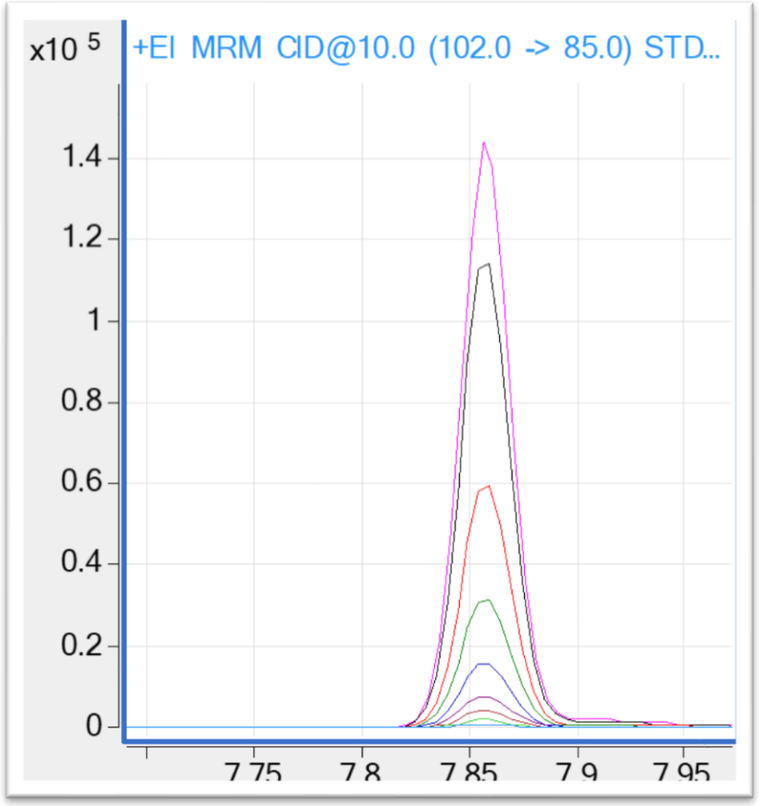
Valsartan

Calibration Curves



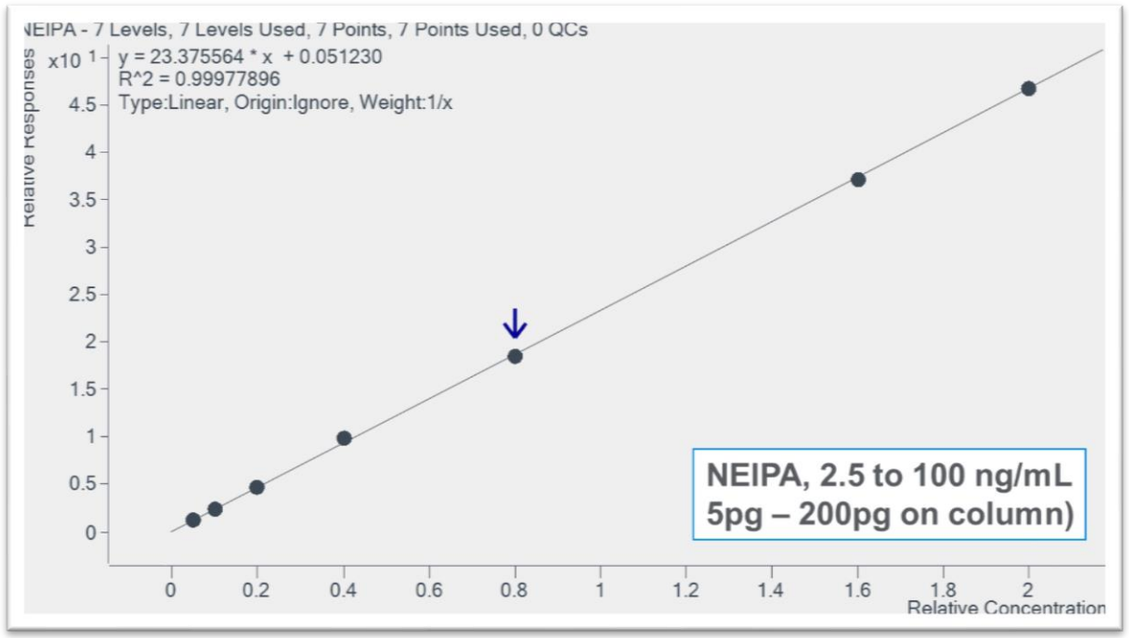


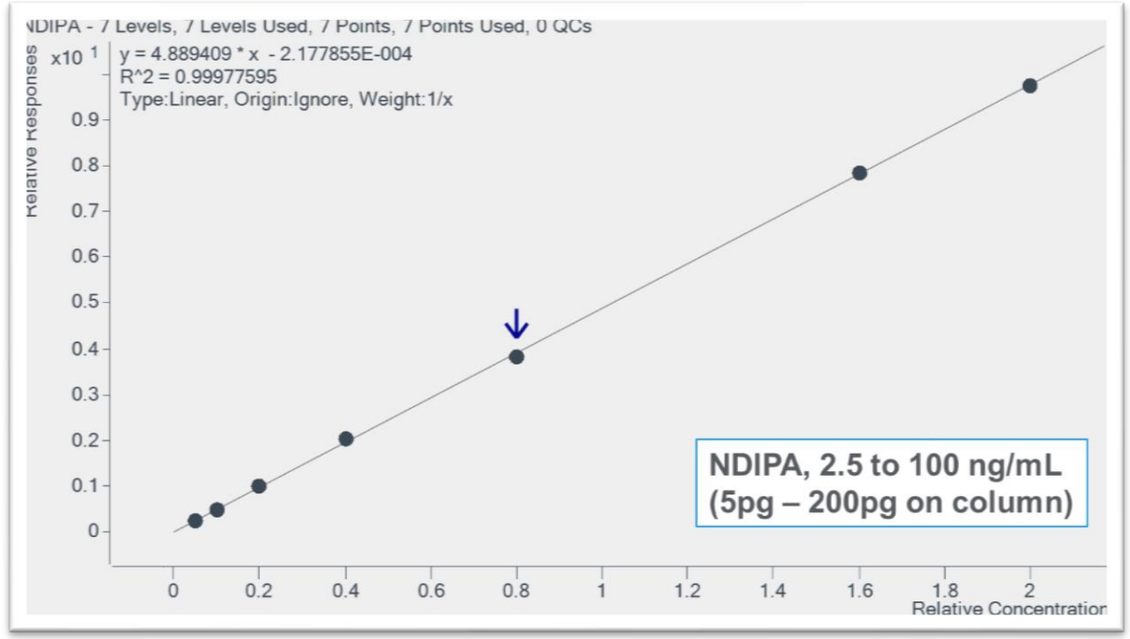


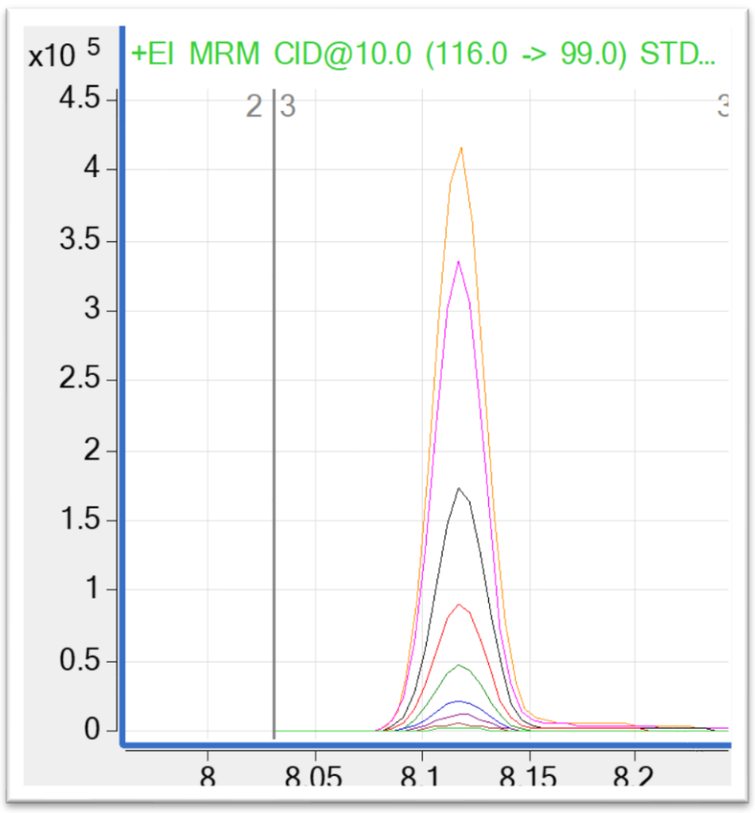


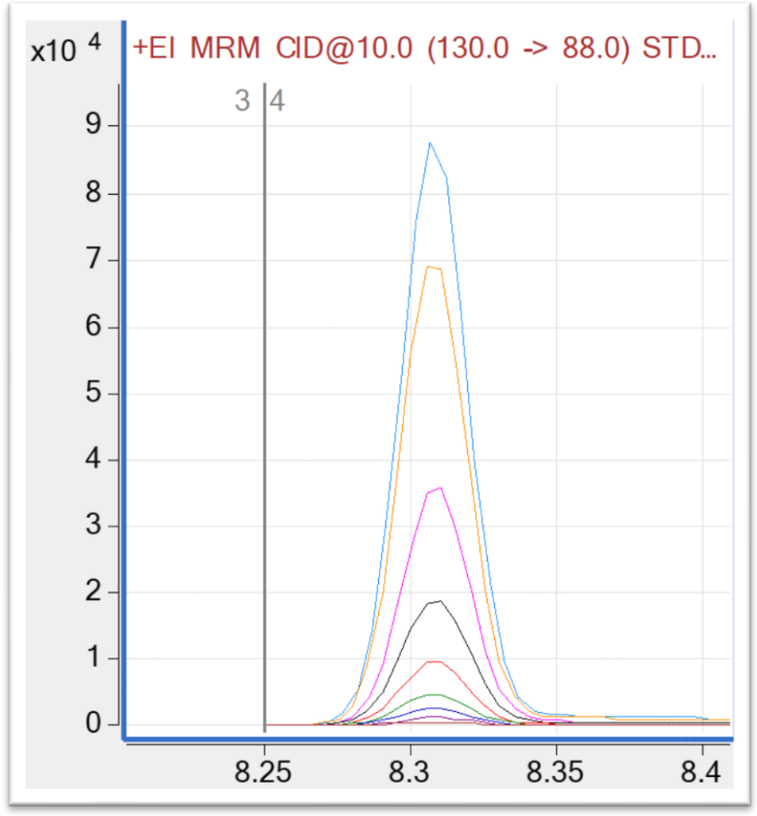
Valsartan

Calibration Curves









Valsartan

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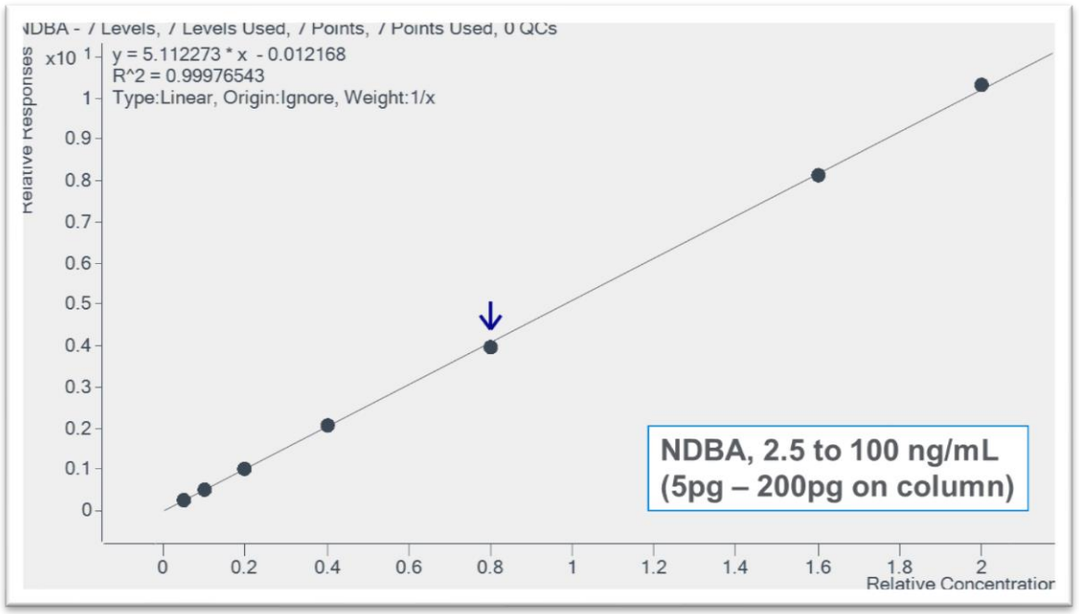
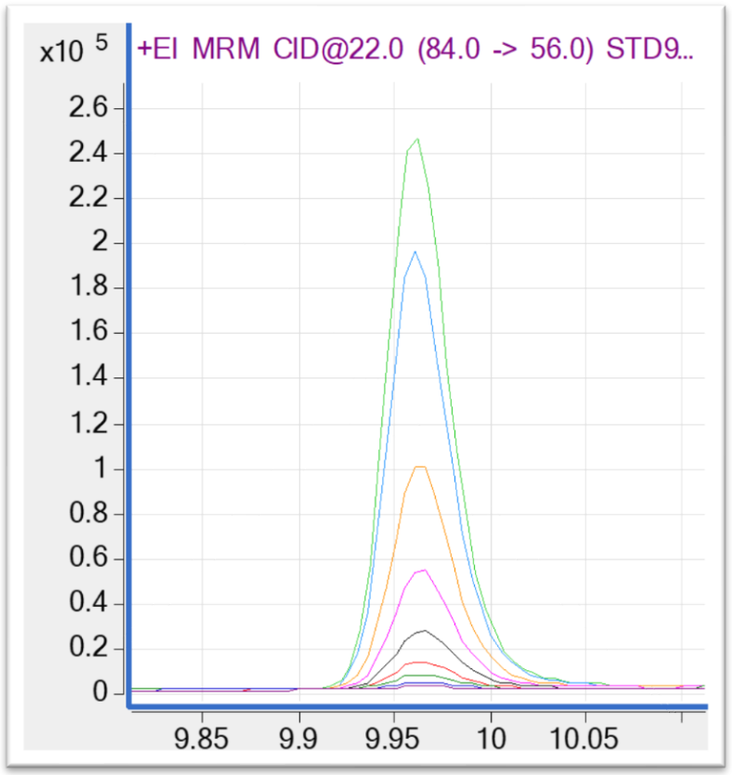
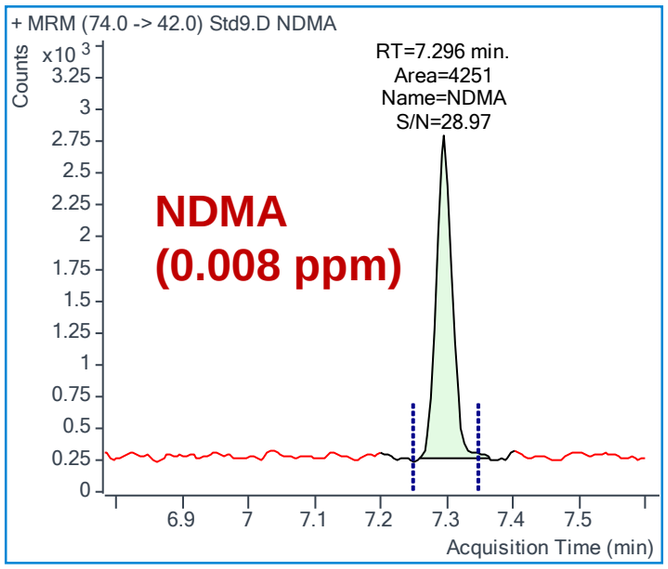
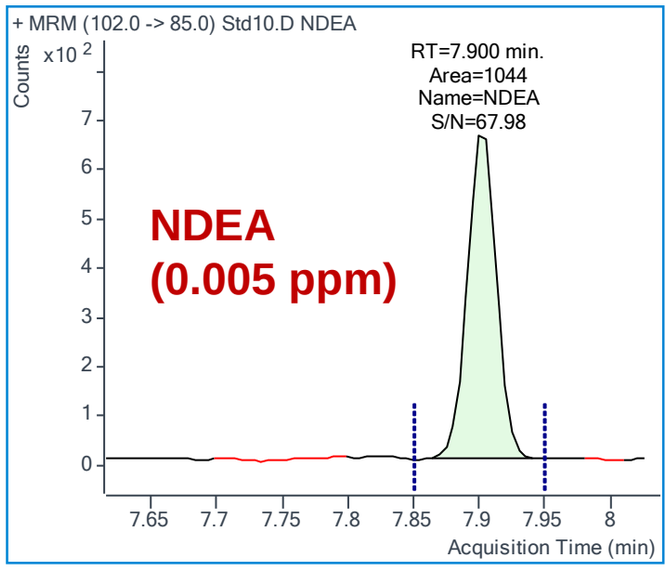
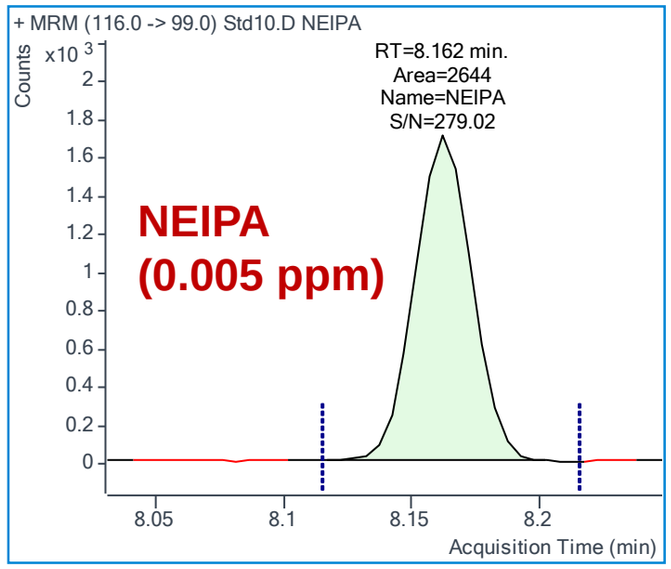


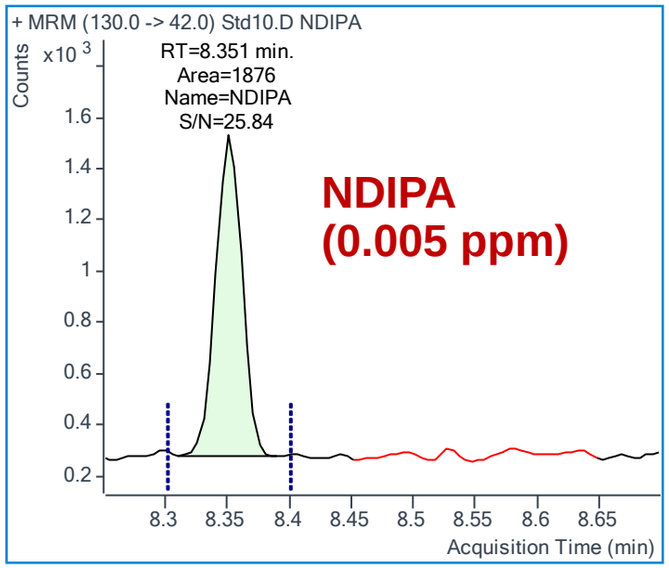


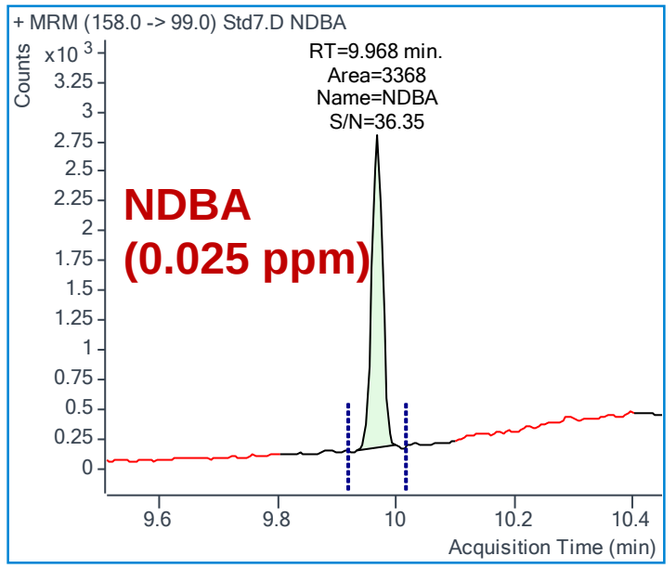
Response at FDA Specified LOQ







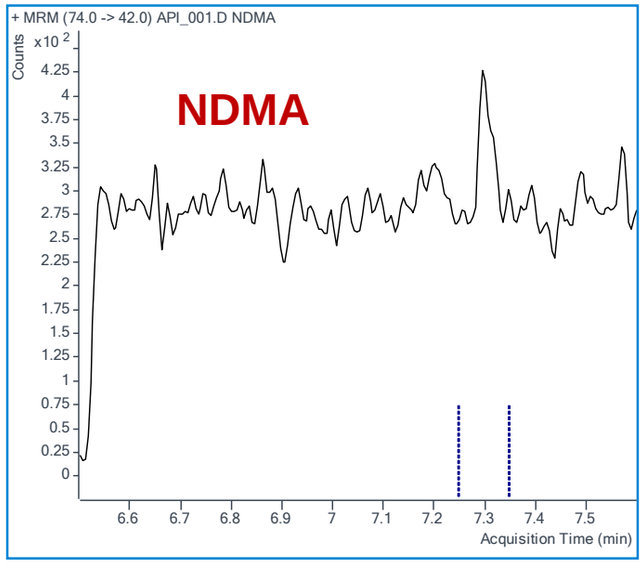


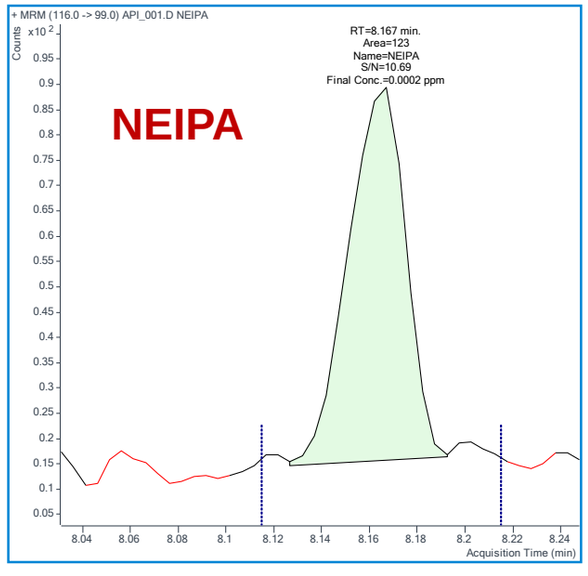


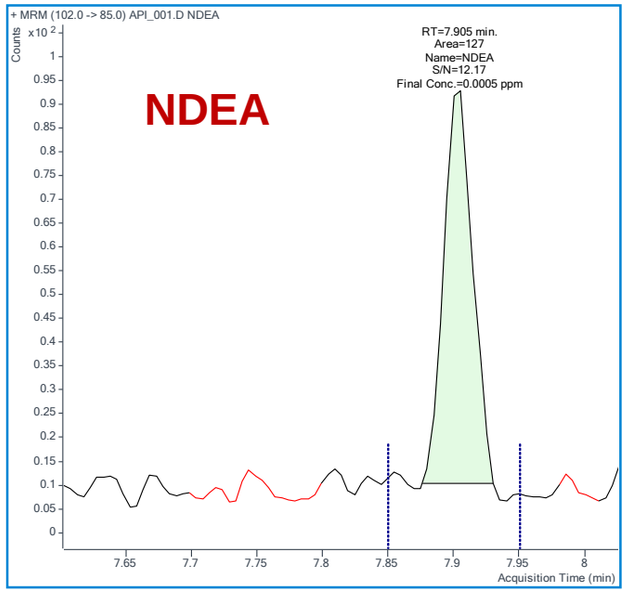
S/N at FDA LOQ

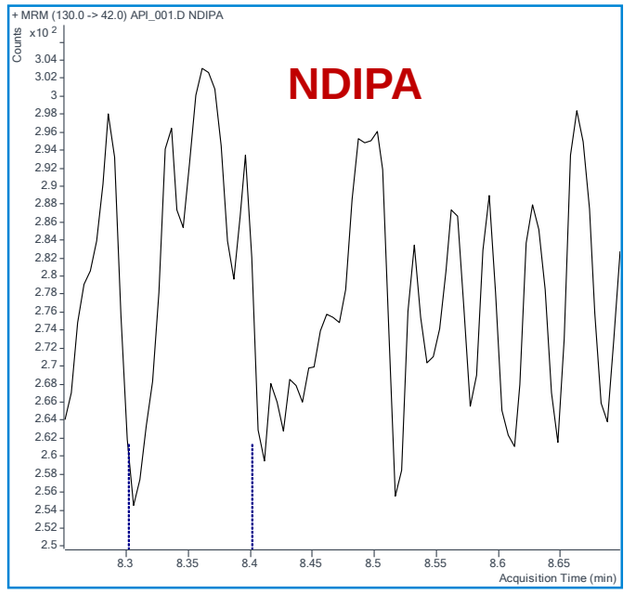
Name	S/N
NDMA	28.97
NDEA	67.98
NEIPA	279.02
NDIPA	25.84
NDBA	36.35

Sample Results for Valsartan API, Extraction 1







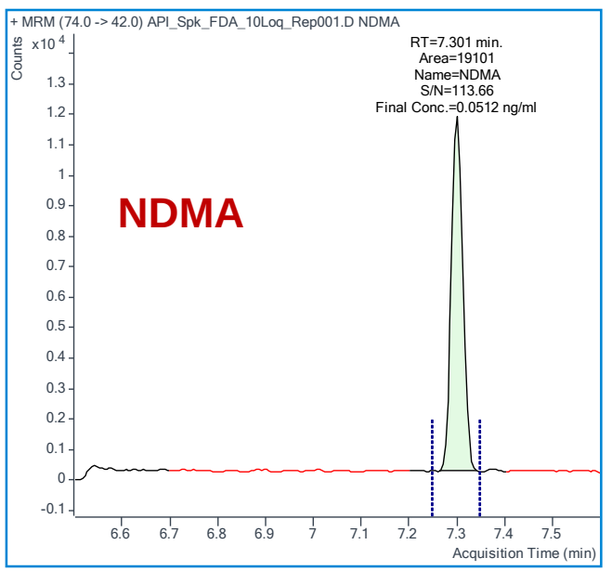


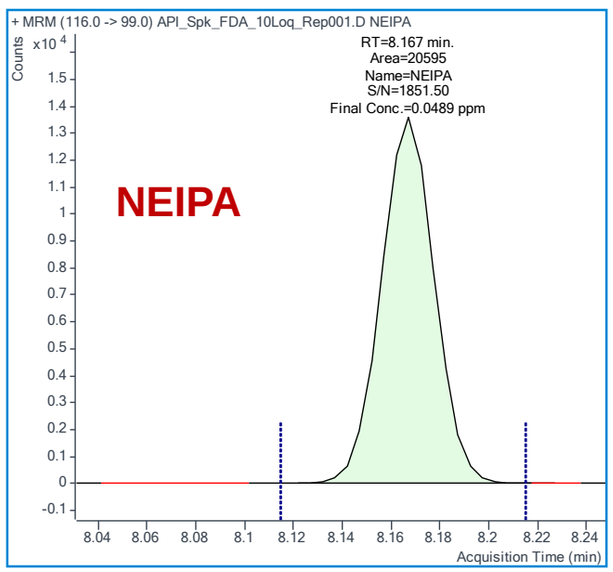
Sample Results

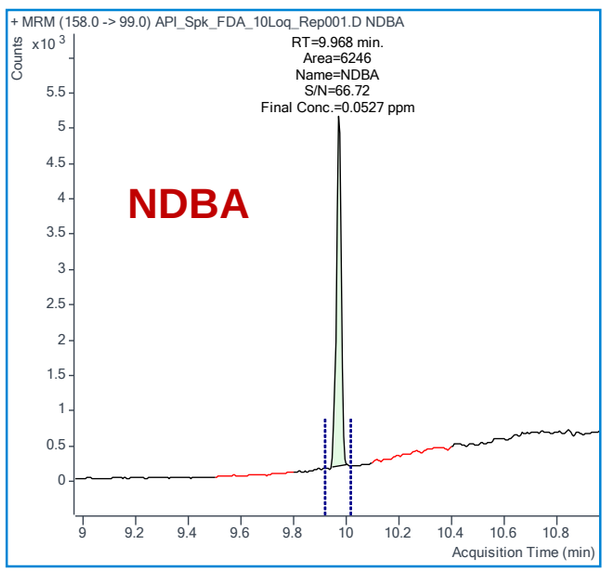
Name	FDA LOQs (ppm)	LOQ Obtained, (ppm)	Sample Results (ppm)
NDMA	0.008	0.0025	ND
NDEA	0.005	0.0005	0.0005
NEIPA	0.005	0.00025	0.0002
NDIPA	0.005	0.0025	ND
NDBA	0.025	0.008	BLQ

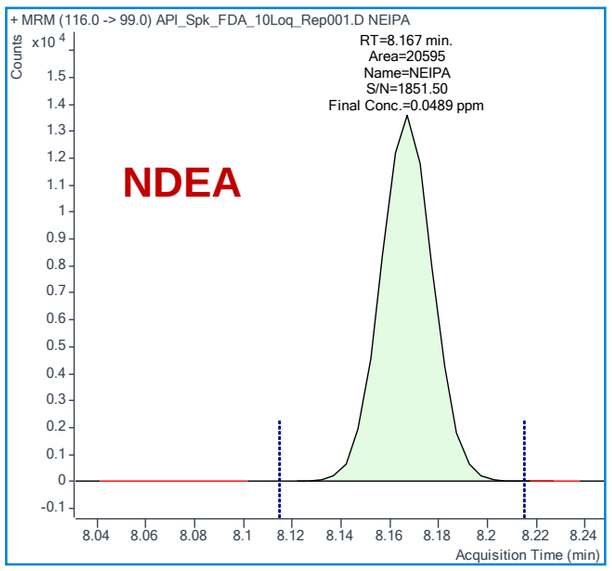
BLQ = Below Limit of Quantitation

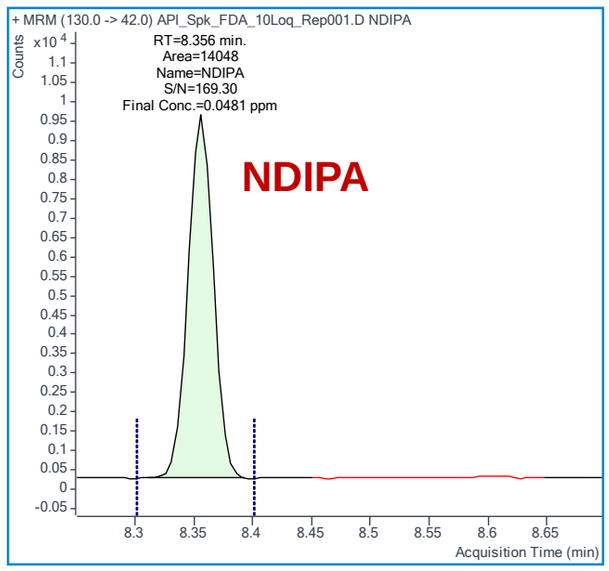
Representative Recovery % of Nitrosamine Impurities in Valsartan at 0.05 ppm











Sample Recovery (0.05 ppm)

Name	Spiking Level (ppm)	Sample Results (ppm)	Recovery (%)
NDMA	0.05	0.051	102
NDEA	0.05	0.049	98
NEIPA	0.05	0.049	98
NDIPA	0.05	0.048	96
NDBA	0.05	0.053	106

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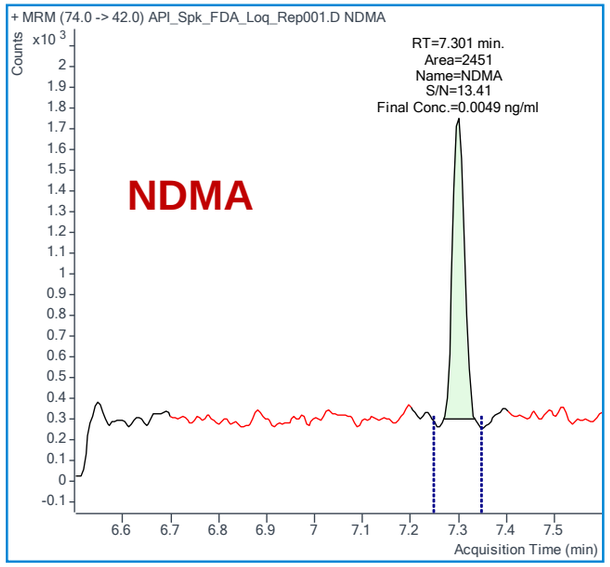
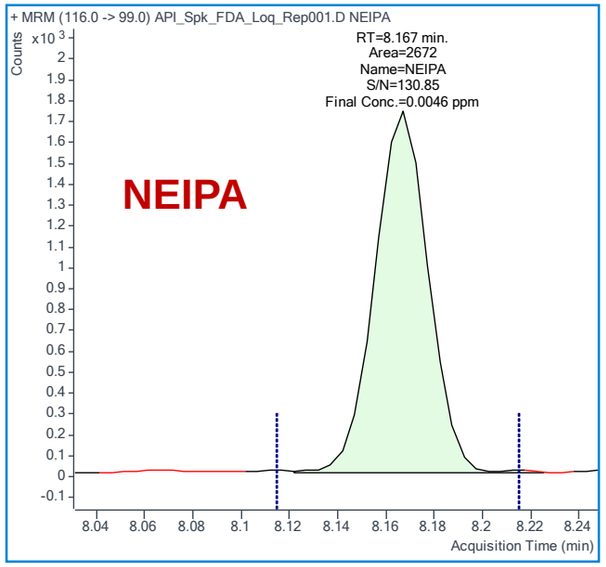
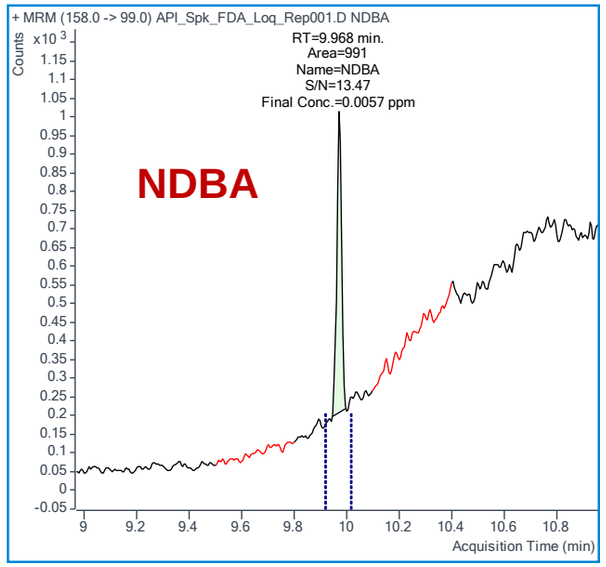
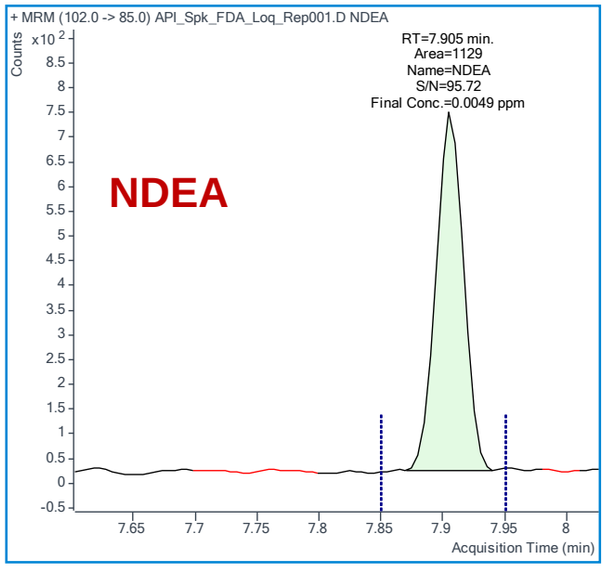
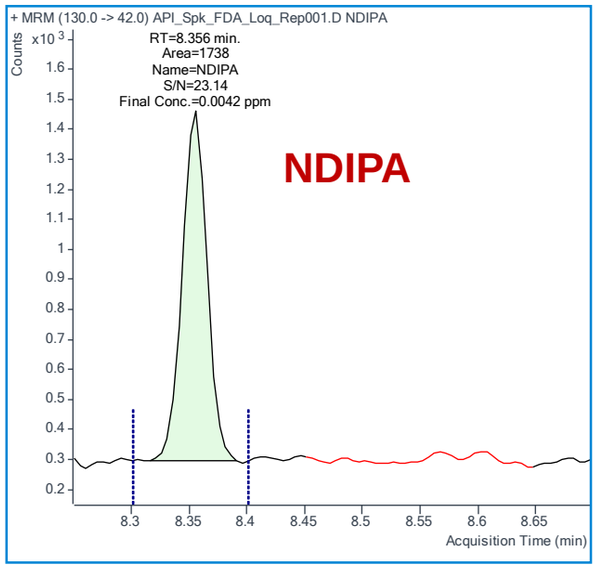
Representative Recovery % of Nitrosamine Impurities in Valsartan at 0.005 ppm











Sample Recovery (0.005 ppm)

Name	Spiking Level (ppm)	Sample Results (ppm)	Recovery (%)
NDMA	0.005	0.0049	98
NDEA	0.005	0.0049	98
NEIPA	0.005	0.0046	92
NDIPA	0.005	0.0042	84
NDBA	0.005	0.0057	114

Valsartan

LC/MS Method for Analysis

Instrument Method

Chromatographic Condition:

Mobile Phase A: 0.2 % Formic Acid in Water

Mobile Phase B: Methanol

Sample Diluent: Water: Methanol 95:5

Flow Rate: 0.5mL/min

Injection Volume: 20µL

Column Used: Infinity Lab Poroshell HPH C18 3 x 150mm 4µm (P/N 693970-502T)

Column Temperature: 40°C

Gradient Program:

Time (Min)	Mobile Phase A	Mobile Phase B	Flow Rate(mL/min)
0	95	5	0.5
5	70	30	0.5
18	30	70	0.4
19	5	95	0.5
22	5	95	0.5
22.1	95	5	0.5
24	95	5	0.5

Table1: Chromatographic Gradient Program for analysis

Post Run Time: 1 minutes

Instrument	Agilent 6470 /Ultivo Triple Quadrupole mass spectrometer
Ion source	Atmospheric Pressure Chemical Ionization (APCI)
MS/MS mode	MRM
Ion mode	Positive
Drying gas temperature	300 °C
Drying gas flow	6 L/min
Nebulizer pressure	55 psi
APCI heater	350 °C
APCI needle positive	4 µA
Capillary voltage, positive	3000 V
MS1/MS2 resolution	0.7/0.7 (unit/unit)

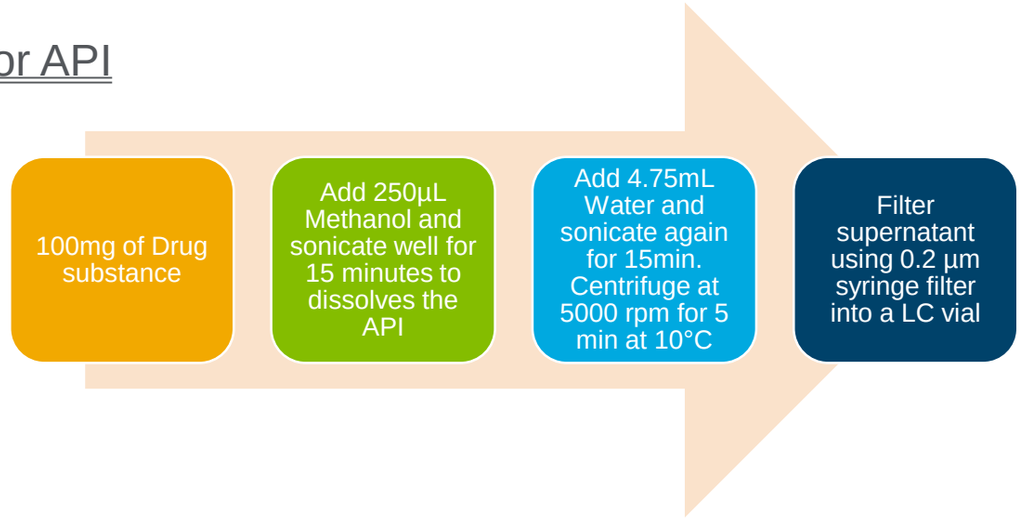
Compound	Precursor Ion (m/z)	Product Ion (m/z)	Retention Time(min)	Retention Time Window (Min)	Fragmentor (V)	Collision Energy(V)	Polarity
NDEA	103.1	75.1	3.484	1.5	85	8	+
NDEA	103.1	47.1	3.484	1.5	85	16	+
NDMA	75.1	58	1.143	1.24	65	10	+
NDMA	75.1	43.1	1.143	1.24	65	17	+
NMBA	147.1	44.2	2.247	1.2	50	7	+
NMBA	147.1	87.2	2.247	1.2	50	7	+
NEIPA	117.1	75.1	4.325	1.0	70	7	+
NEIPA	117.1	47.1	4.325	1.0	70	15	+
NDIPA	131.1	89.1	4.916	1.0	50	5	+
NDIPA	131.1	43.1	4.916	1.0	50	7	+
NDBA	159.1	57.2	6.096	1.0	70	7	+
NDBA	159.1	41.1	6.096	1.0	70	24	+

Calibrations

0.1 ng/mL to 100 ng/mL

Sample Preparation

For API

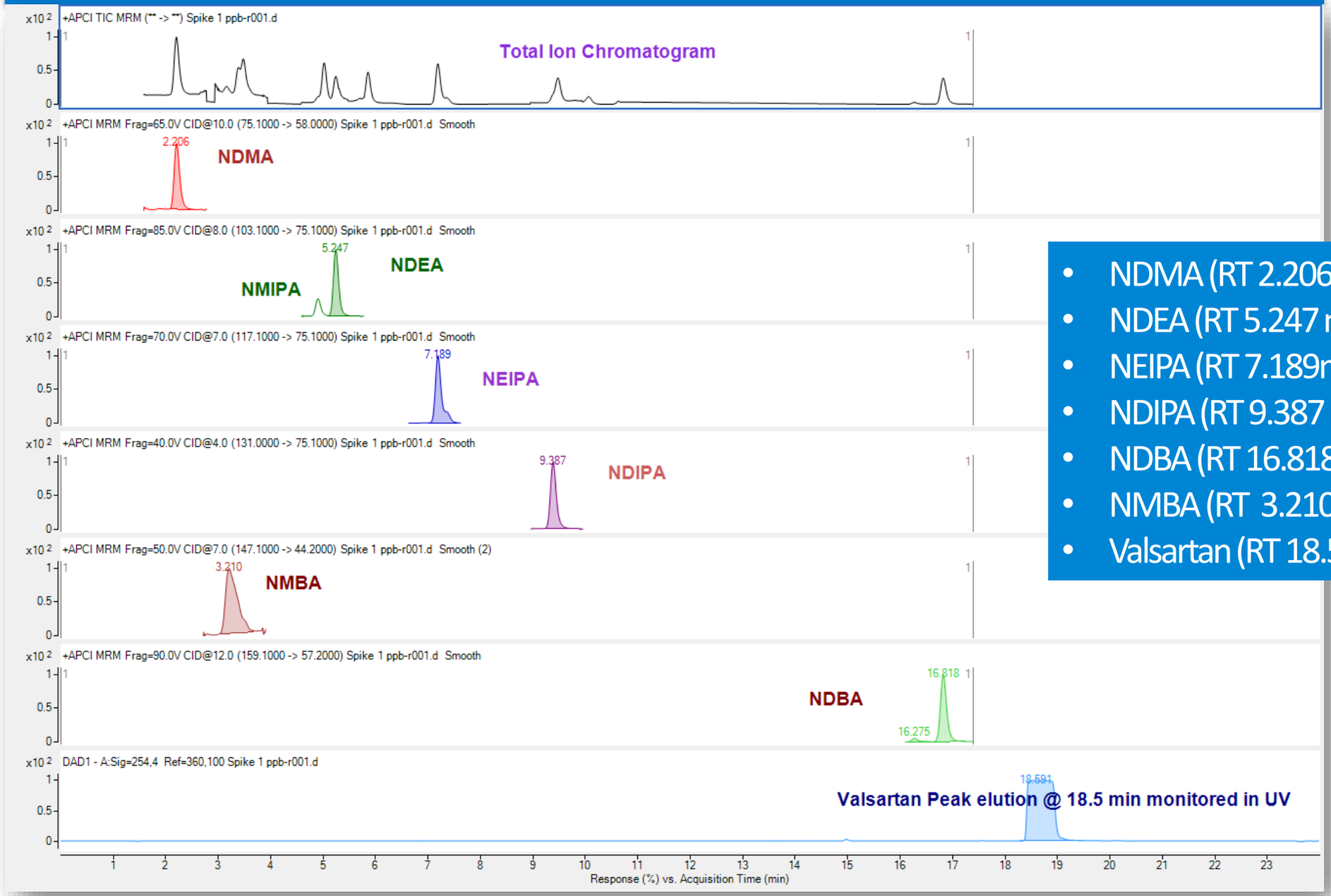


System Suitability

The coefficient of determination (R^2) of the linear calibration curve should be ≥ 0.990.
The S/N ratio of the 1 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 1 ng/mL standard should be ≤ 10

Valsartan

Results for 6 nitrosamine impurities at 20 ng/mL in Valsartan API



- NDMA (RT 2.206 min)
- NDEA (RT 5.247 min)
- NEIPA (RT 7.189min)
- NDIPA (RT 9.387 min)
- NDBA (RT 16.818 min)
- NMBA (RT 3.210 min)
- Valsartan (RT 18.591min)

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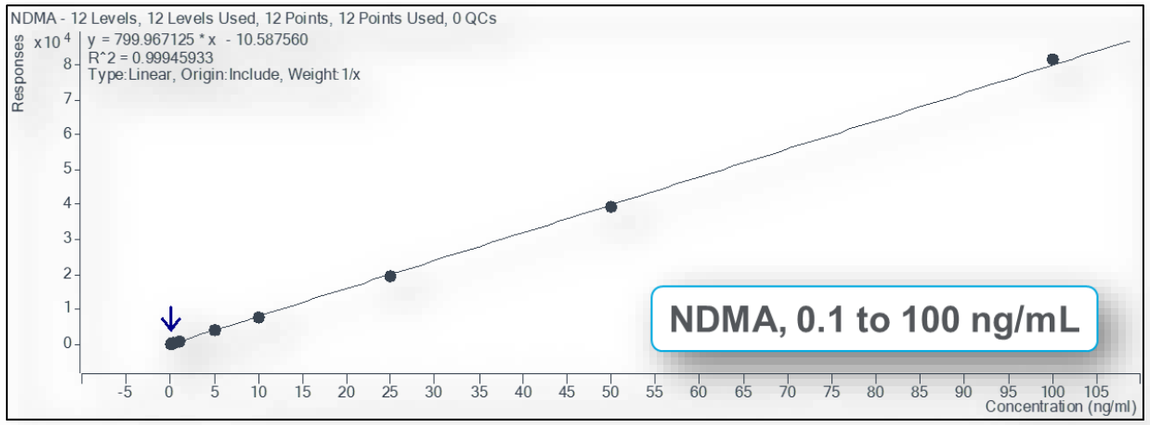
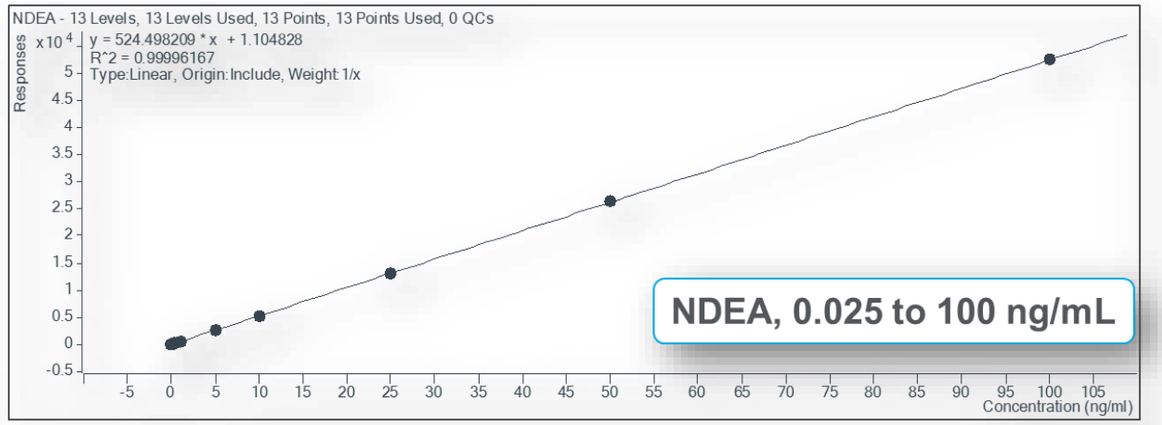
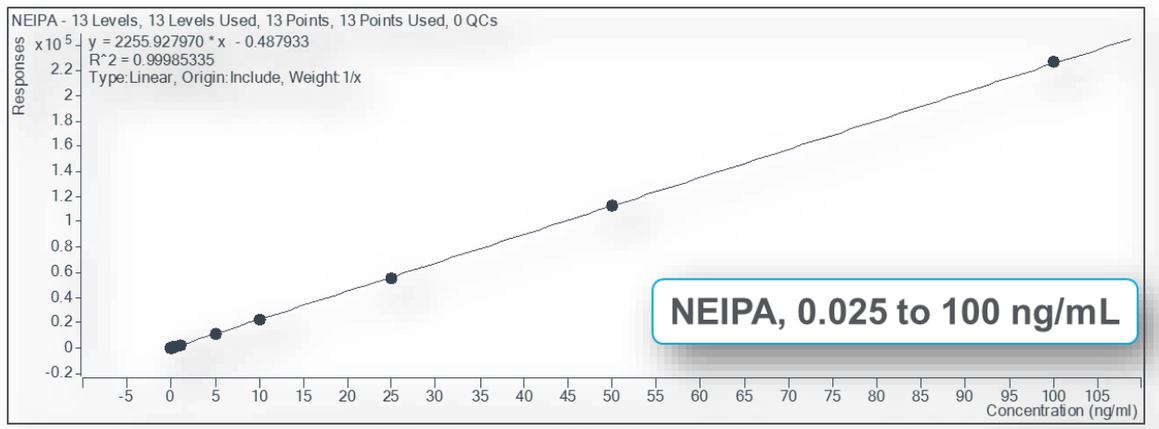
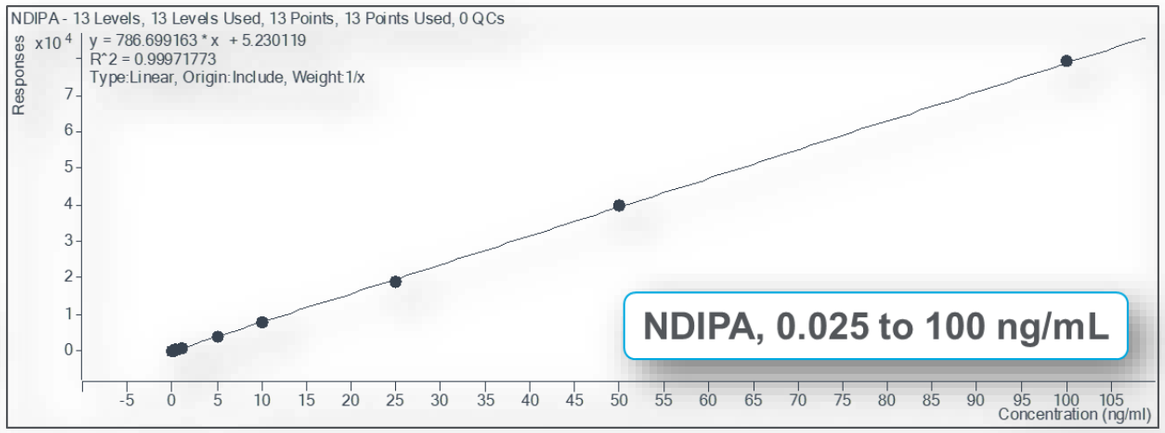
Valsartan

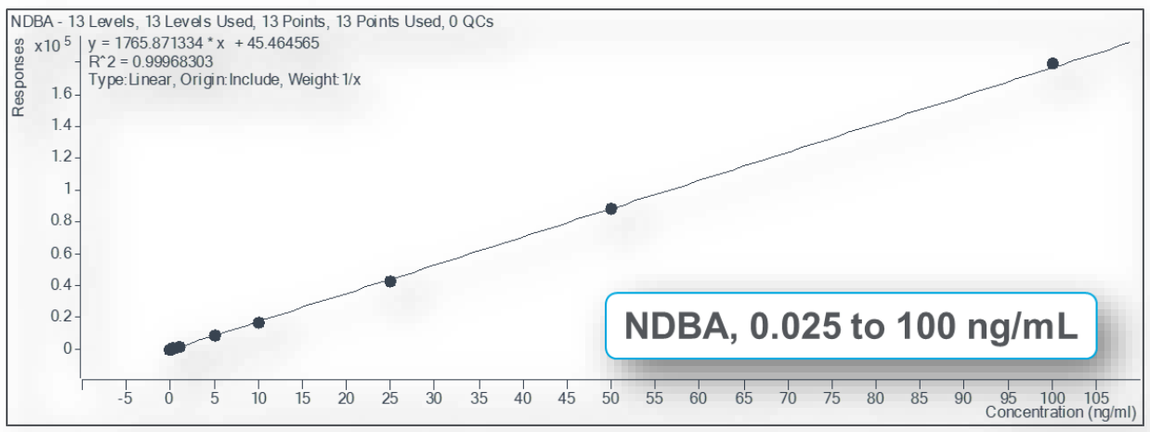
Calibration Curves

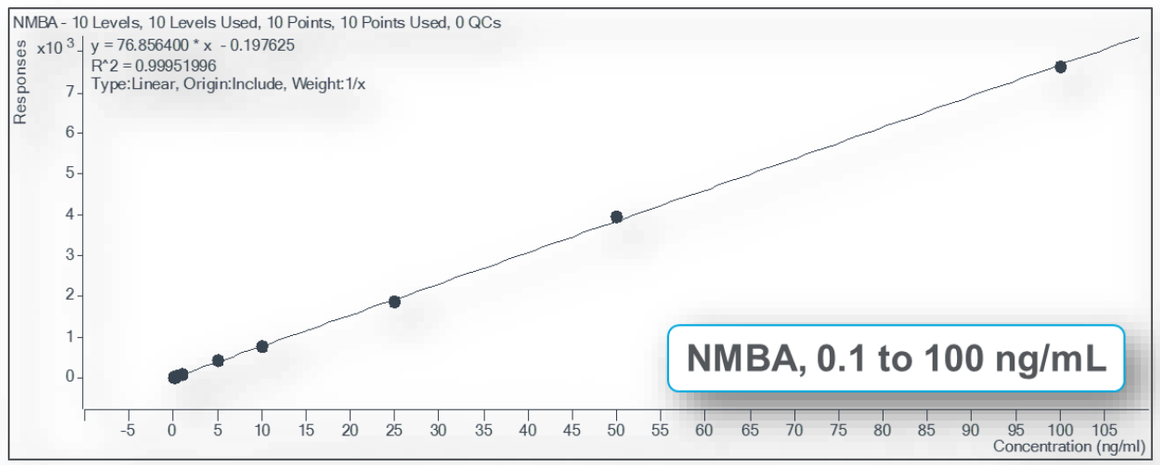












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Representative Recovery % of Nitrosamine Impurities

@ 1ng/mL (0.05ppm) concentration using 20mg/mL sample size

S.No.	Nitrosamine Impurities	Average Recovery Percentage
1	NDMA	110
2	NMBA	117
3	NDEA	108
4	NEIPA	103
5	NDIPA	104
6	NDBA	101

Note: Use of corresponding internal standards for each nitrosamines may further help in any recovery issue.

Benefits of Agilent LC/TQ	
Optimized methods	• Optimized method for valsartan drug substance • Detect and quantify nitrosamine impurities limits per published FDA regulatory testing method guidance
Scalable application	• Best precision = best ion ratios = best quant results; Rugged ion source design
Sample prep	• Sample preparation as per EDQM guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument • Efficient quant review with MassHunter • Data Integrity

GC/MS Method for Analysis

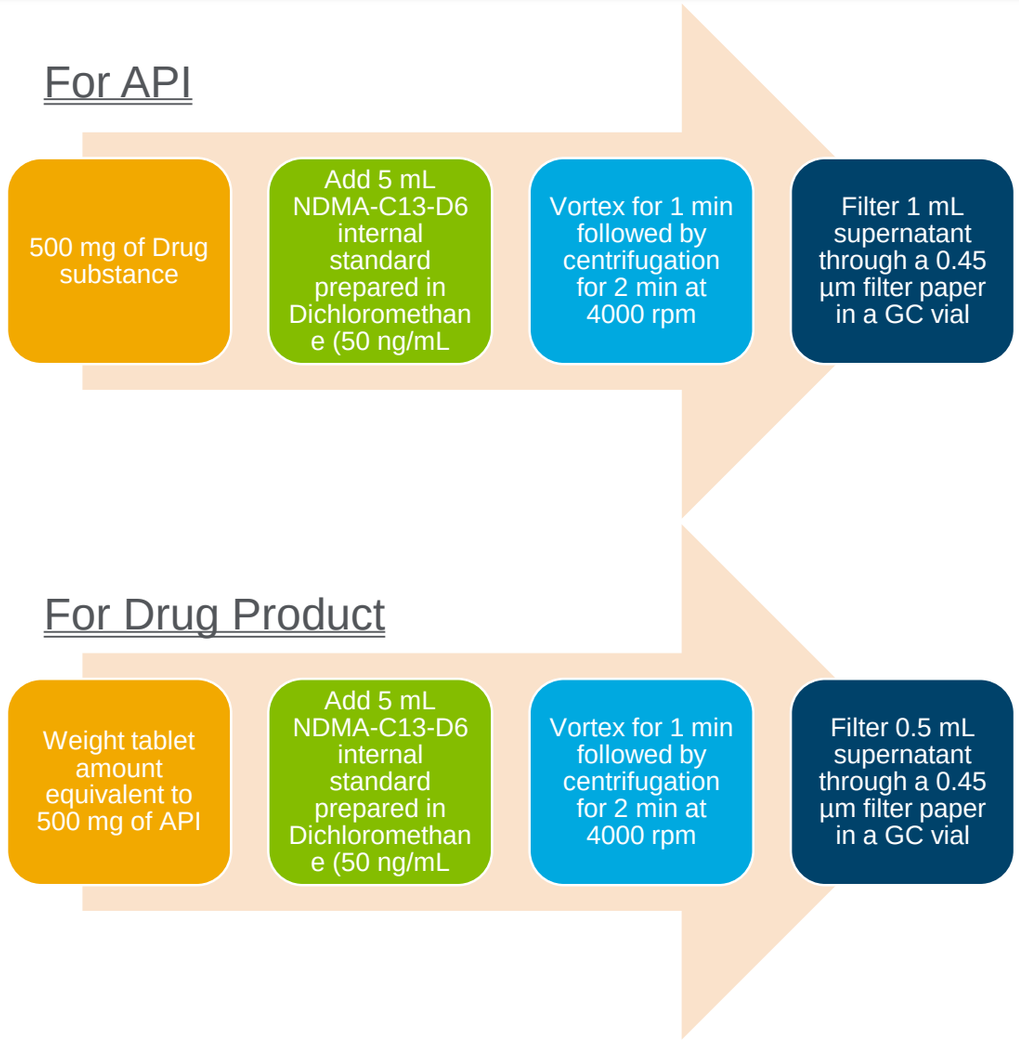
Instrument Method

ALS	GC	MS
Injection Volume: 2µL	Carrier Gas: He 1mL/min	EI Mode

Parameter	Value
MMI injection mode	Pulsed splitless: 12.285 psi until 0.5 min
Inlet temperature	250 °C
Oven temperature program	40 °C (0.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)
Total run time	12.33 min
MS transfer line temperature	250 °C

Parameter	Value	
Source temperature	250 °C	
Quadrupole temperature	Q1 and Q2 = 150 °C	
MS1 and MS2 resolution	All compounds Unit	
Collision gas flow	Nitrogen at 1.5 mL/min,	
Quenching gas flow	Helium at 4 mL/min	
Quant./qual. transitions (FDA method)	Start time: 6.5 min NDMA	74 → 44, CE 15, dwell 150 ms 74 → 42, CE 20, dwell 50 ms NDMA:C13-d_6 82 →48, CE 20, dwell 100 ms
	Start time: 7.60 min NDEA	102 →85, CE 10 V, dwell 150 ms 102 →56, CE 18 V, dwell 150 ms
	Start time: 8.03 min NEIPA	116 →99, CE 10 V, dwell 150 ms 71 →56, CE 10 V, dwell 150 ms
	Start time: 8.25 min NDIPA	130 →88, CE 10 V, dwell 150 ms 130 →42, CE 10 V, dwell 150 ms
	Start time: 8.70 min NDBA	158 →99, CE 10 V, dwell 150 ms 84 →56, CE 22 V, dwell 150 ms

Sample Preparation



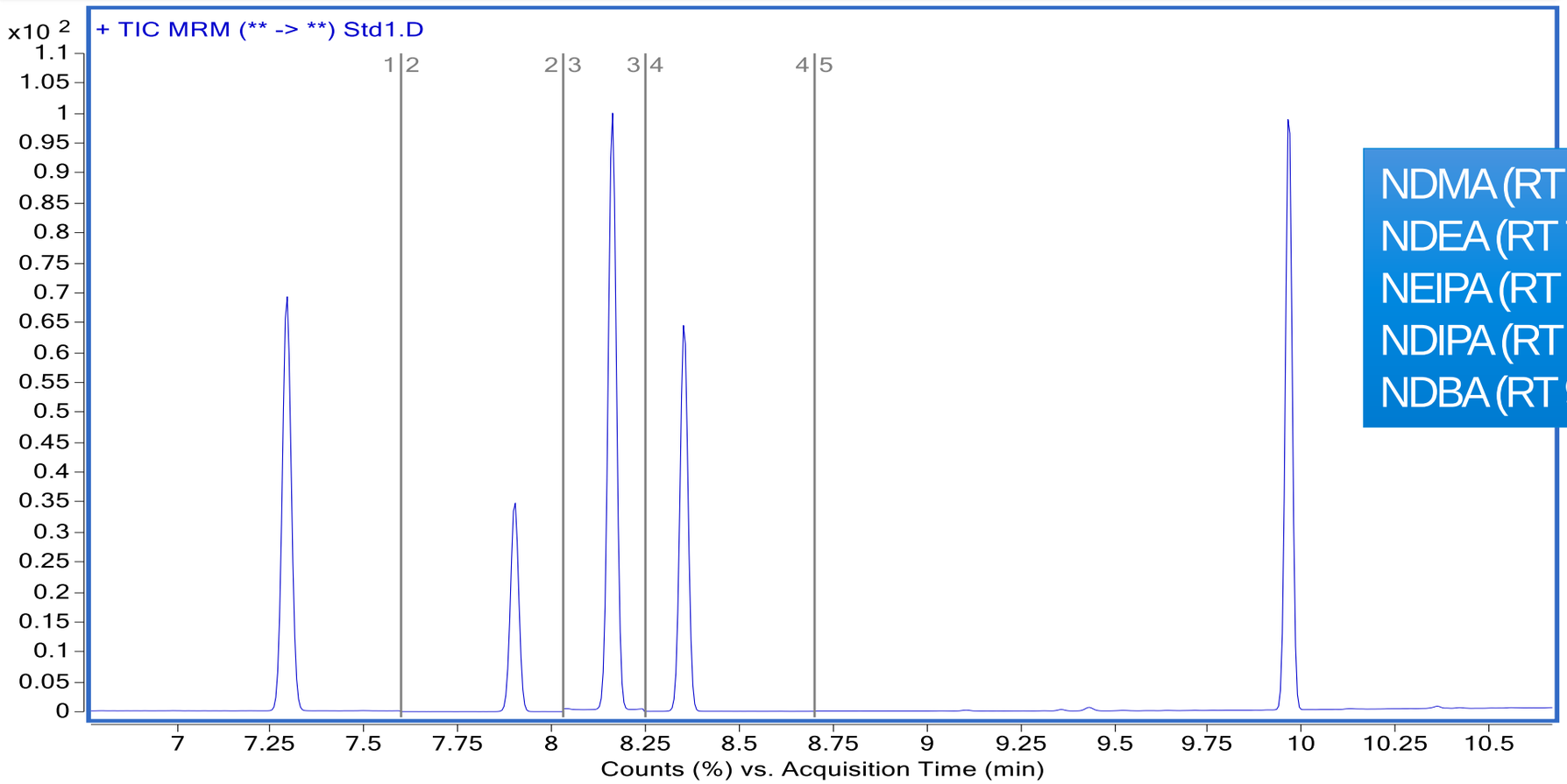
Calibrations

2.5 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 40 ng/ml, 80 ng/ml and 100 ng/ml each prepared in Dichloromethane containing 50 ng/mL of NDMA –C13-D6

System Suitability

The coefficient of determination (R2) of the linear calibration curve should be ≥ 0.998.
The S/N ratio of the 5 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 40 ng/mL standard should be ≤ 5

Results for 5 nitrosamine impurities at 100 ng/mL in Losartan API

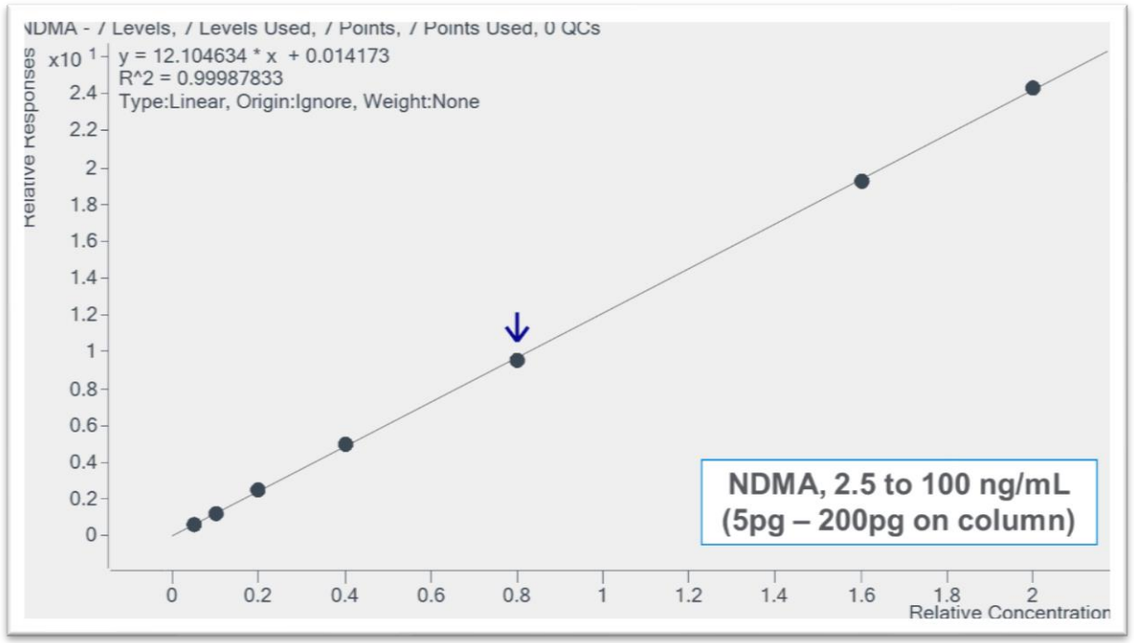


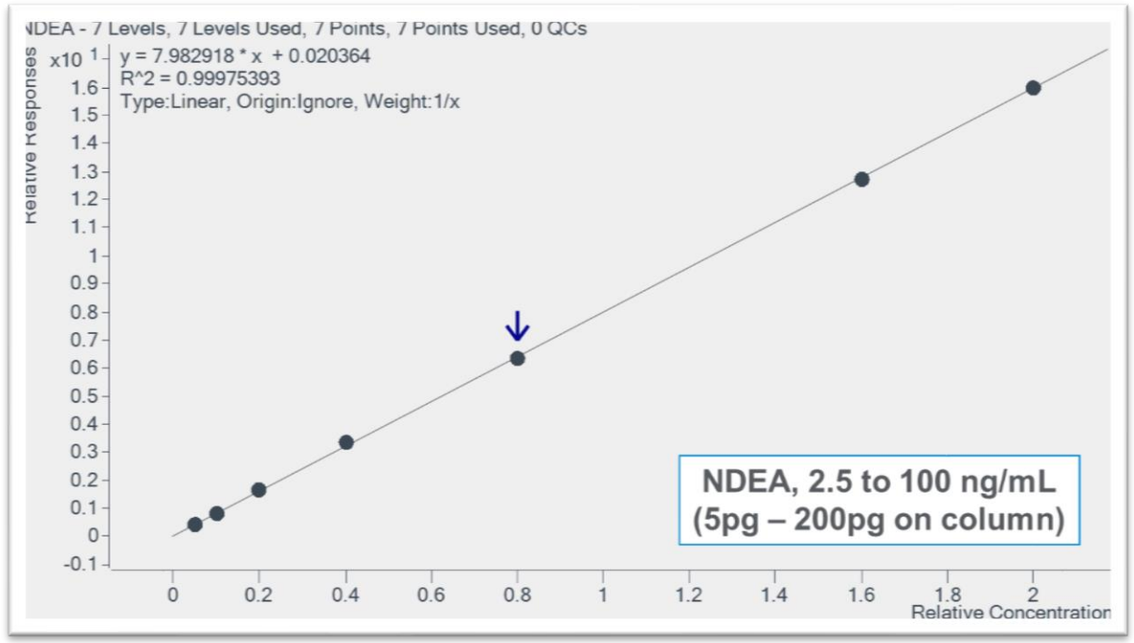
NDMA (RT 7.294 min)
NDEA (RT 7.903 min)
NEIPA (RT 8.157 min)
NDIPA (RT 8.346 min)
NDBA (RT 9.777 min)

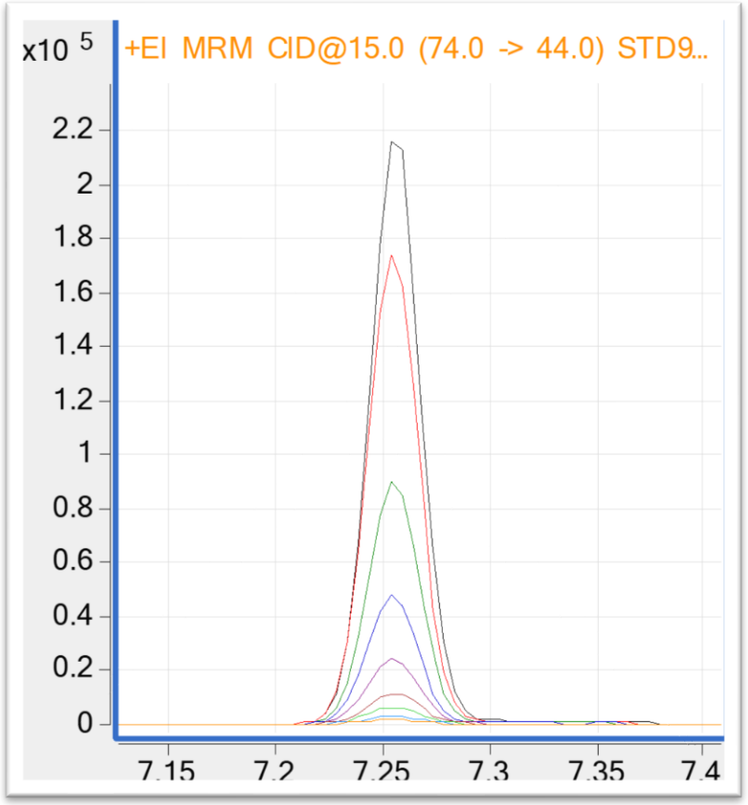
Benefits Agilent GC/TQ	
Optimized methods	• Optimized method for both API and Formulation • Compatible with stringent FDA regulations
Scalable application	• Best precision = best ion ratios = best quant results Rugged ion source design • Retention Time Locking for reproducible methods over time and between labs
Sample prep	• Sample preparation as per FDA guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument. • Efficient Quant review with MassHunter • Data Integrity

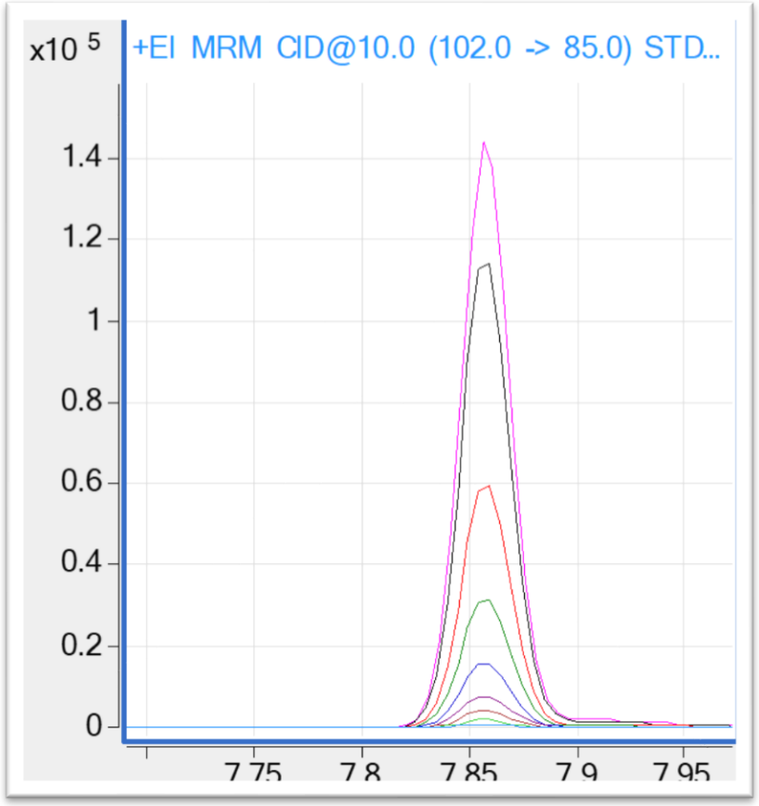
Losartan

Calibration Curves



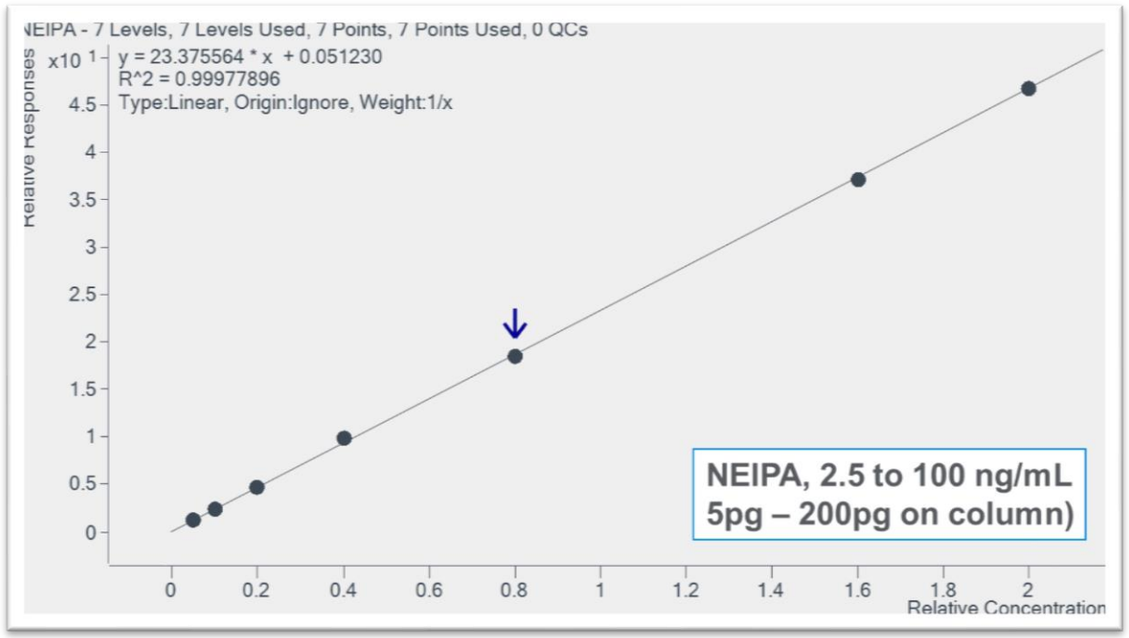


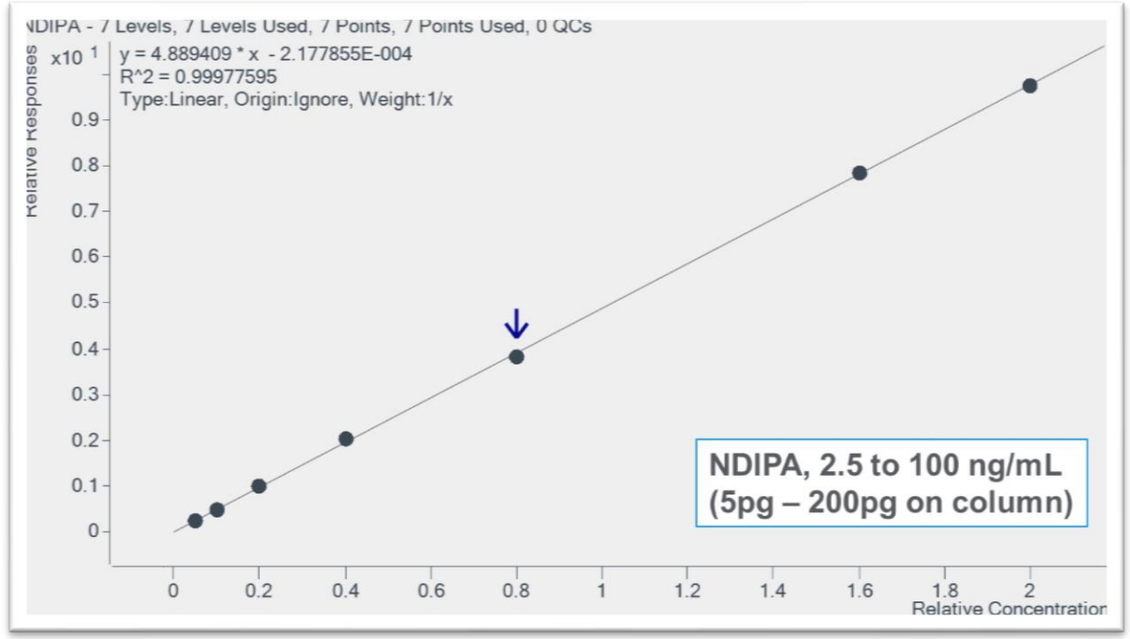


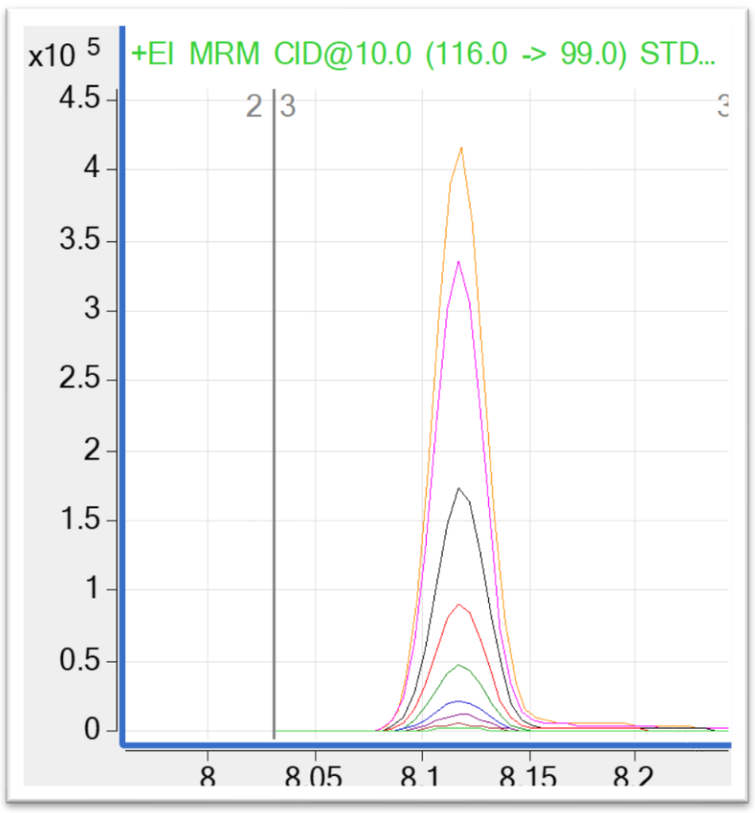


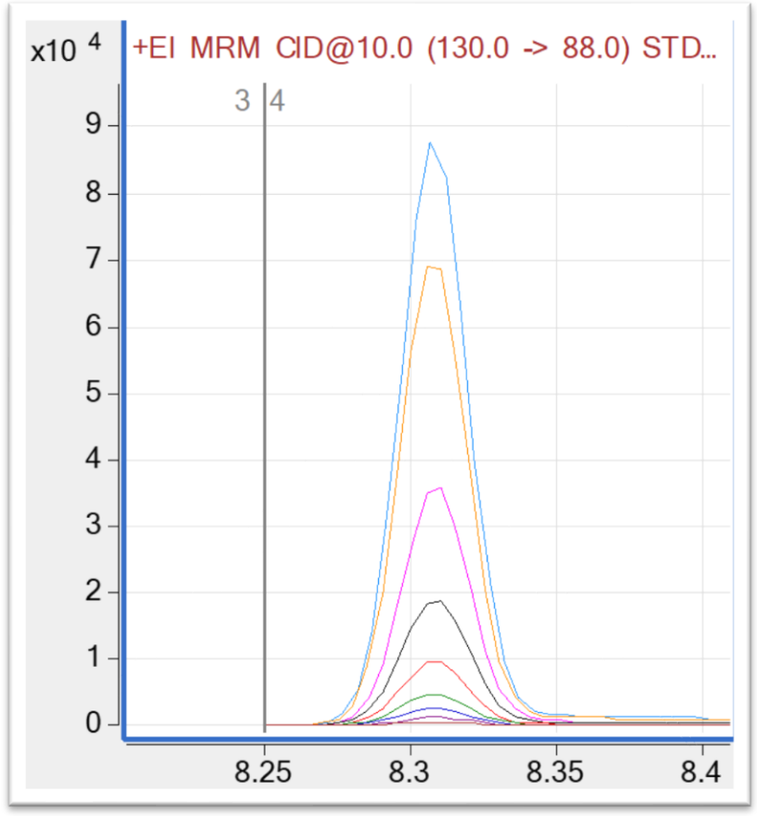
Losartan

Calibration Curves

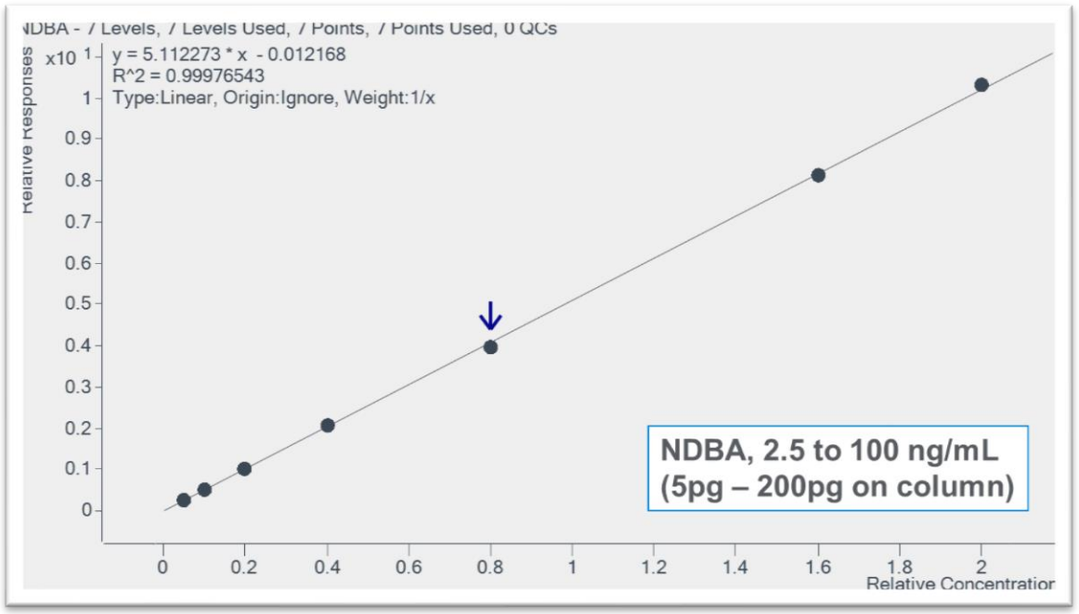


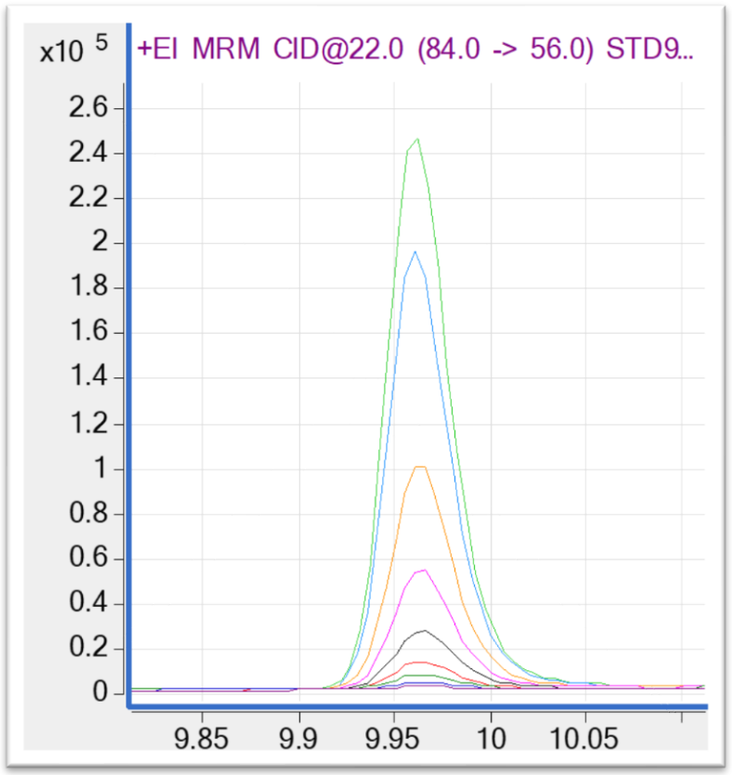






Calibration Curves





Losartan

LC/MS Method for Analysis

Instrument Method

Chromatographic Condition:

Mobile Phase A: 0.2 % Formic Acid in Water

Mobile Phase B: Methanol

Sample Diluent: Water: Methanol/ 95:5 (v/v)

Flow Rate: 0.25mL/min

Injection Volume: 20µL

Column Used: Zorbax Eclipse Plus Phenyl-Hexyl, RRHD 2.1 x 100mm 1.8µm (P/N 959758-912)

Column Temperature: 40°C

Gradient Program:

Time (Min)	Mobile Phase A	Mobile Phase B	Flow Rate(mL/min)
0	95	5	0.25
5	75	25	0.25
13	45	55	0.25
20	45	55	0.4
20.1	5	95	0.4
23	5	95	0.25
23.1	95	5	0.25
25	95	5	0.25

Table1: Chromatographic Gradient Program for analysis

Post Run Time: 2 minutes

Instrument	Agilent 6470 /Ultivo Triple Quadrupole mass spectrometer
Ion source	Atmospheric Pressure Chemical Ionization (APCI)
MS/MS mode	MRM
Ion mode	Positive
Drying gas temperature	300 °C
Drying gas flow	6 L/min
Nebulizer pressure	55 psi
APCI heater	350 °C
APCI needle positive	4 µA
Capillary voltage, positive	3000 V
MS1/MS2 resolution	0.7/0.7 (unit/unit)

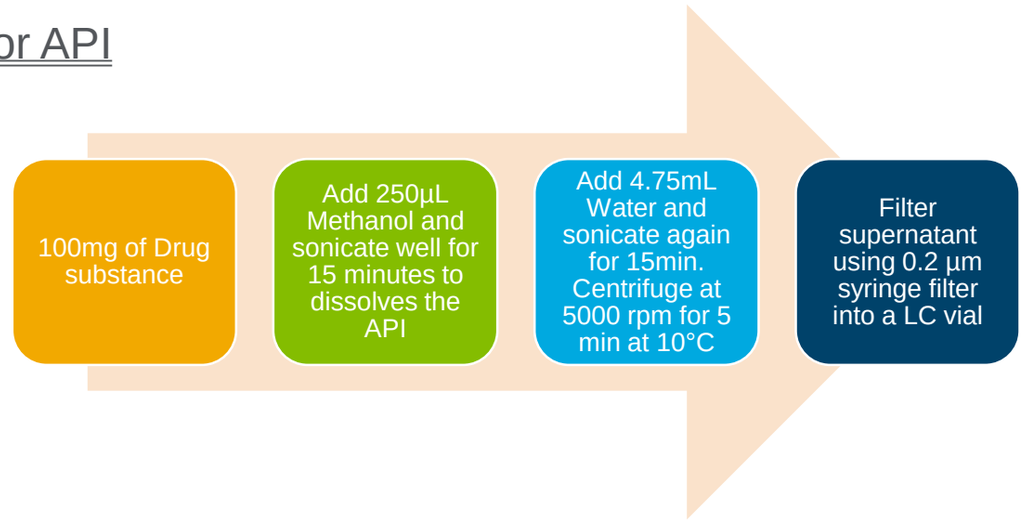
Compound	Precursor Ion (m/z)	Product Ion (m/z)	Retention Time(min)	Retention Time Window (Min)	Fragmentor (V)	Collision Energy(V)	Polarity
NDEA	103.1	75.1	3.484	1.5	85	8	+
NDEA	103.1	47.1	3.484	1.5	85	16	+
NDMA	75.1	58	1.143	1.24	65	10	+
NDMA	75.1	43.1	1.143	1.24	65	17	+
NMBA	147.1	44.2	2.247	1.2	50	7	+
NMBA	147.1	87.2	2.247	1.2	50	7	+
NEIPA	117.1	75.1	4.325	1.0	70	7	+
NEIPA	117.1	47.1	4.325	1.0	70	15	+
NDIPA	131.1	89.1	4.916	1.0	50	5	+
NDIPA	131.1	43.1	4.916	1.0	50	7	+
NDBA	159.1	57.2	6.096	1.0	70	7	+
NDBA	159.1	41.1	6.096	1.0	70	24	+

Calibrations

0.05/ 0.1 ng/mL to 25 ng/mL

Sample Preparation

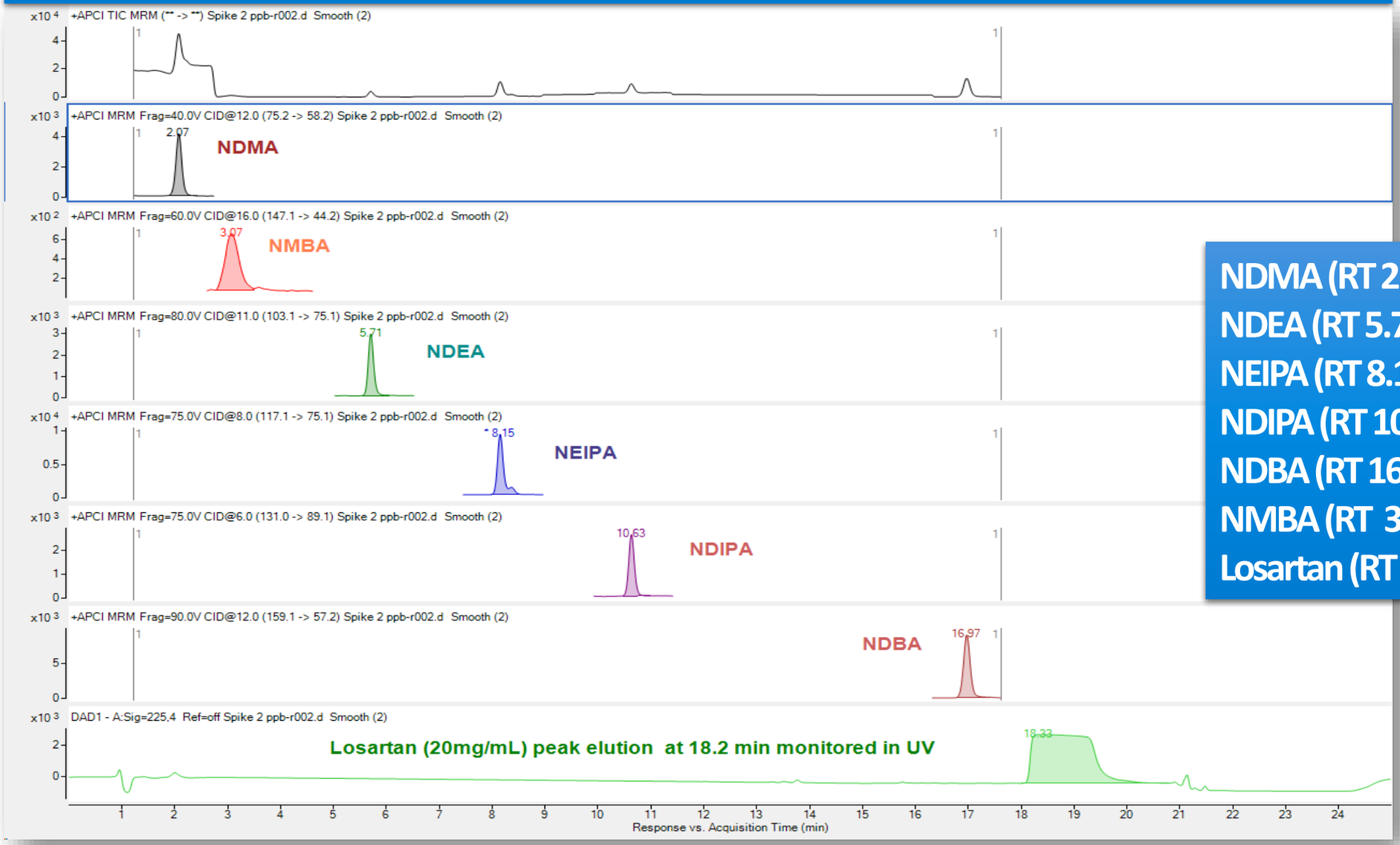
For API



System Suitability

The coefficient of determination (R^2) of the linear calibration curve should be ≥ 0.990.
The S/N ratio of the 1 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 1 ng/mL standard should be ≤ 10

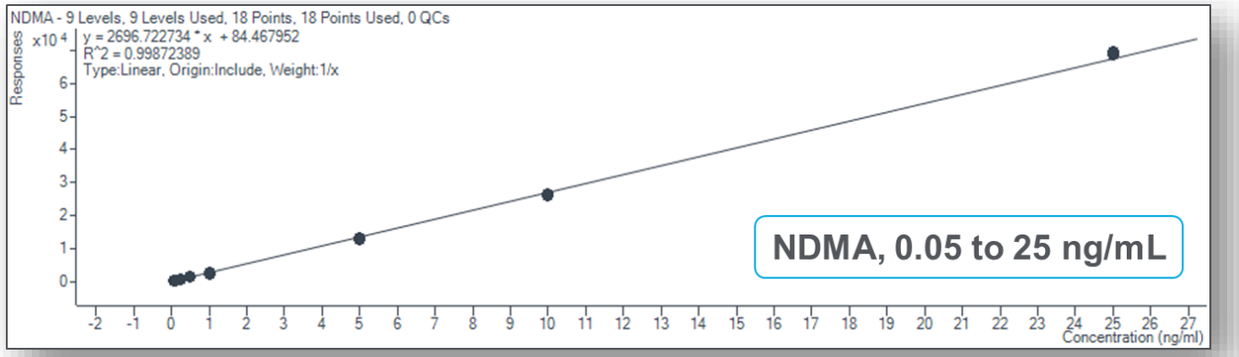
Results for 6 Nitrosamine impurities at 20 ng/mL in Losartan API

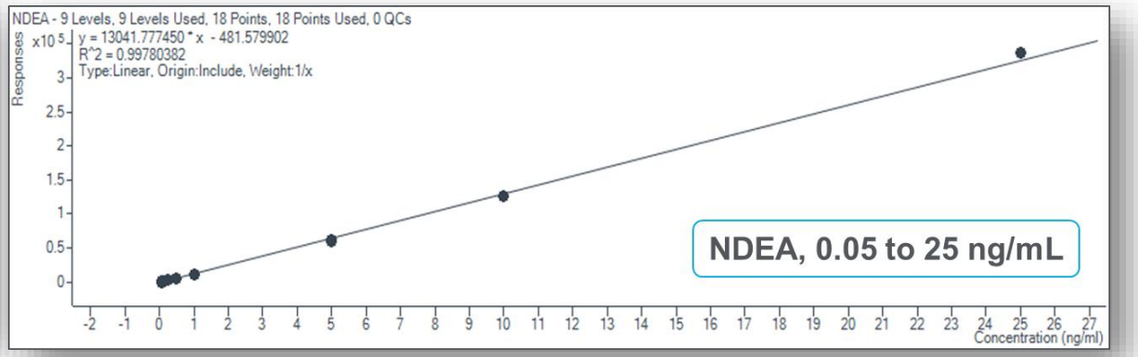


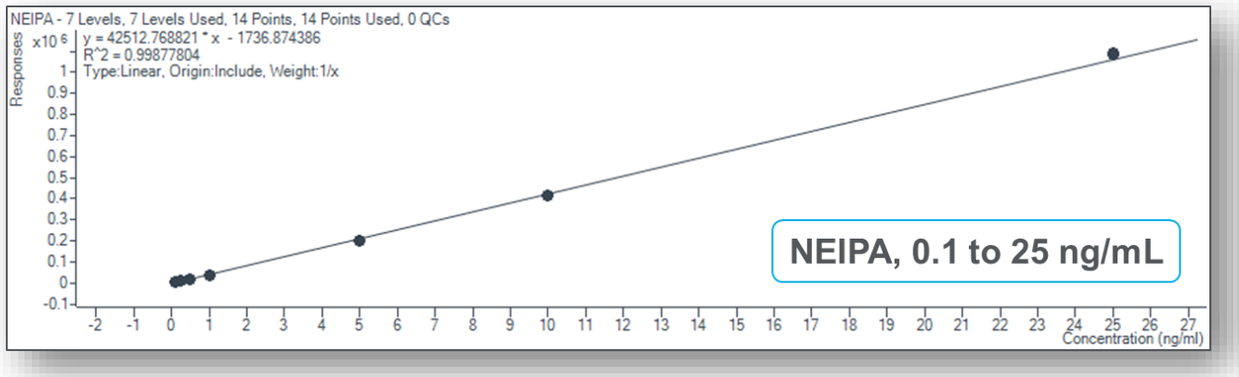
NDMA (RT 2.07 min)
NDEA (RT 5.71 min)
NEIPA (RT 8.15min)
NDIPA (RT 10.63min)
NDBA (RT 16.97 min)
NMBA (RT 3.07 min)
Losartan (RT 18.3min)

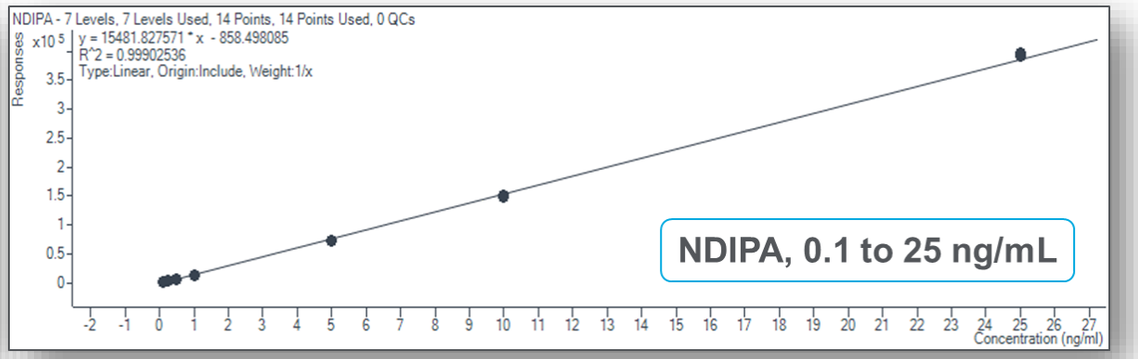
Losartan

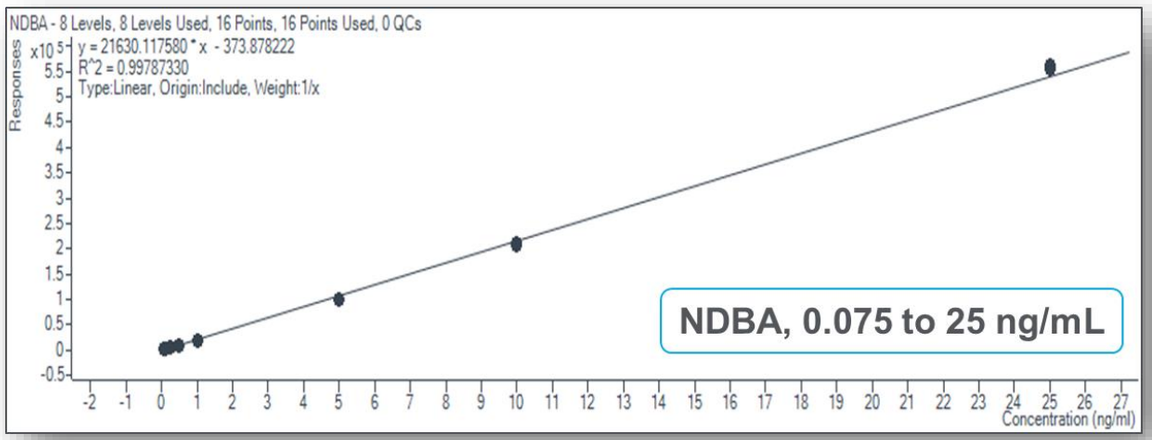
Calibration Curves

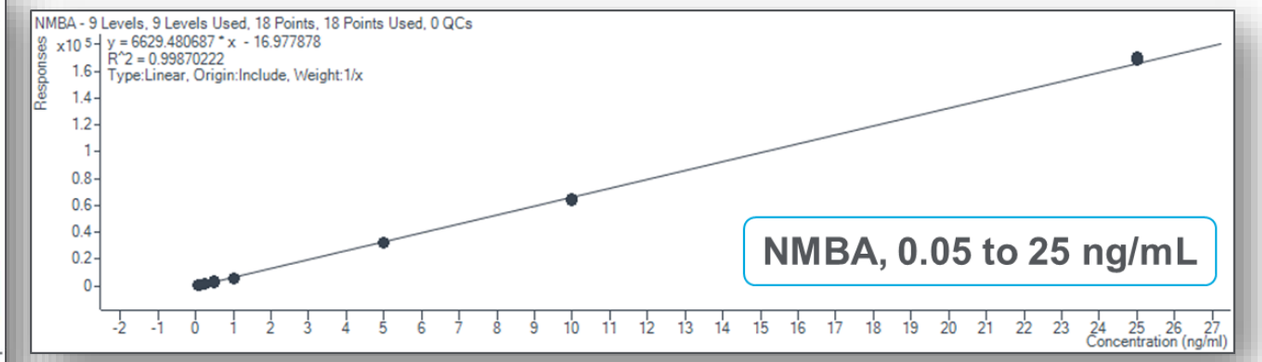












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Losartan

Representative Recovery % of Nitrosamine Impurities

@ 1ng/mL (0.05ppm) and 2ng/mL (0.1ppm) concentrations using 20mg/mL sample size

Nitrosamine Impurity	Concentration (ng/mL)	Recovery %
NDMA	2	110
NMBA	1	113
NDEA	1	103
NEIPA	1	100
NDIPA	1	98
NDBA	2	91

Note:
1.The Losartan sample used for recovery study was containing NDMA and NDBA at some concentration levels so recovery was established at 2ng/mL spike for these two impurities.
2.Use of corresponding internal standards for each nitrosamines may further help in any recovery issue.

Benefits of Agilent LC/TQ	
Optimized methods	• Optimized method for losartan drug substance • Detect and quantify nitrosamine impurities limits per published FDA regulatory testing method guidance
Scalable application	• Best precision = best ion ratios = best quant results; Rugged ion source design
Sample prep	• Sample preparation as per EDQM guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument • Efficient Quant review with MassHunter • Data Integrity

GC/MS Method for Analysis

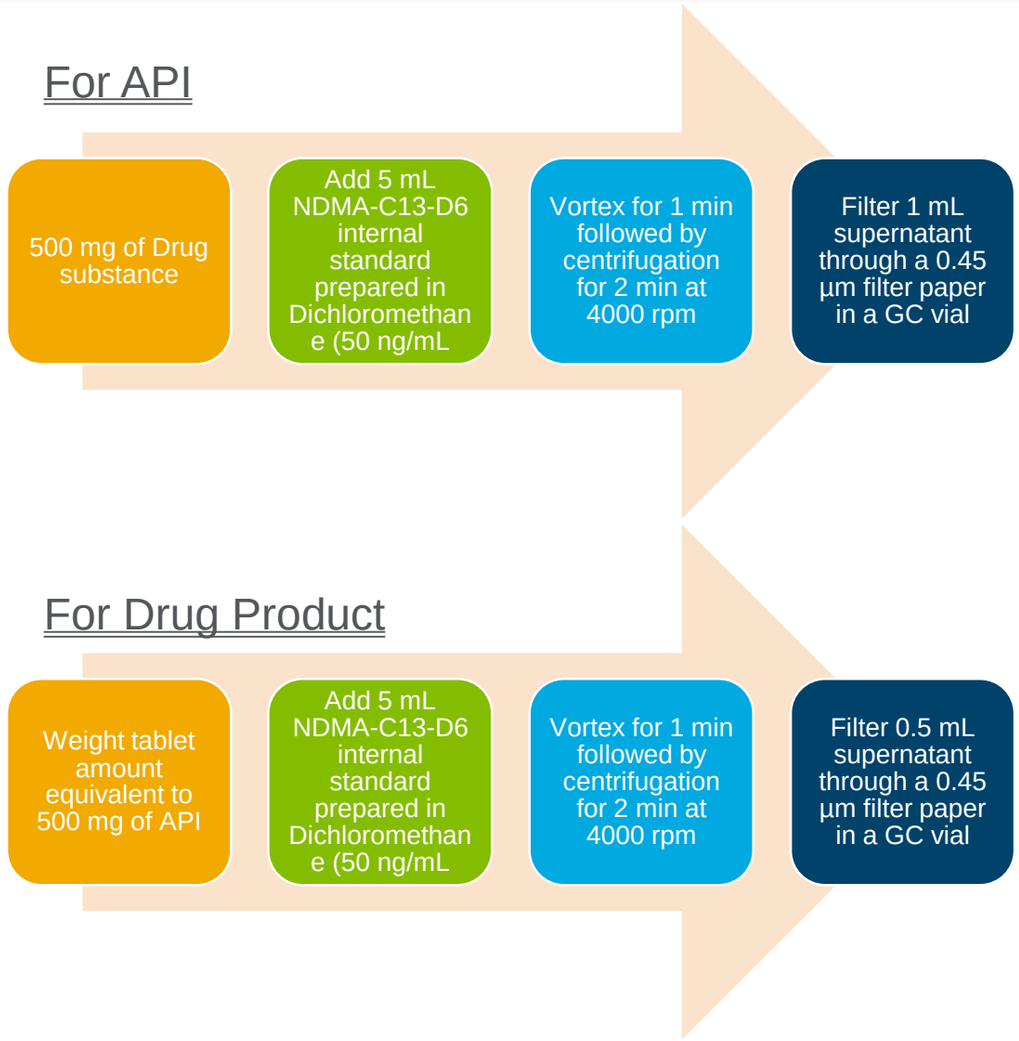
Instrument Method

ALS	GC	MS
Injection Volume: 2µL	Carrier Gas: He 1mL/min	EI Mode

Parameter	Value
MMI injection mode	Pulsed splitless: 12.285 psi until 0.5 min
Inlet temperature	250 °C
Oven temperature program	40 °C (0.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)
Total run time	12.33 min
MS transfer line temperature	250 °C

Parameter	Value	
Source temperature	250 °C	
Quadrupole temperature	Q1 and Q2 = 150 °C	
MS1 and MS2 resolution	All compounds Unit	
Collision gas flow	Nitrogen at 1.5 mL/min,	
Quenching gas flow	Helium at 4 mL/min	
Quant./qual. transitions (FDA method)	Start time: 6.5 min NDMA	74 → 44, CE 15, dwell 150 ms 74 → 42, CE 20, dwell 50 ms NDMA:C13-d_6 82 →48, CE 20, dwell 100 ms
	Start time: 7.60 min NDEA	102 →85, CE 10 V, dwell 150 ms 102 →56, CE 18 V, dwell 150 ms
	Start time: 8.03 min NEIPA	116 →99, CE 10 V, dwell 150 ms 71 →56, CE 10 V, dwell 150 ms
	Start time: 8.25 min NDIPA	130 →88, CE 10 V, dwell 150 ms 130 →42, CE 10 V, dwell 150 ms
	Start time: 8.70 min NDBA	158 →99, CE 10 V, dwell 150 ms 84 →56, CE 22 V, dwell 150 ms

Sample Preparation



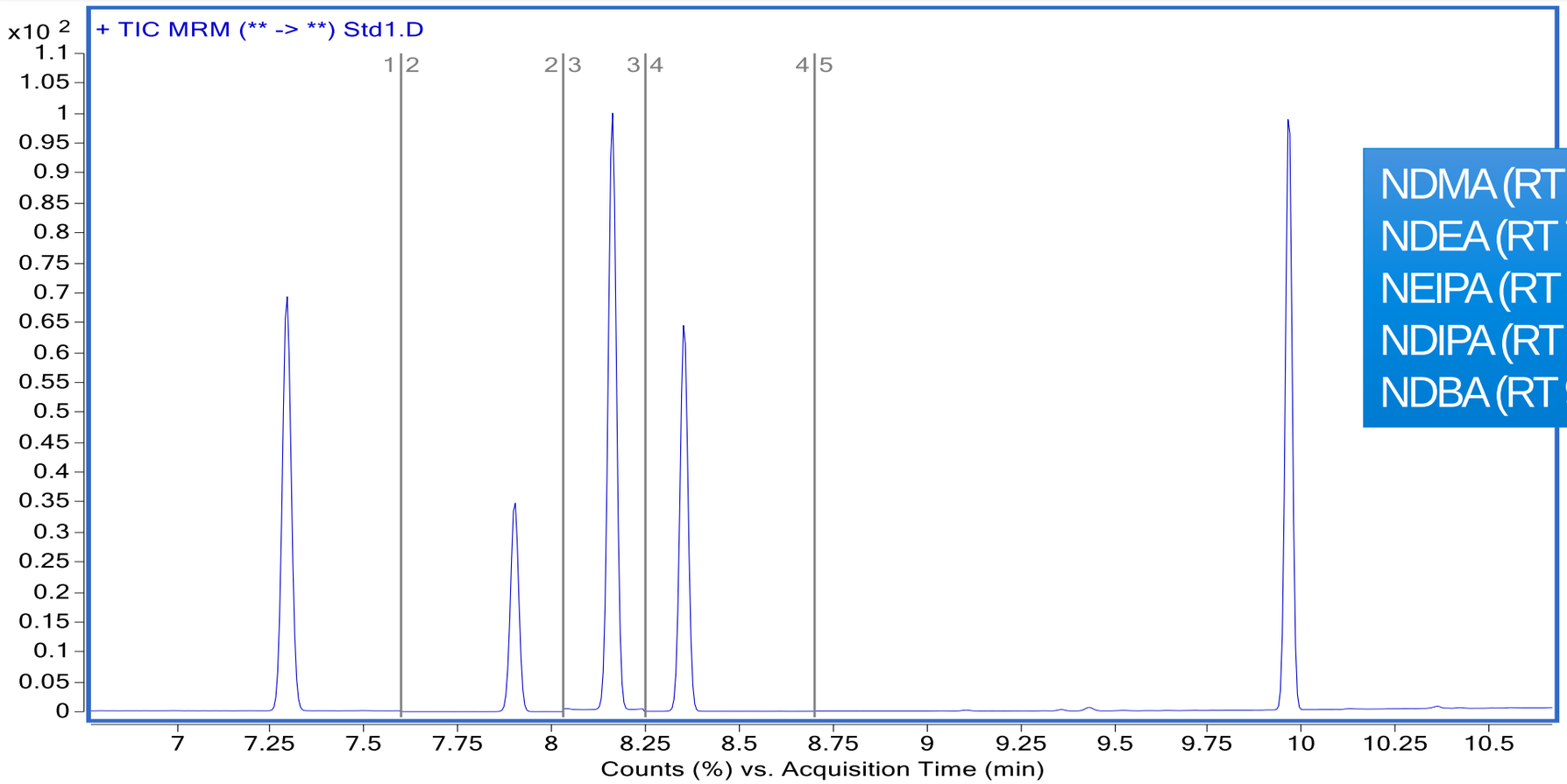
Calibrations

2.5 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 40 ng/ml, 80 ng/ml and 100 ng/ml each prepared in Dichloromethane containing 50 ng/mL of NDMA –C13-D6

System Suitability

The coefficient of determination (R2) of the linear calibration curve should be ≥ 0.998.
The S/N ratio of the 5 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 40 ng/mL standard should be ≤ 5

Results for 5 nitrosamine impurities at 100 ng/mL in Telmisartan API

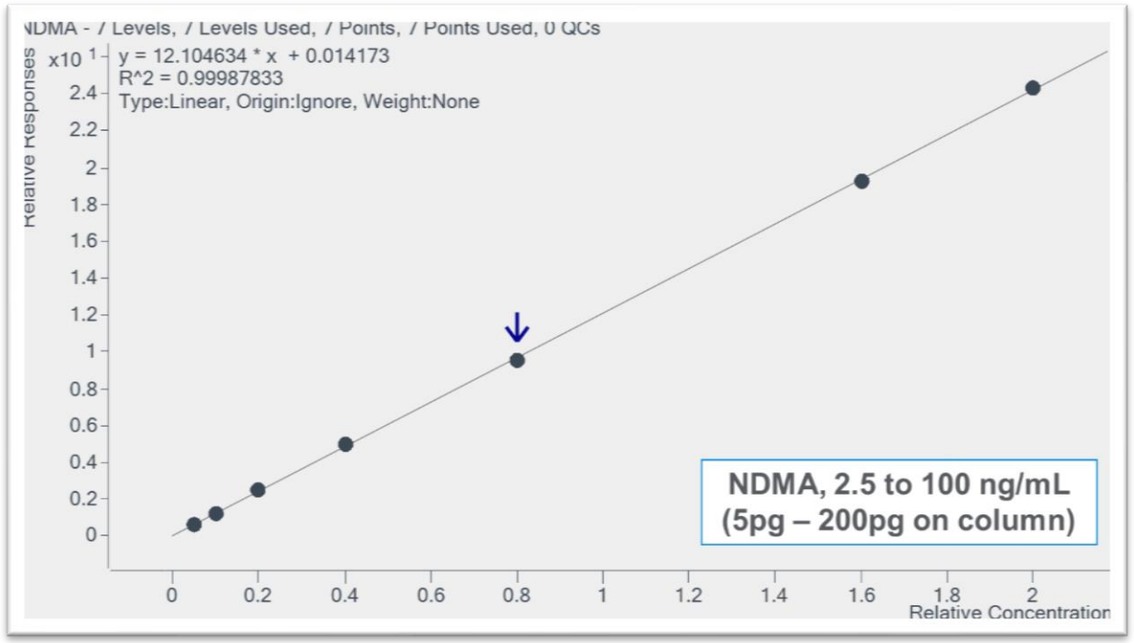


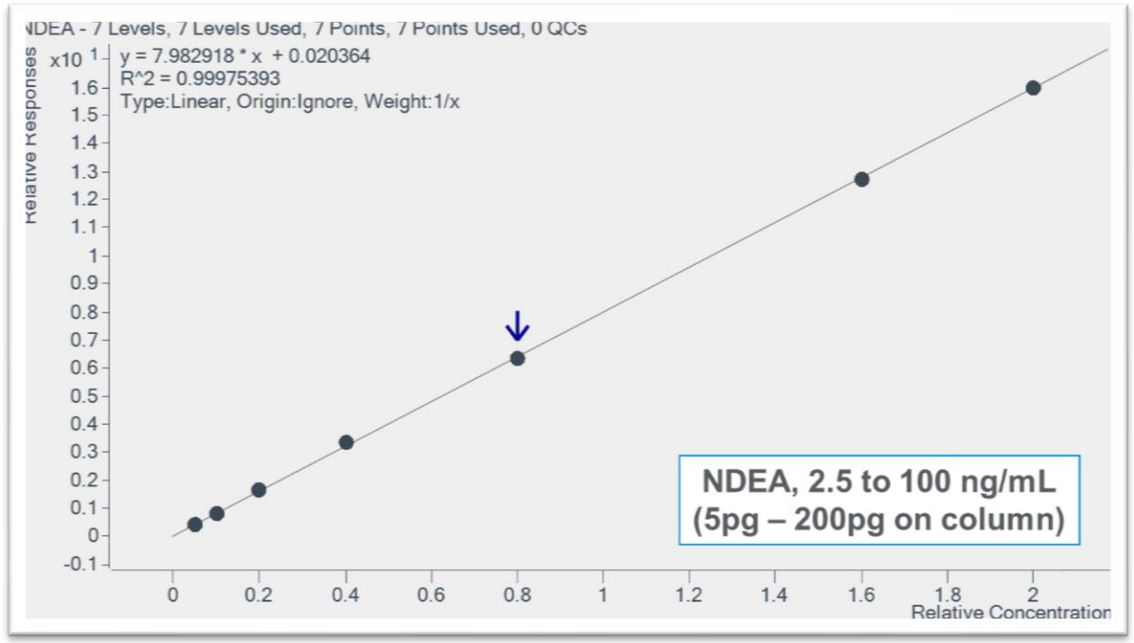
NDMA (RT 7.294 min)
NDEA (RT 7.903 min)
NEIPA (RT 8.157 min)
NDIPA (RT 8.346 min)
NDBA (RT 9.777 min)

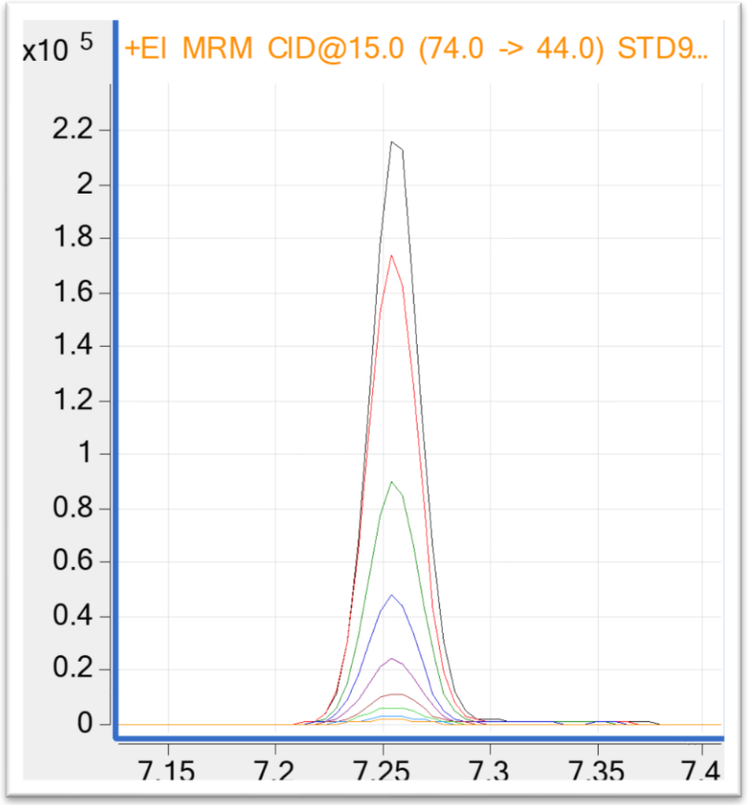
Benefits Agilent GC/TQ	
Optimized methods	• Optimized method for both API and Formulation • Compatible with stringent FDA regulations
Scalable application	• Best precision = best ion ratios = best quant results Rugged ion source design • Retention Time Locking for reproducible methods over time and between labs
Sample prep	• Sample preparation as per FDA guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument. • Efficient Quant review with MassHunter • Data Integrity

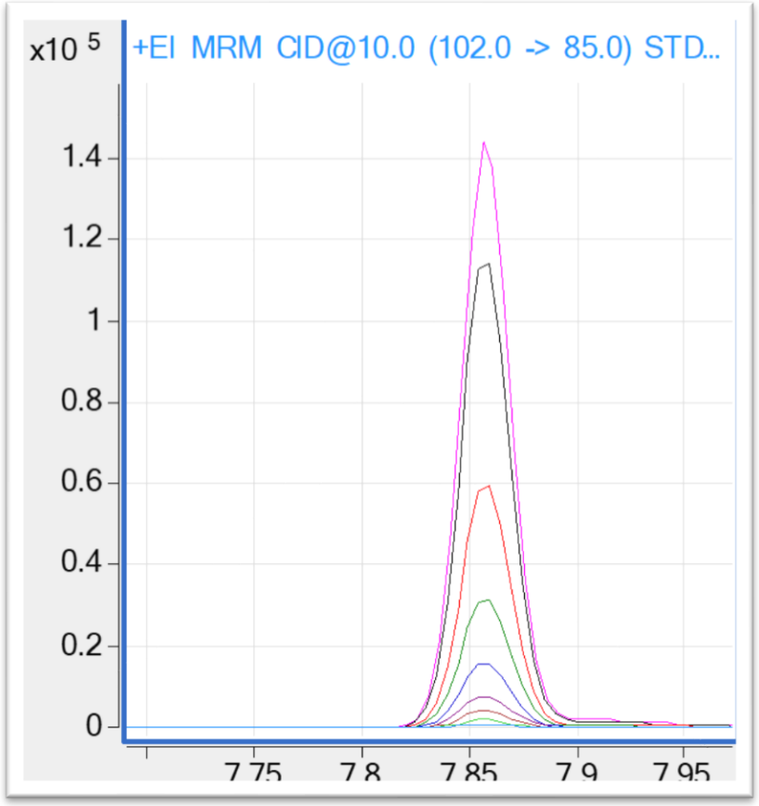
Telmisartan

Calibration Curves

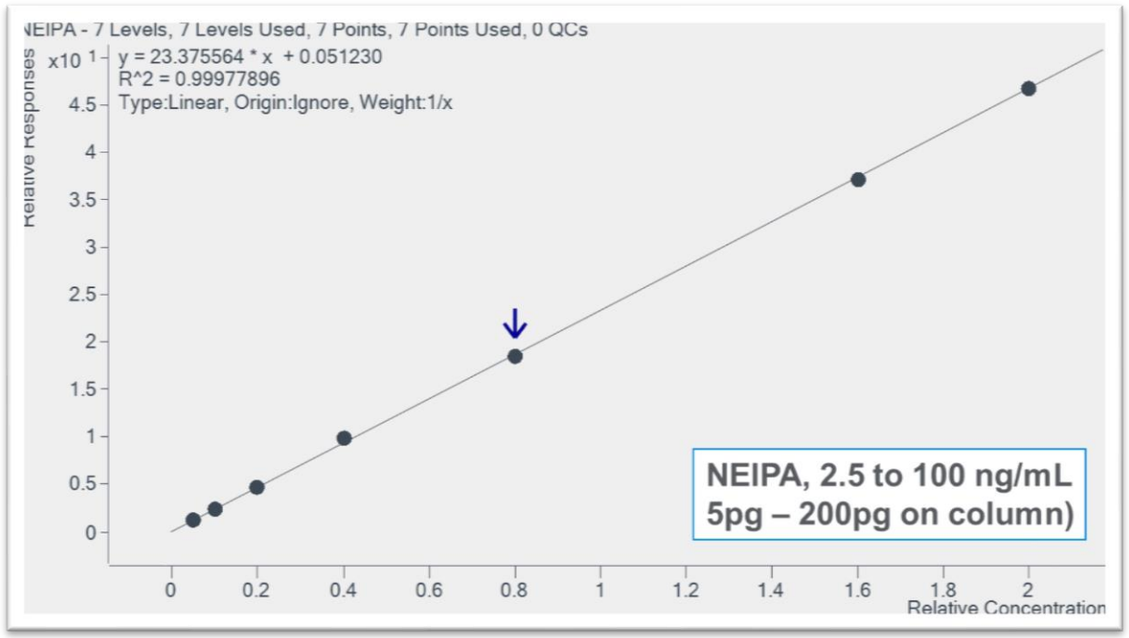


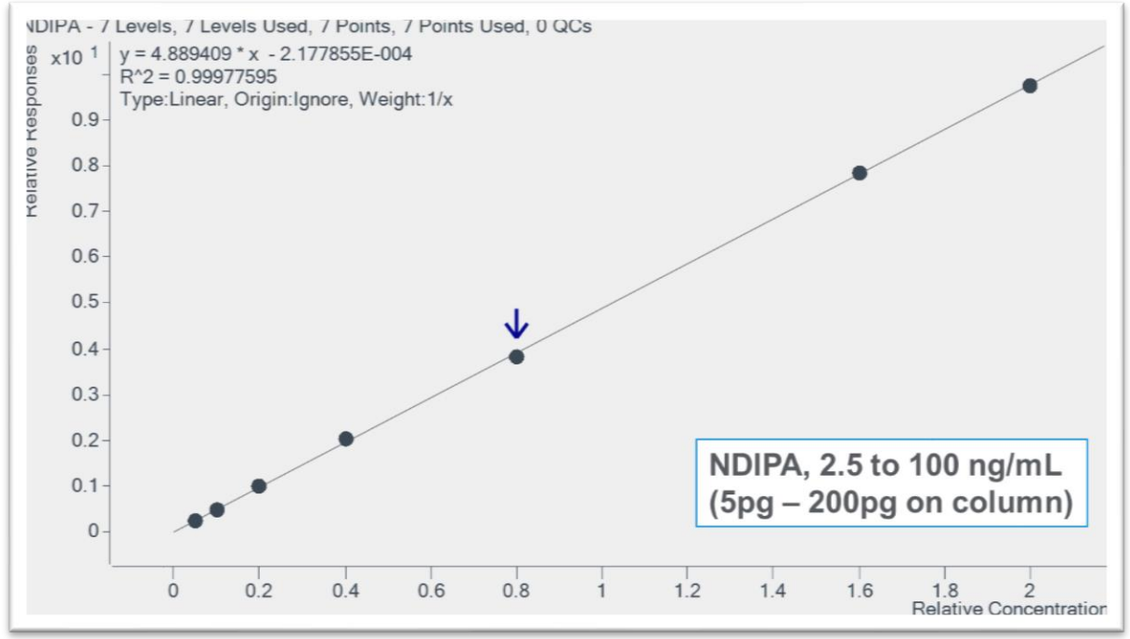


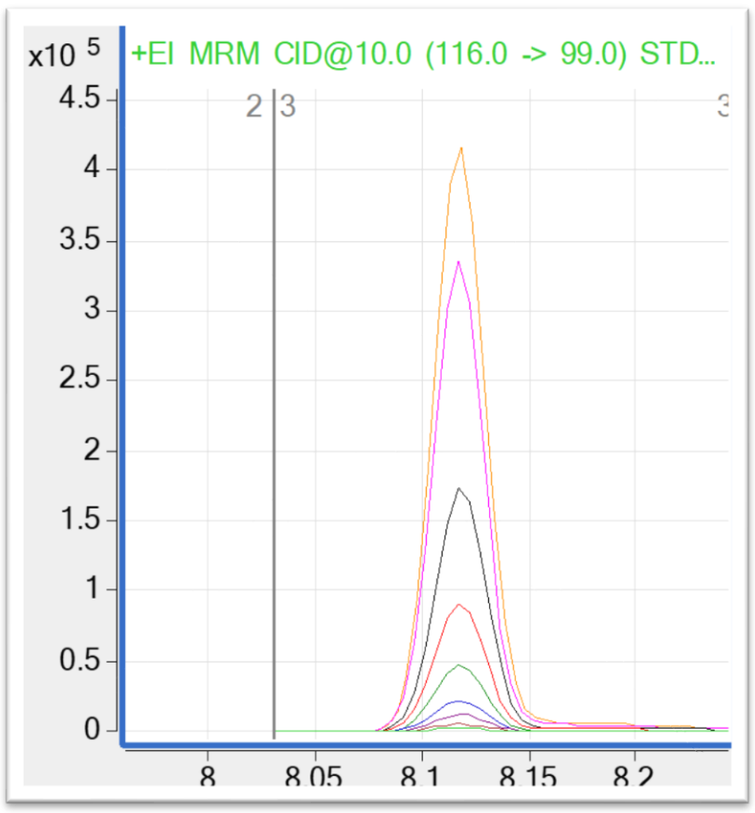


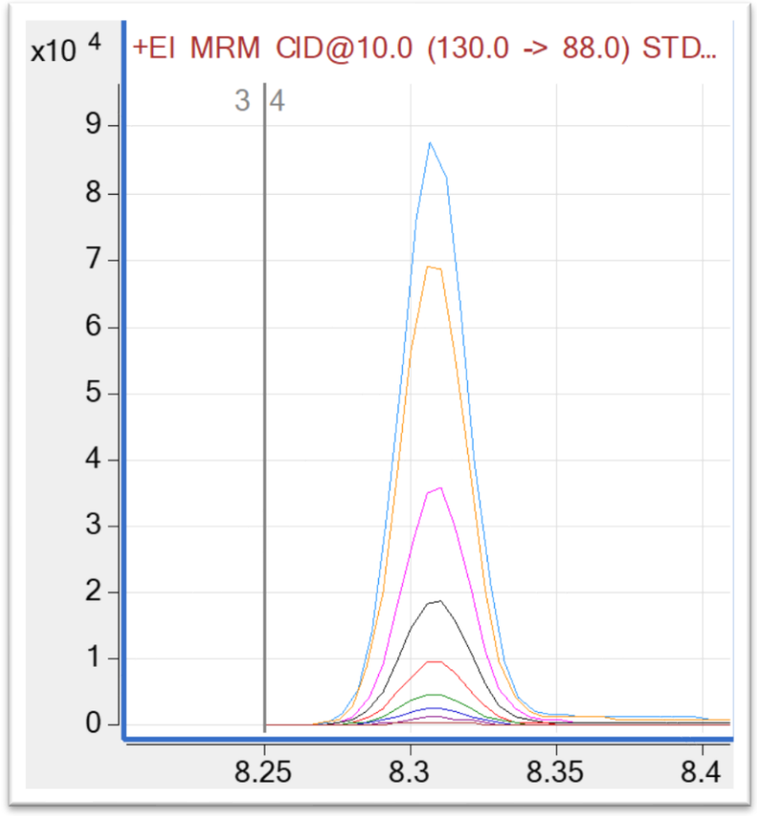


Calibration Curves

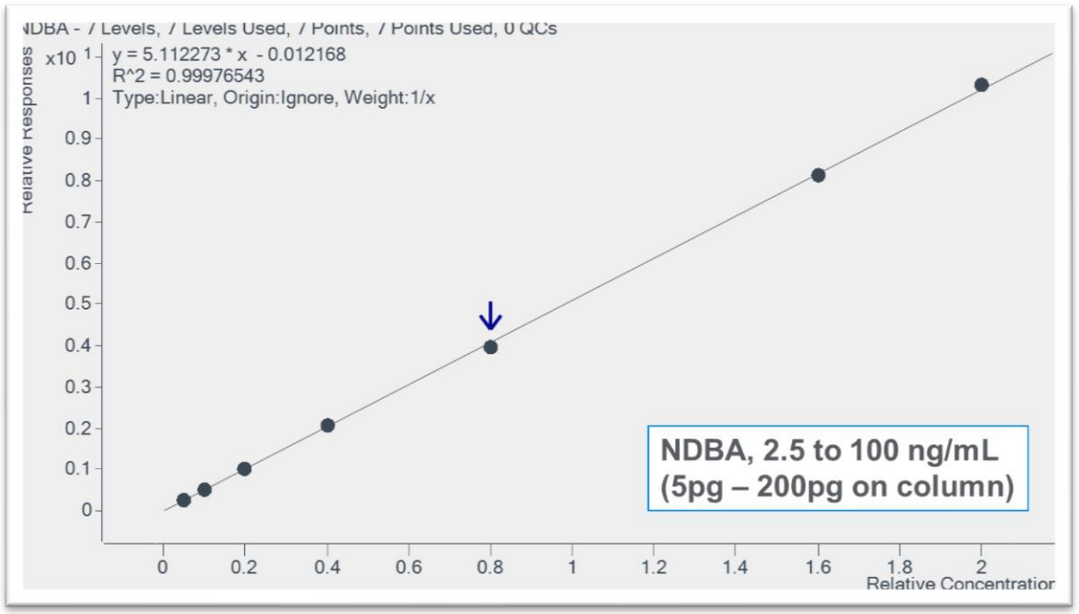


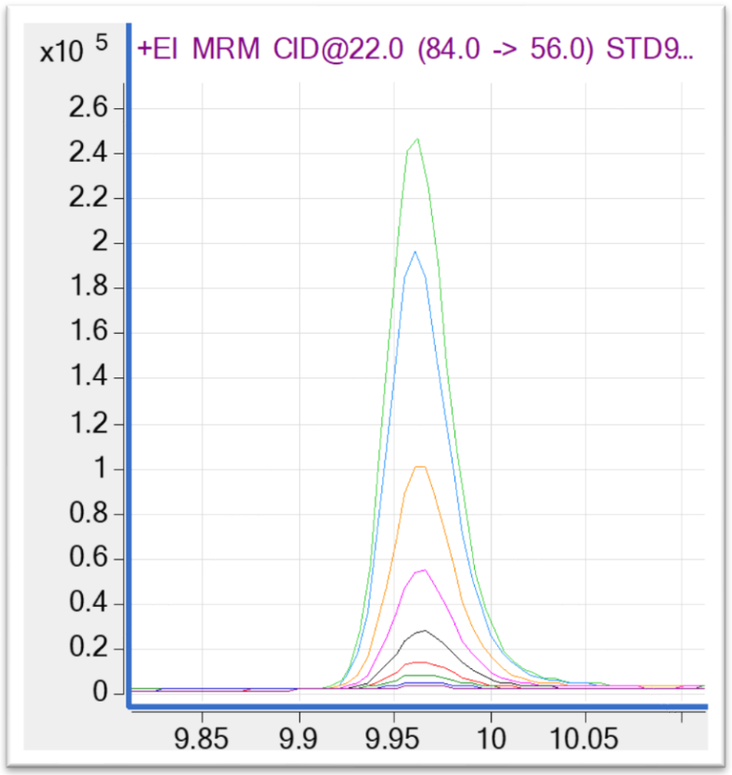




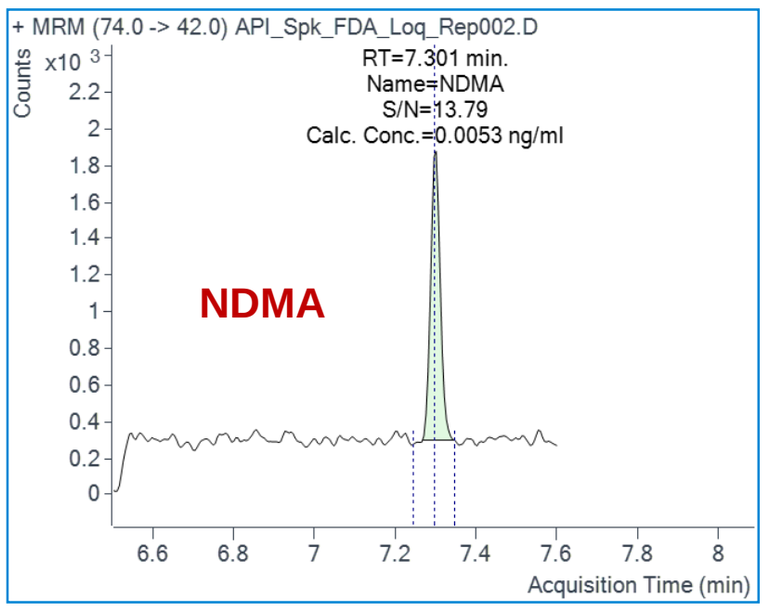


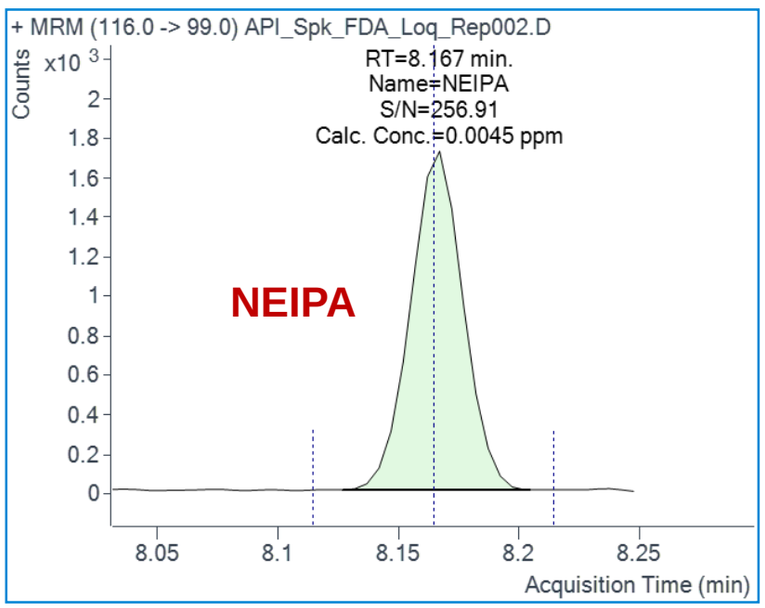
Calibration Curves

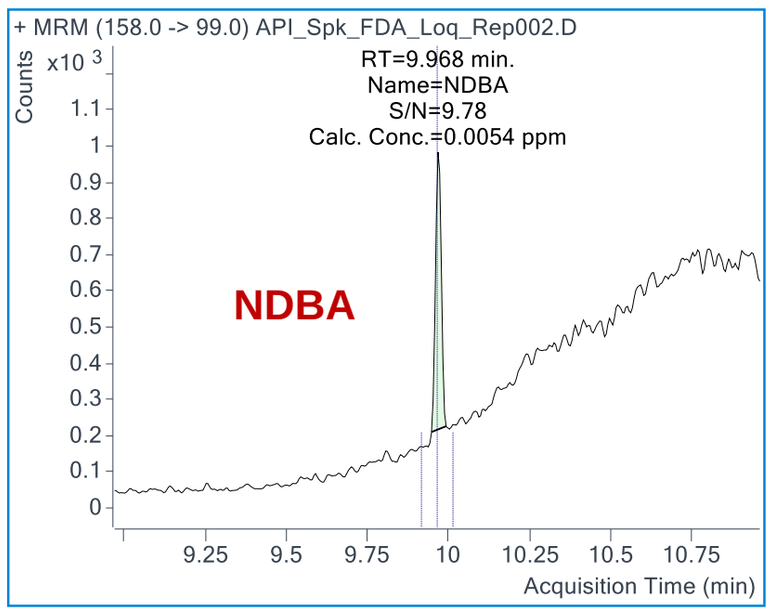


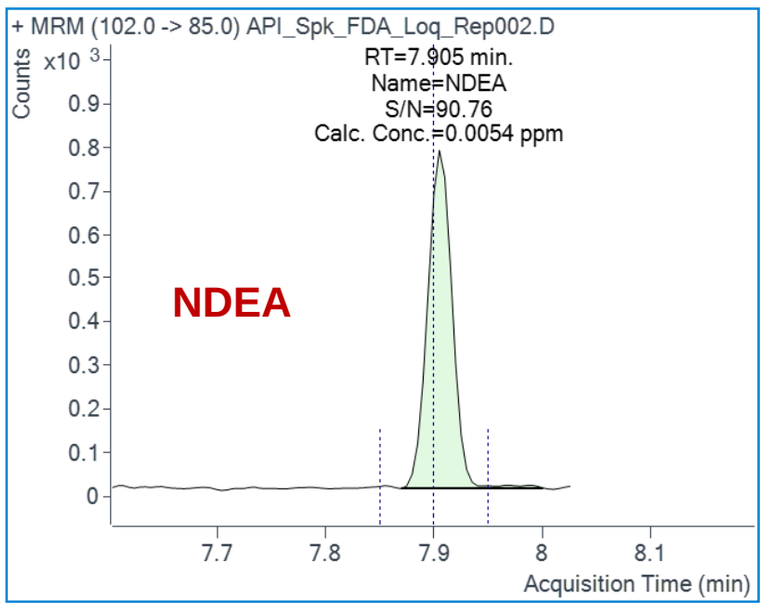


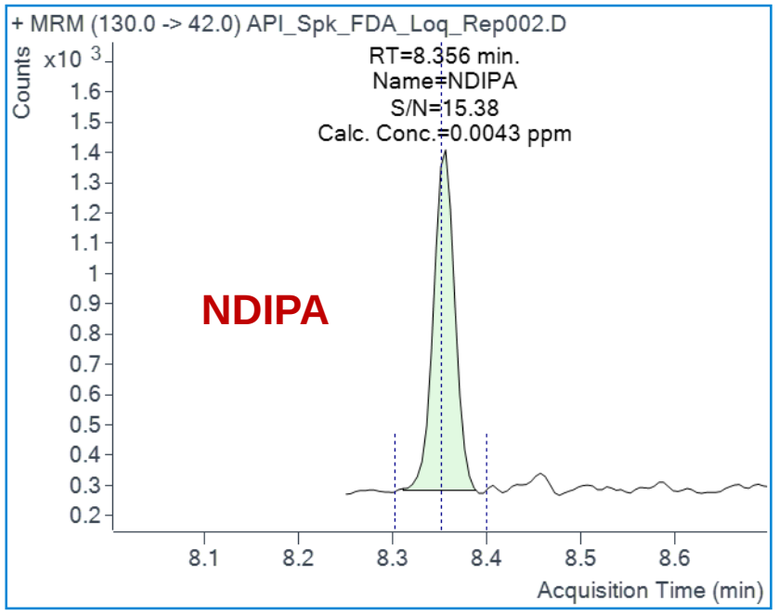
Representative Recovery % of Nitrosamine Impurities in Telmisartan at 0.005 ppm











Compound	Spiking Level (ppm)	Sample Results (ppm)	Recovery (%)
NDMA	0.005	0.0053	106
NDEA	0.005	0.0054	108
NEIPA	0.005	0.0045	90
NDIPA	0.005	0.0043	86
NDBA	0.005	0.0054	108

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Telmisartan

LC/MS Method for Analysis

Instrument Method

Mobile phase A:	0.1 % formic acid in water
Mobile phase B:	0.1 % formic acid in Methanol
Multisampler temperature:	10°C
Injection volume:	20 µL
Analytical column:	Agilent Zorbax Eclipse Plus C18 150*3.0mm 3.5micron (P/N:959963-302)
Column temperature:	40 °C
Flow rate:	0.3 mL/min

Gradient

Time (min)	% A	% B	Flow (mL/min)
0	95	5	0.3
3.0	95	5	0.3
7.0	40	60	0.3
11.0	5	95	0.3
15.0	5	95	0.3
15.1	95	5	0.3
18.0	95	5	0.3

Instrument	Agilent 6470 Triple Quadrupole mass spectrometer
Ion source	Atmospheric Pressure Chemical Ionization (APCI)
MS/MS mode	MRM
Ion mode	Positive
Drying gas temperature	300 °C
Drying gas flow	6 L/min
Nebulizer pressure	35 psi
APCI heater	350 °C
APCI needle positive	4 µA
Capillary voltage, positive	4000 V
MS1/MS2 resolution	0.7/0.7 (unit/unit)
Dwell time	50 ms

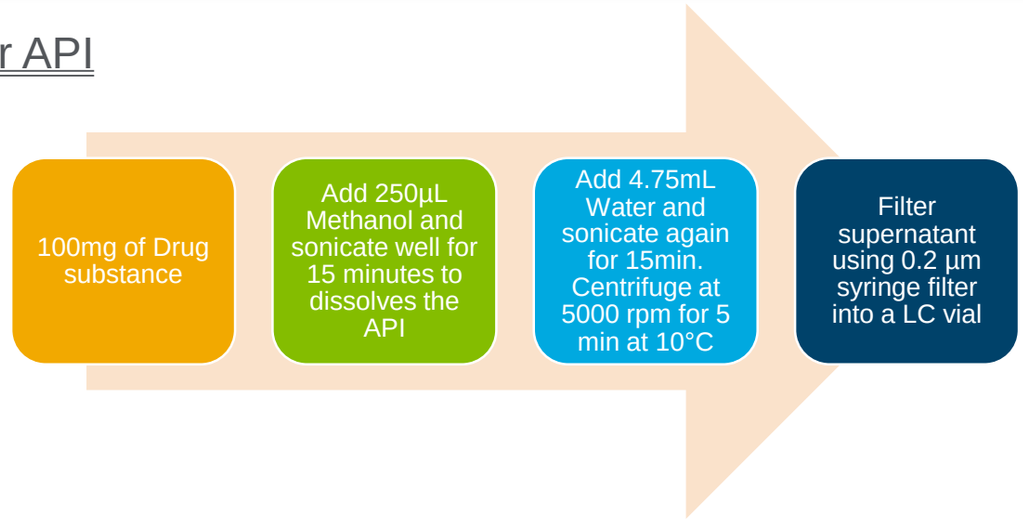
Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy(V)	CAV(V)	Polarity
NDMA(Quantifier)	75.1	43.1	100	17	5	+
NDMA (Qualifier)	75.1	58.1	75	11	5	+
NMBA(Quantifier)	147.1	117.4	60	4	3	+
NMBA(Qualifier)	147.1	44.2	60	12	3	+
NDEA(Quantifier)	103.1	75.1	80	9	3	+
NDEA(Qualifier)	103.1	47.1	80	17	3	+
NEIPA(Quantifier)	117.1	75.1	75	8	3	+
NEIPA(Qualifier)	117.1	47.1	75	18	8	+
NDIPA(Quantifier)	131.1	89.1	75	6	3	+
NDIPA(Qualifier)	131.1	43.1	75	12	8	+
NDBA(Quantifier)	159.1	57.2	81	12	5	+
NDBA(Qualifier)	159.1	41.1	81	22	5	+

Calibrations

0.1 ng/mL to 100 ng/mL

Sample Preparation

For API



System Suitability

The coefficient of determination (R^2) of the linear calibration curve should be ≥ 0.990.
The S/N ratio of the 1 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 1 ng/mL standard should be ≤ 10

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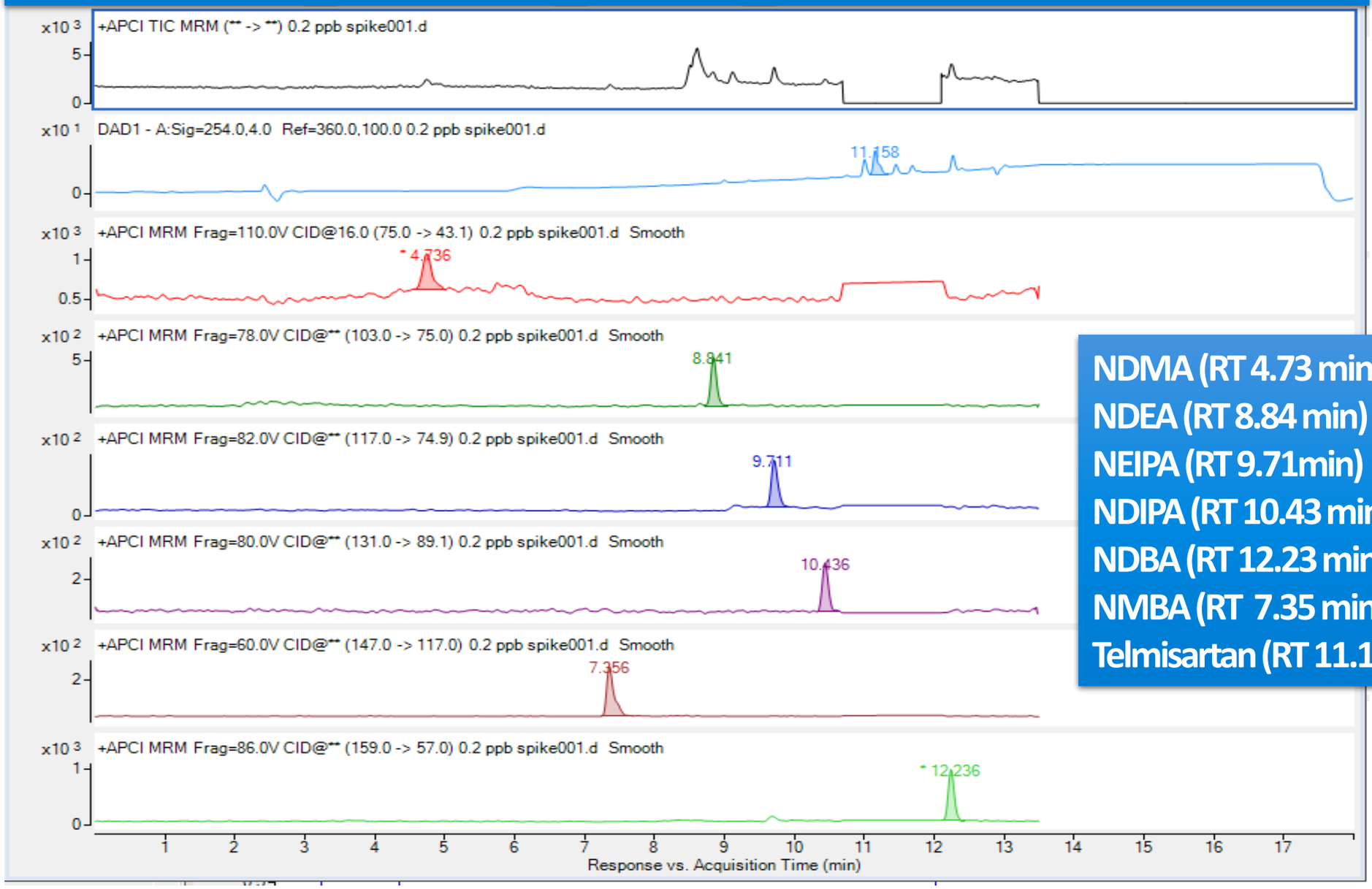
Agilent

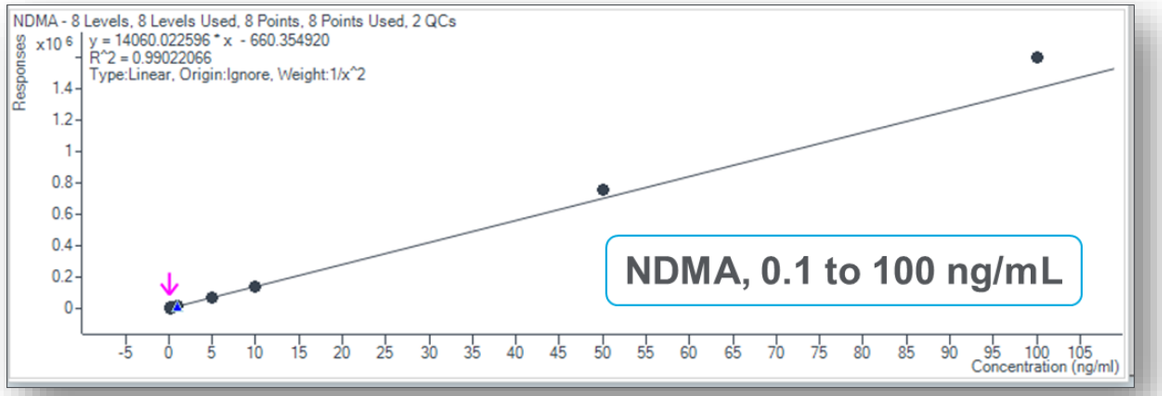
Results for 6 nitrosamine impurities at 20 ng/mL in Telmisartan API

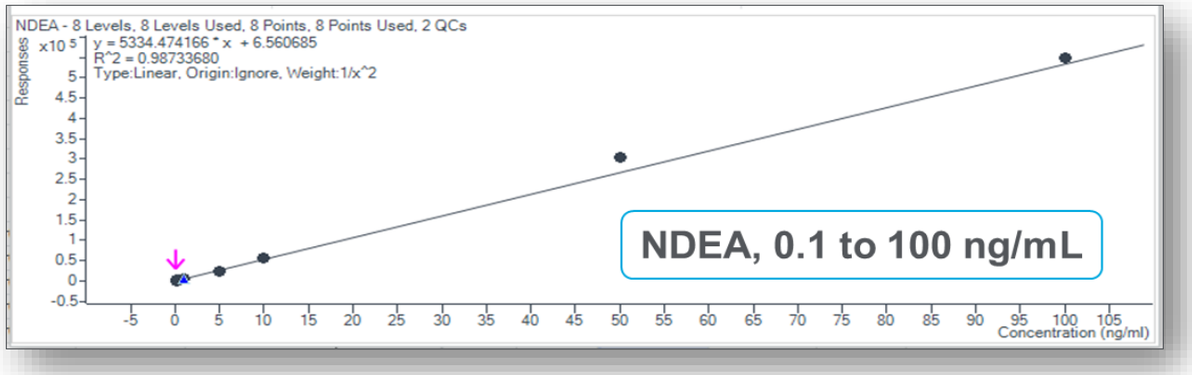


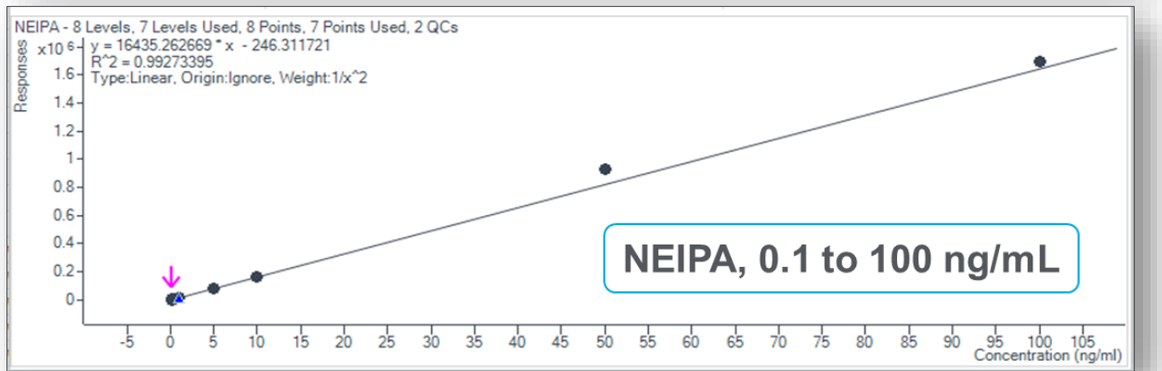
NDMA (RT 4.73 min)
NDEA (RT 8.84 min)
NEIPA (RT 9.71min)
NDIPA (RT 10.43 min)
NDBA (RT 12.23 min)
NMBA (RT 7.35 min)
Telmisartan (RT 11.158 min)

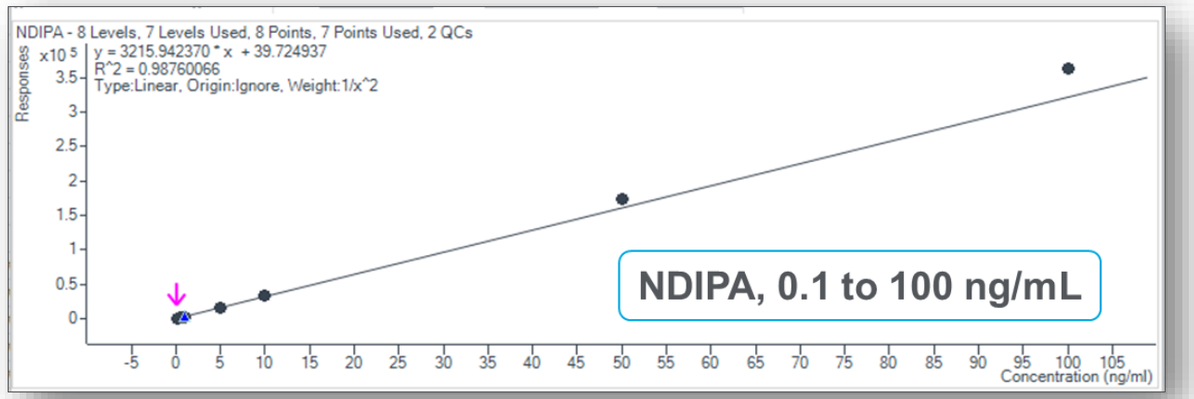
Telmisartan

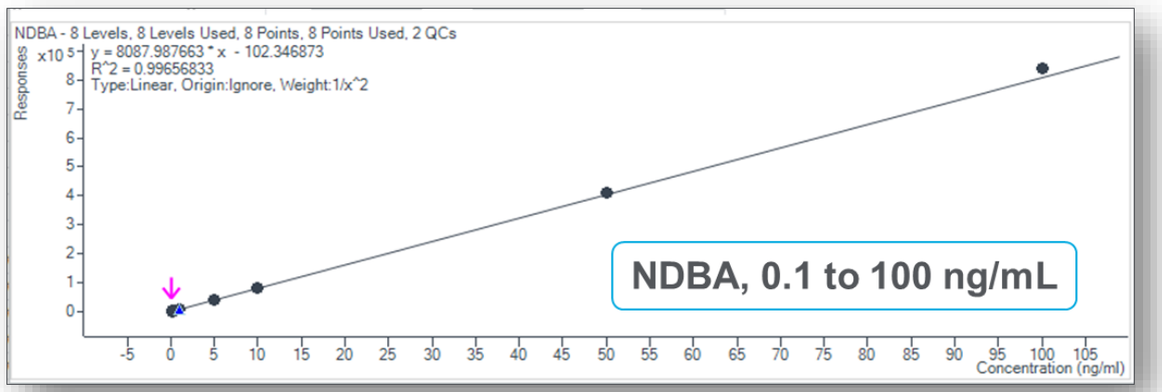
Calibration Curves

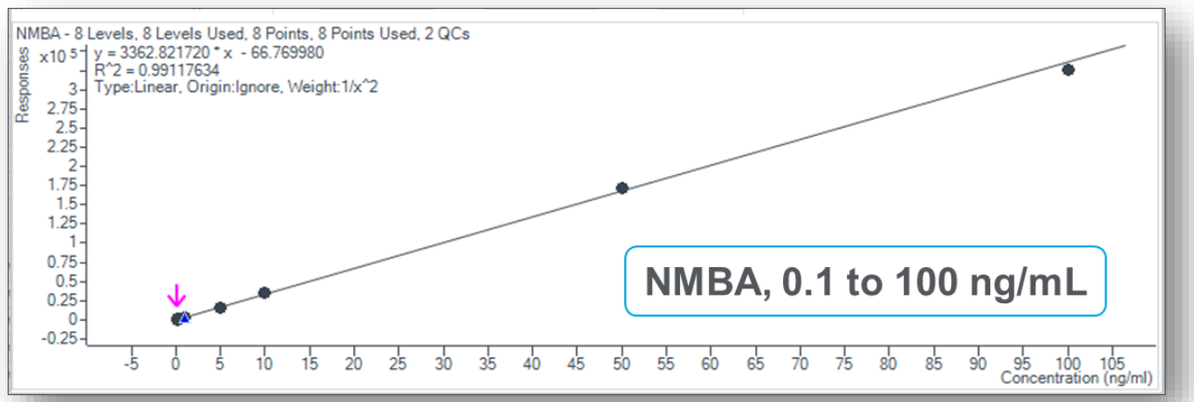












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Representative Recovery % of Nitrosamine Impurities

@ 0.2ng/mL (0.004ppm) concentration using 50mg/mL sample size

S.No.	Nitrosamine Impurities	Average Recovery%
1	NDMA	101.7
2	NMBA	104.3
3	NDEA	117.7
4	NEIPA	91.1
5	NDIPA	95.6
6	NDBA	91.1

Note: Use of corresponding internal standards for each nitrosamines may further help in any recovery issue.

Benefits of Agilent LC/TQ	
Optimized methods	• Optimized method for telmisartan drug substance • Detect and quantify nitrosamine impurities limits per published FDA regulatory testing method guidance
Scalable application	• Best precision = best ion ratios = best quant results; Rugged ion source design
Sample prep	• Sample preparation as per EDQM guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument • Efficient Quant review with MassHunter • Data Integrity

GC/MS Method for Analysis

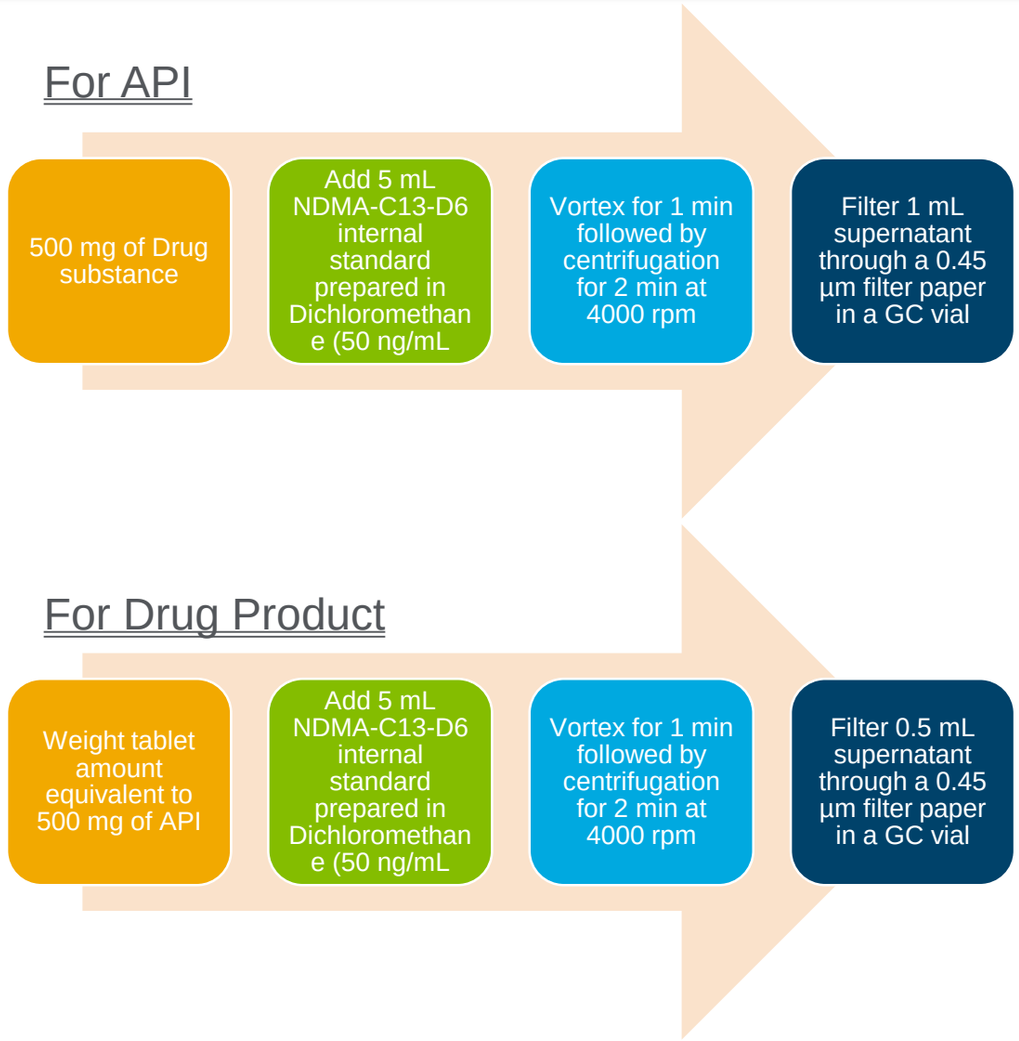
Instrument Method

ALS	GC	MS
Injection Volume: 2µL	Carrier Gas: He 1mL/min	EI Mode

Parameter	Value
MMI injection mode	Pulsed splitless: 12.285 psi until 0.5 min
Inlet temperature	250 °C
Oven temperature program	40 °C (0.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)
Total run time	12.33 min
MS transfer line temperature	250 °C

Parameter	Value	
Source temperature	250 °C	
Quadrupole temperature	Q1 and Q2 = 150 °C	
MS1 and MS2 resolution	All compounds Unit	
Collision gas flow	Nitrogen at 1.5 mL/min,	
Quenching gas flow	Helium at 4 mL/min	
Quant./qual. transitions (FDA method)	Start time: 6.5 min NDMA	74 → 44, CE 15, dwell 150 ms 74 → 42, CE 20, dwell 50 ms NDMA:C13-d_6 82 →48, CE 20, dwell 100 ms
	Start time: 7.60 min NDEA	102 →85, CE 10 V, dwell 150 ms 102 →56, CE 18 V, dwell 150 ms
	Start time: 8.03 min NEIPA	116 →99, CE 10 V, dwell 150 ms 71 →56, CE 10 V, dwell 150 ms
	Start time: 8.25 min NDIPA	130 →88, CE 10 V, dwell 150 ms 130 →42, CE 10 V, dwell 150 ms
	Start time: 8.70 min NDBA	158 →99, CE 10 V, dwell 150 ms 84 →56, CE 22 V, dwell 150 ms

Sample Preparation



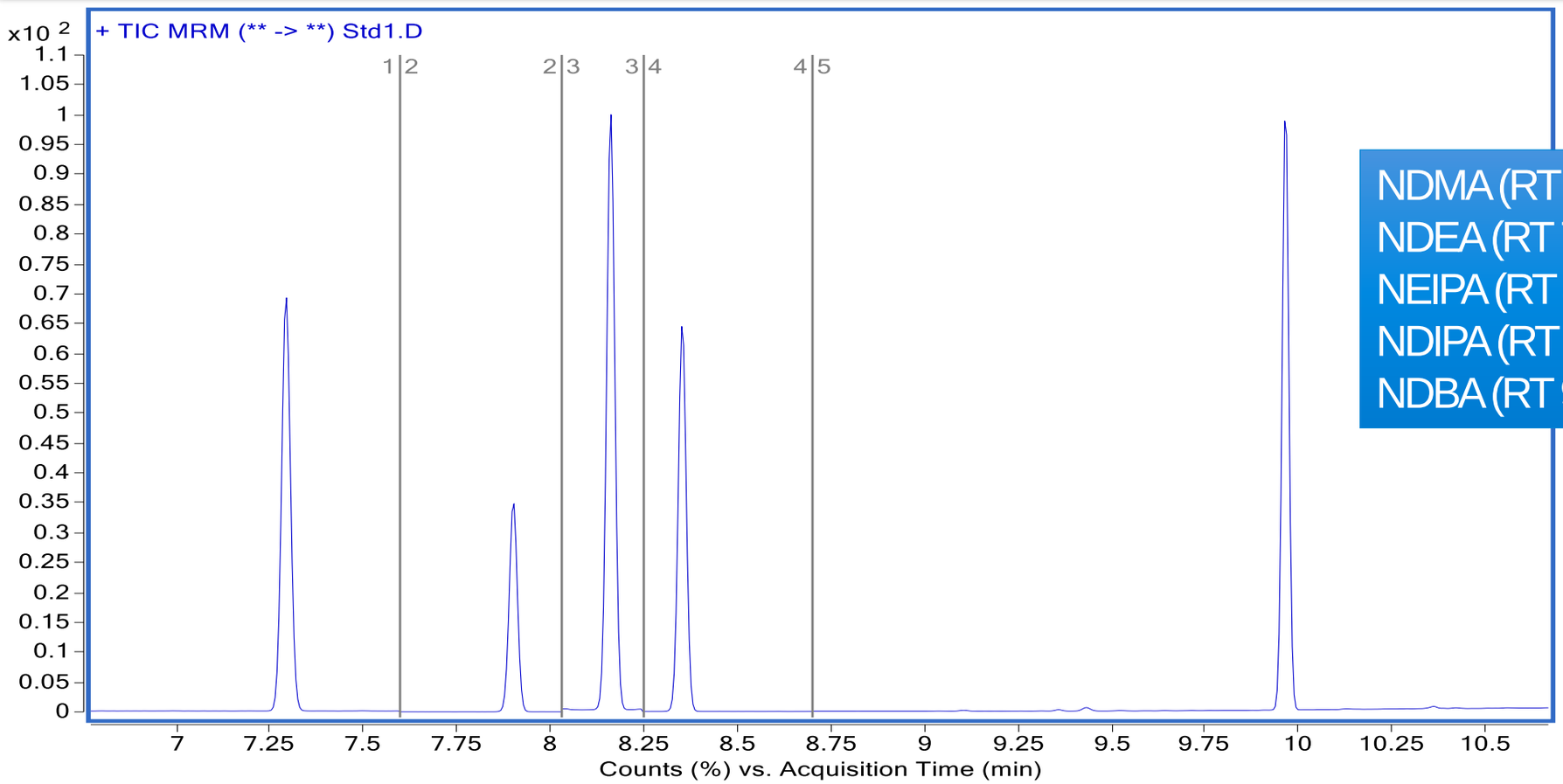
Calibrations

2.5 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 40 ng/ml, 80 ng/ml and 100 ng/ml each prepared in Dichloromethane containing 50 ng/mL of NDMA –C13-D6

System Suitability

The coefficient of determination (R2) of the linear calibration curve should be ≥ 0.998.
The S/N ratio of the 5 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 40 ng/mL standard should be ≤ 5

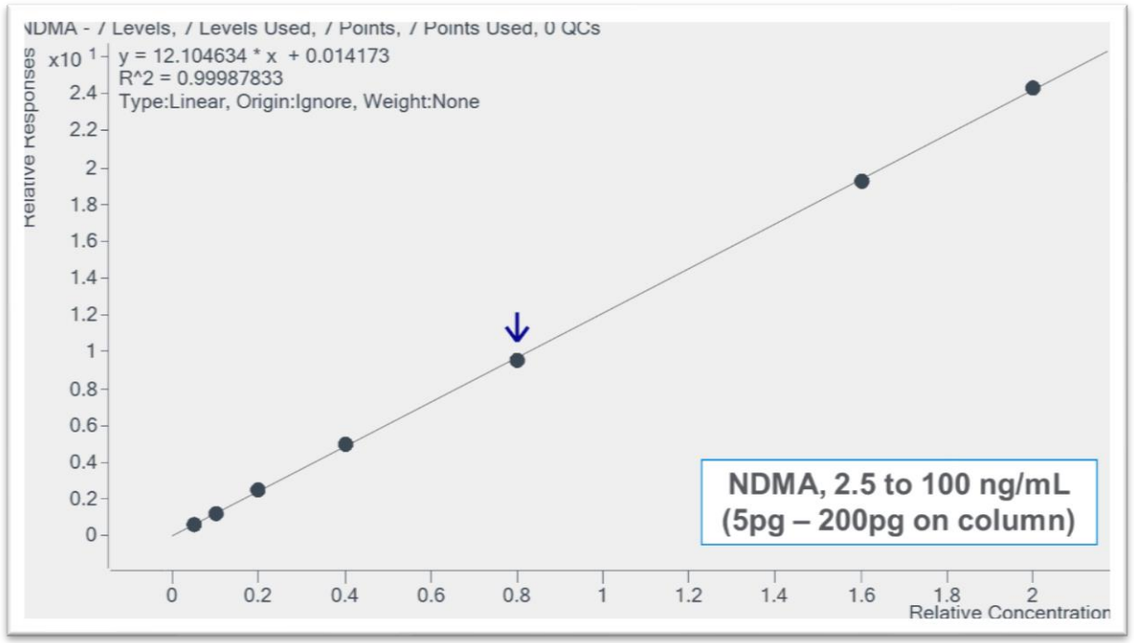
Results for 5 nitrosamine impurities at 100 ng/mL in Candesartan API

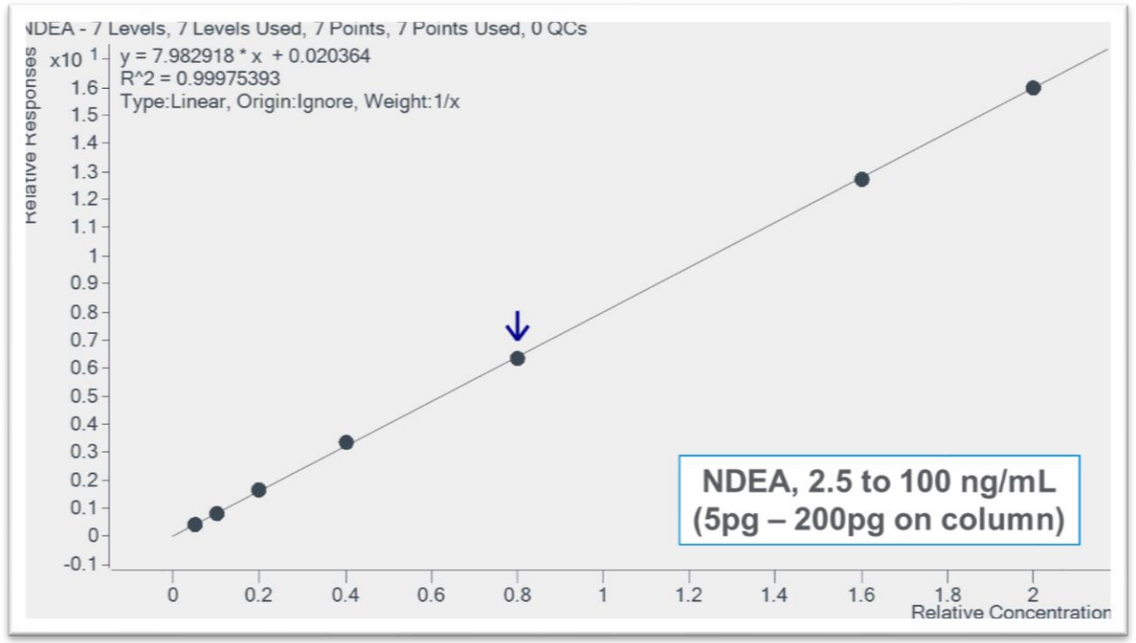


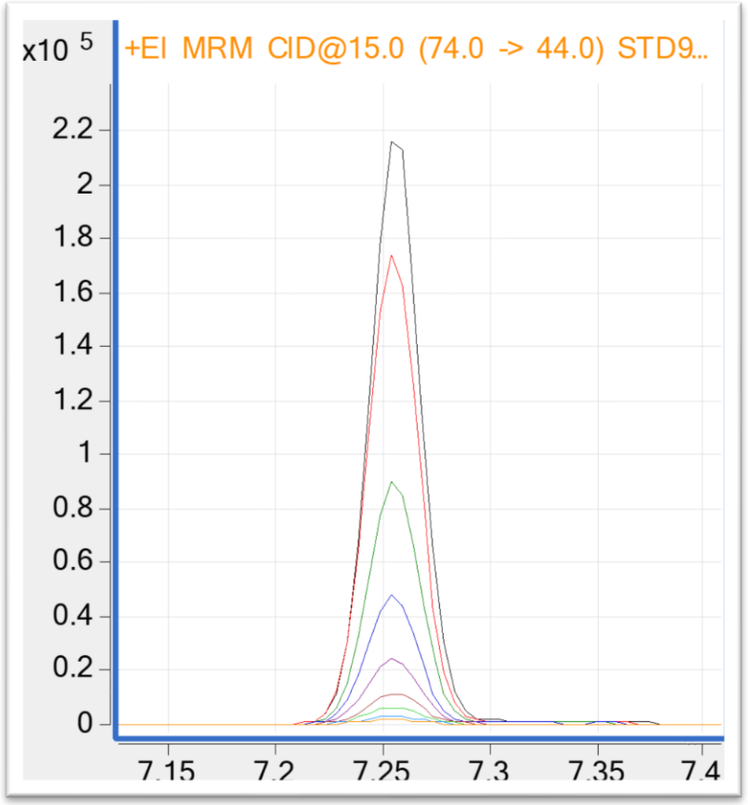
NDMA (RT 7.294 min)
NDEA (RT 7.903 min)
NEIPA (RT 8.157 min)
NDIPA (RT 8.346 min)
NDBA (RT 9.777 min)

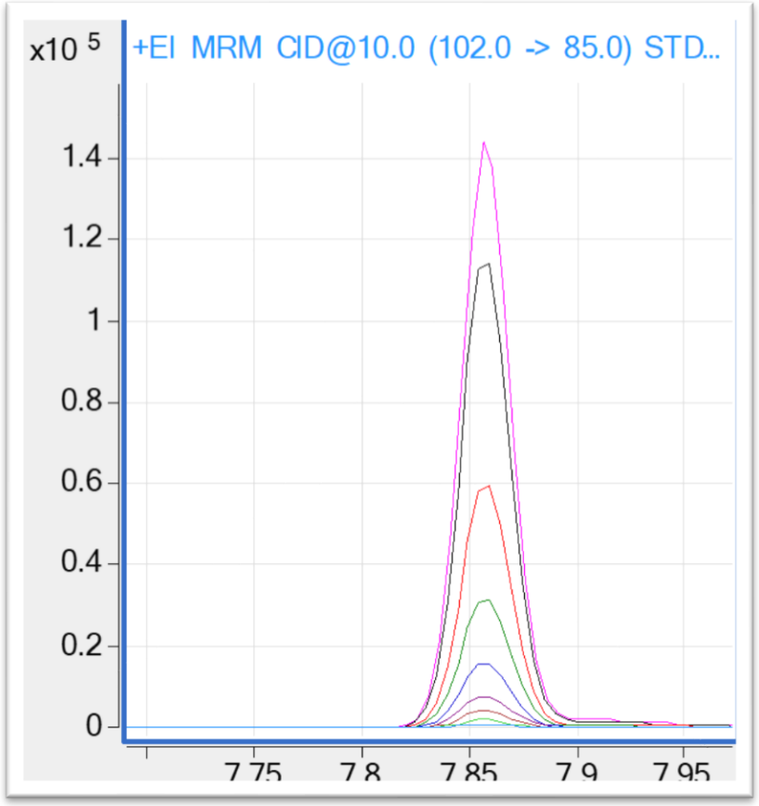
Benefits Agilent GC/TQ	
Optimized methods	• Optimized method for both API and Formulation • Compatible with stringent FDA regulations
Scalable application	• Best precision = best ion ratios = best quant results Rugged ion source design • Retention Time Locking for reproducible methods over time and between labs
Sample prep	• Sample preparation as per FDA guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument. • Efficient Quant review with MassHunter • Data Integrity

Calibration Curves



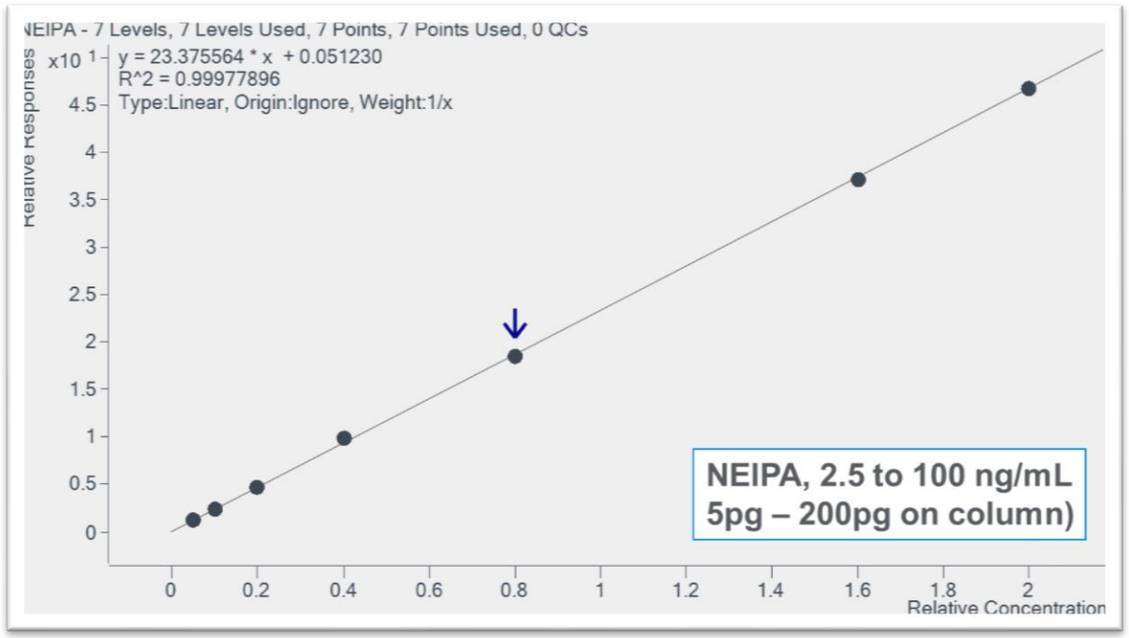


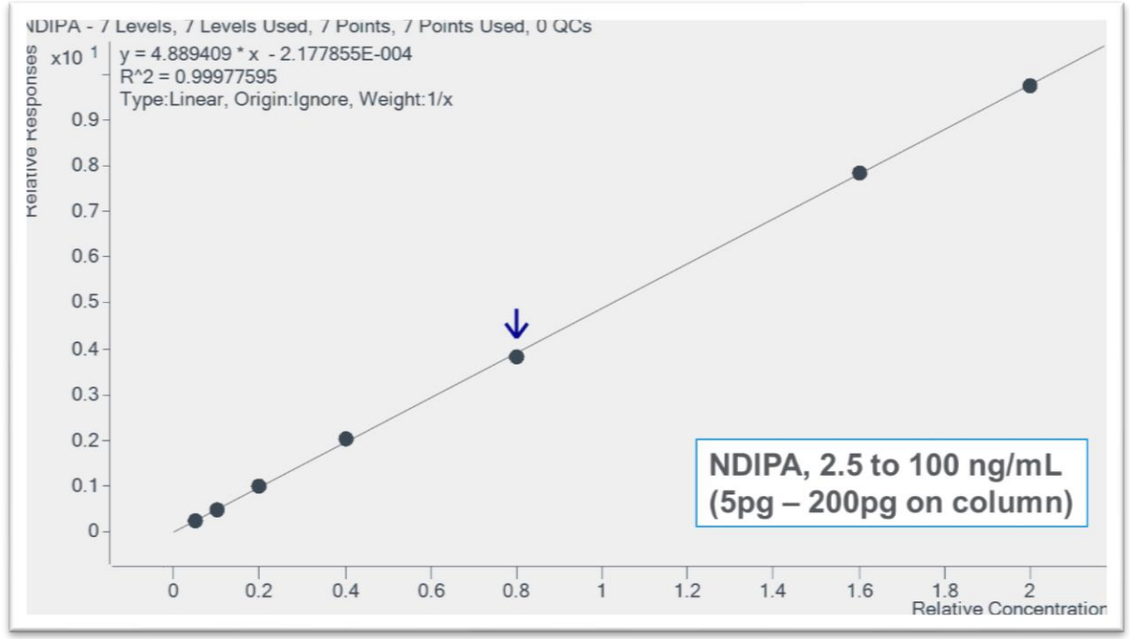


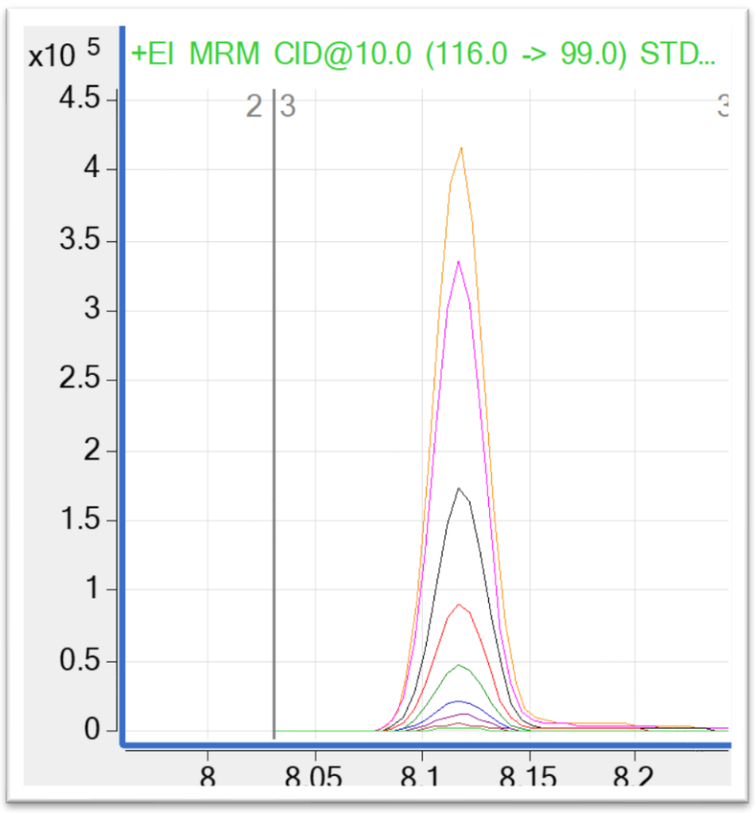


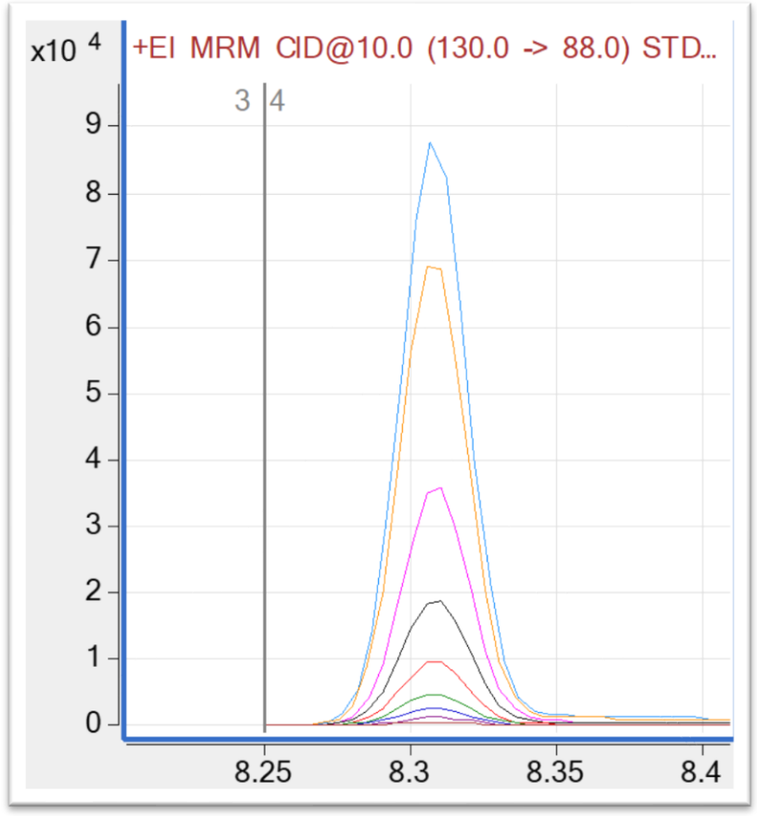
Candesartan

Calibration Curves

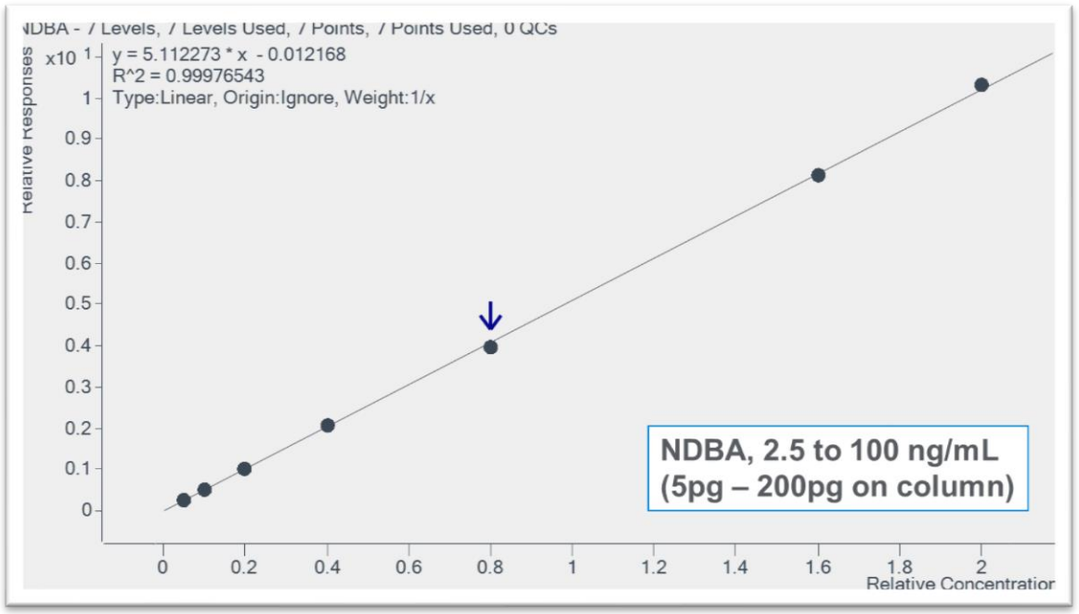


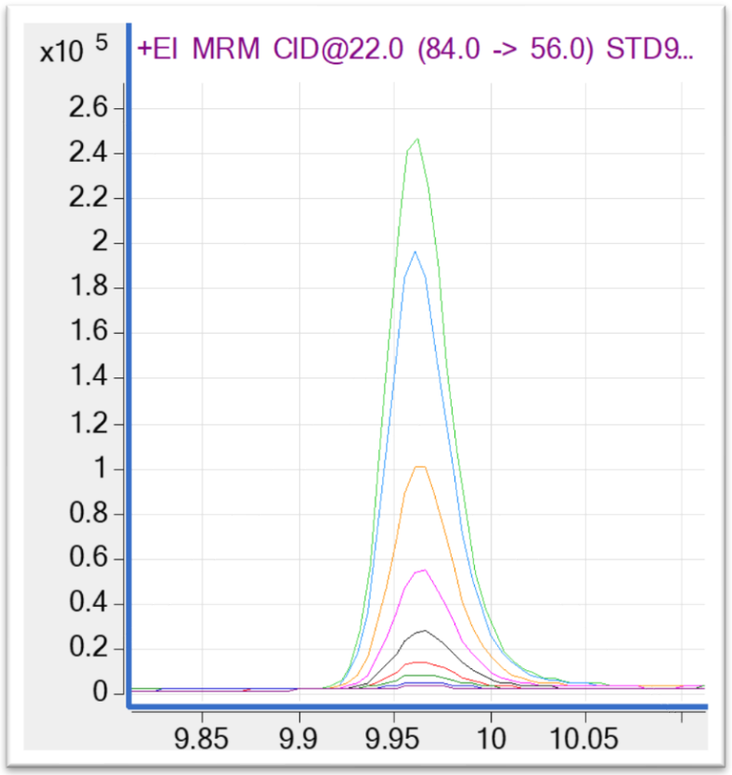




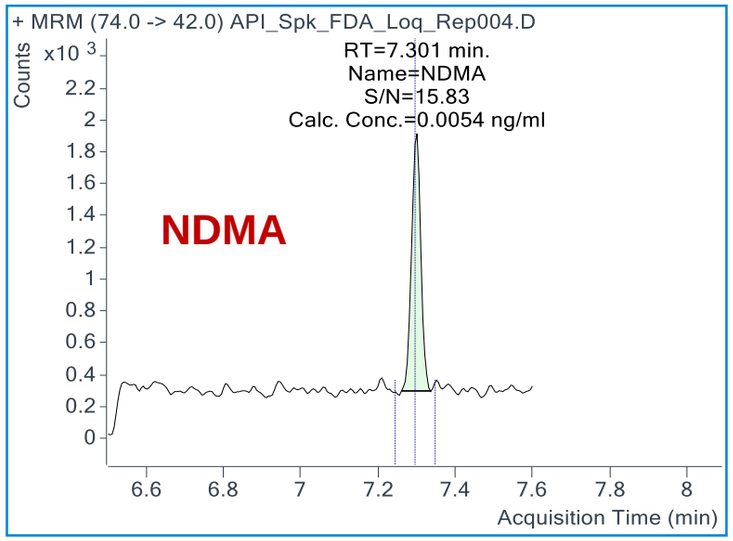


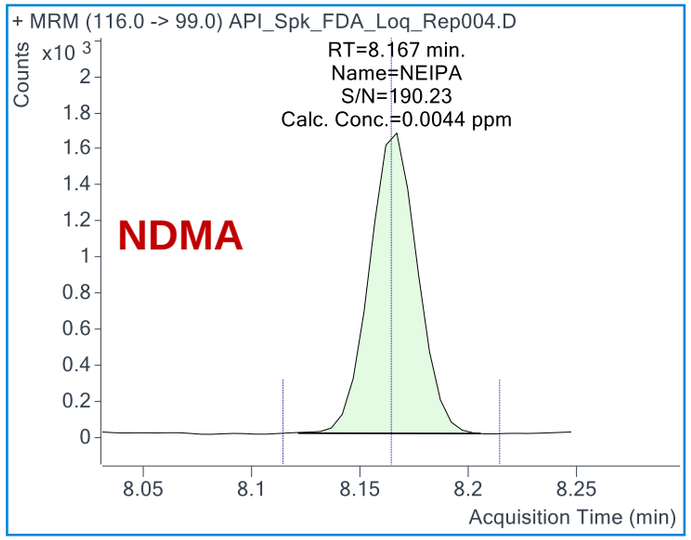
Calibration Curves

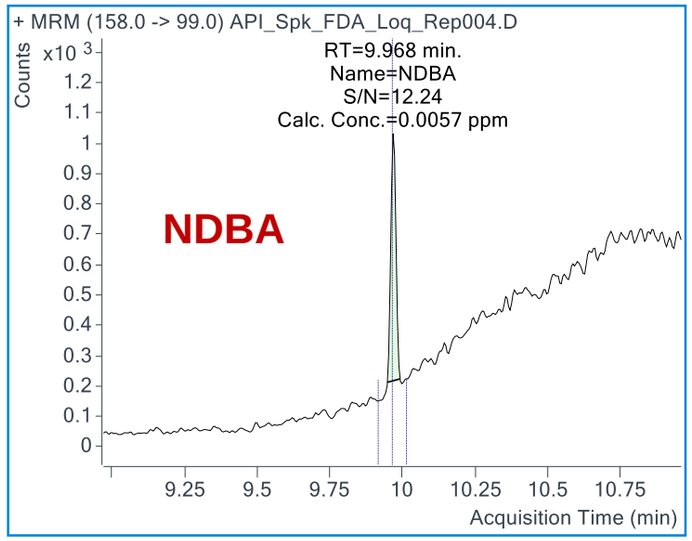


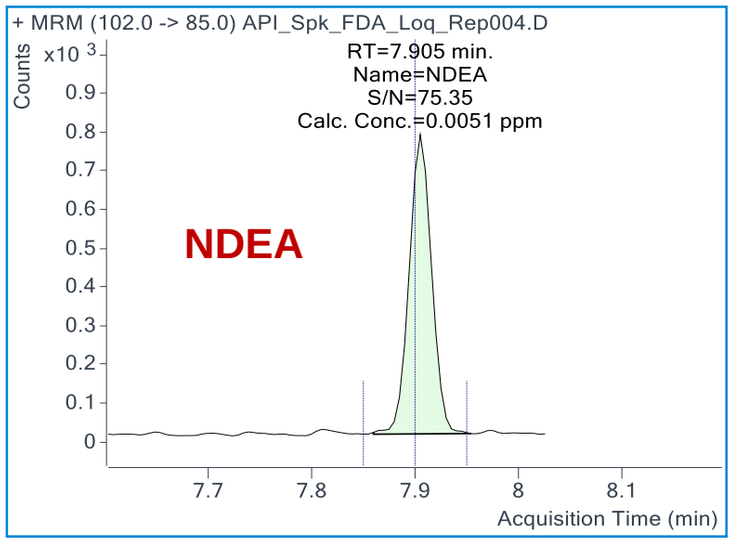


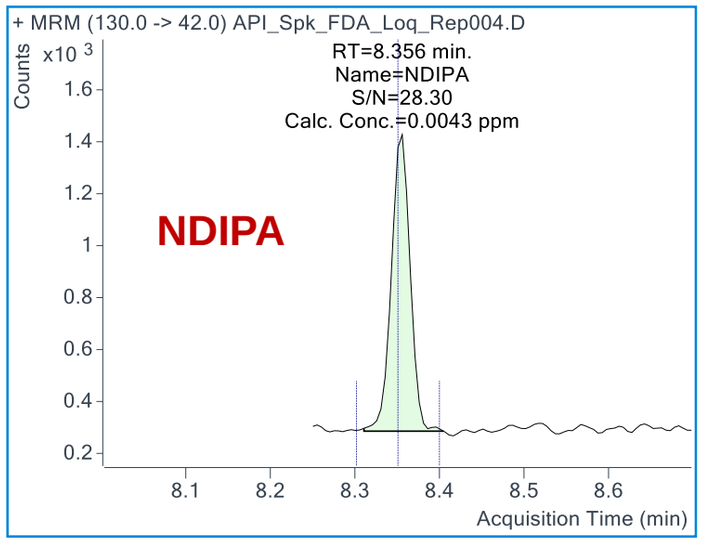
Representative Recovery % of Nitrosamine Impurities in Candesartan at 0.005 ppm











Compound	Spiking Level (ppm)	Sample Results (ppm)	Recovery (%)
NDMA	0.005	0.0054	108
NDEA	0.005	0.0051	102
NEIPA	0.005	0.0044	88
NDIPA	0.005	0.0043	86
NDBA	0.005	0.0057	114

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Candesartan

LC/MS Method for Analysis

Instrument Method

Mobile phase A:	0.2 % formic acid in water
Mobile phase B:	Methanol
Multisampler temperature:	10°C
Injection volume:	20 µL
Analytical column:	Agilent Zorbax Eclipse Plus C18 100*3.0mm 1.8micron (P/N:959758-302)
Column temperature:	40 °C
Flow rate:	0.4 mL/min

Gradient

Time (min)	% A	% B	Flow (mL/min)
0	95	5	0.4
2.0	95	5	0.4
5.5	40	60	0.4
9.0	5	95	0.4
11.0	5	95	0.4
11.1	95	5	0.4
14.0	95	5	0.4

Instrument	Agilent 6470 Triple Quadrupole mass spectrometer
Ion source	Atmospheric Pressure Chemical Ionization (APCI)
MS/MS mode	MRM
Ion mode	Positive
Drying gas temperature	300 °C
Drying gas flow	6 L/min
Nebulizer pressure	35 psi
APCI heater	350 °C
APCI needle positive	4 µA
Capillary voltage, positive	4000 V
MS1/MS2 resolution	0.7/0.7 (unit/unit)
Dwell time	50 ms

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy(V)	CAV(V)	Polarity
NDMA(Quantifier)	75.1	43.1	100	17	5	+
NDMA (Qualifier)	75.1	58.1	75	11	5	+
NMBA(Quantifier)	147.1	117.4	60	4	3	+
NMBA(Qualifier)	147.1	44.2	60	12	3	+
NDEA(Quantifier)	103.1	75.1	80	9	3	+
NDEA(Qualifier)	103.1	47.1	80	17	3	+
NEIPA(Quantifier)	117.1	75.1	75	8	3	+
NEIPA(Qualifier)	117.1	47.1	75	18	8	+
NDIPA(Quantifier)	131.1	89.1	75	6	3	+
NDIPA(Qualifier)	131.1	43.1	75	12	8	+
NDBA(Quantifier)	159.1	57.2	81	12	5	+
NDBA(Qualifier)	159.1	41.1	81	22	5	+

Calibrations

0.1 ng/mL to 100 ng/mL

Sample Preparation

For API

100mg of Drug substance → Add 250µL Methanol and sonicate well for 15 minutes to dissolves the API → Add 4.75mL Water and sonicate again for 15min. Centrifuge at 5000 rpm for 5 min at 10°C → Filter supernatant using 0.2 µm syringe filter into a LC vial

System Suitability

The coefficient of determination (R^2) of the linear calibration curve should be ≥ 0.990.
The S/N ratio of the 1 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 1 ng/mL standard should be ≤ 10

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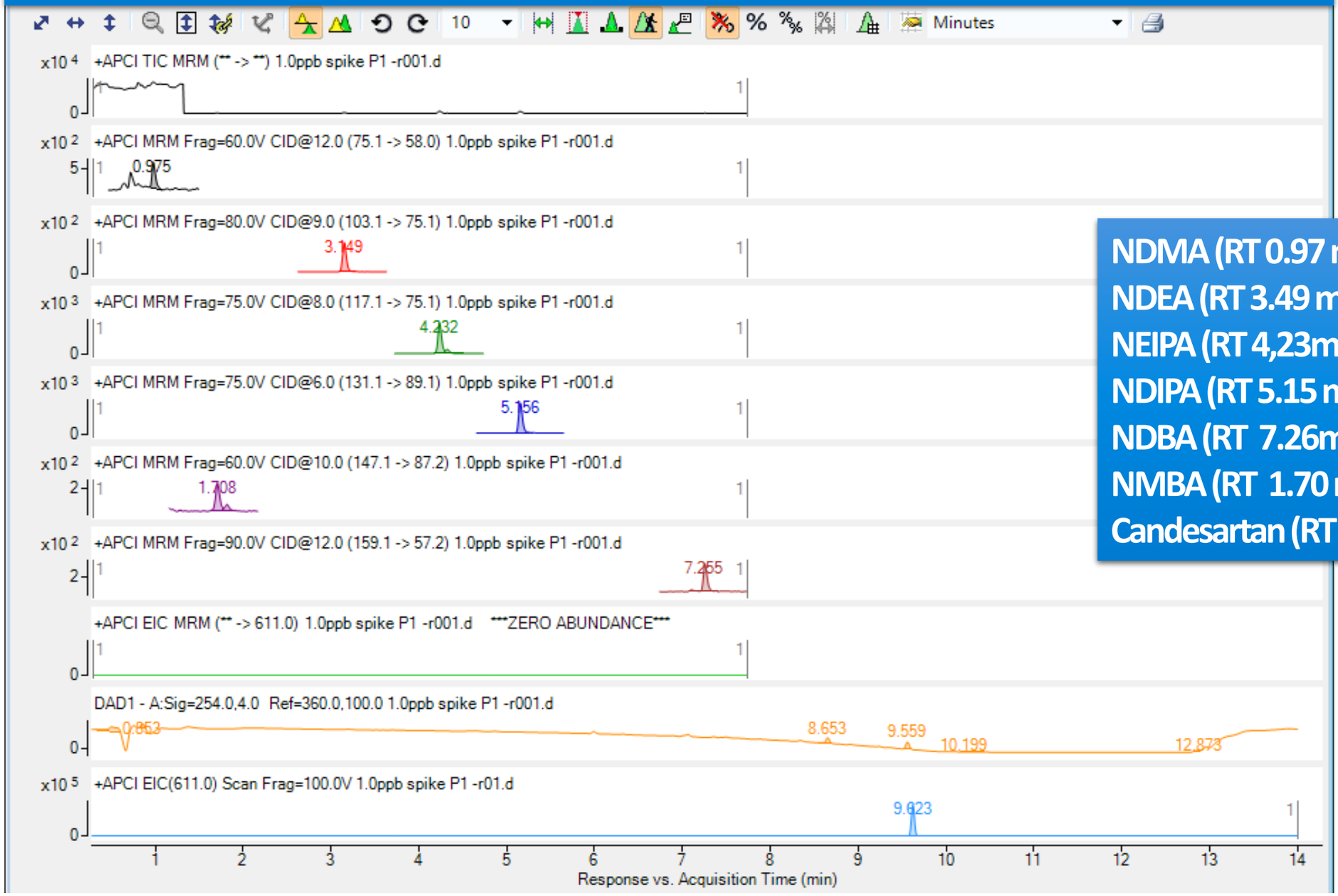
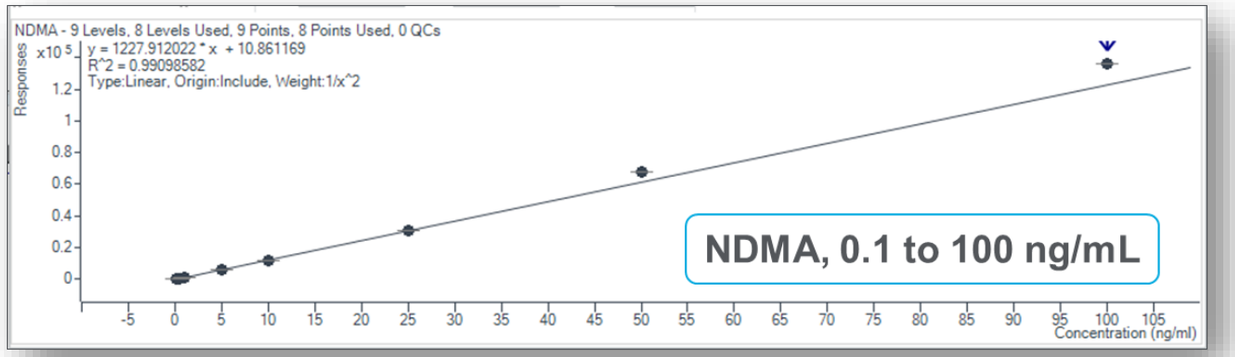
Results for 6 nitrosamine impurities at 20 ng/mL in Candesartan API

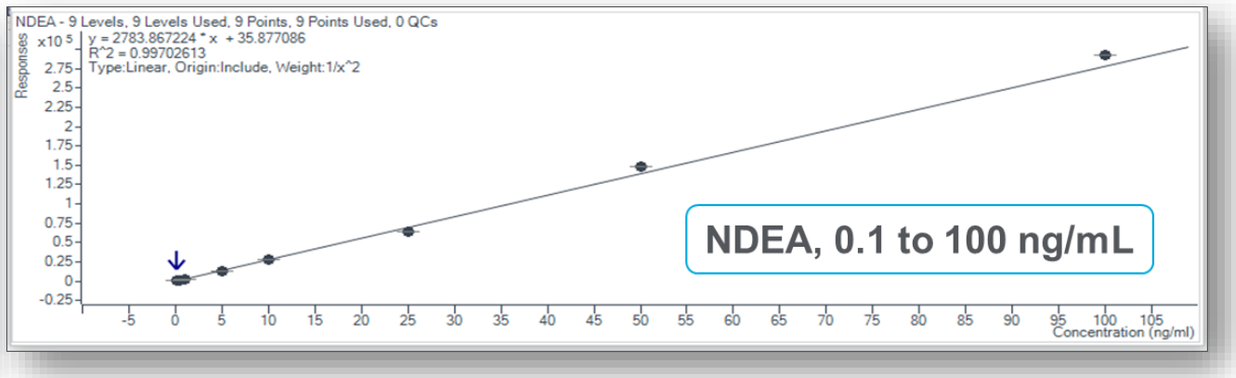


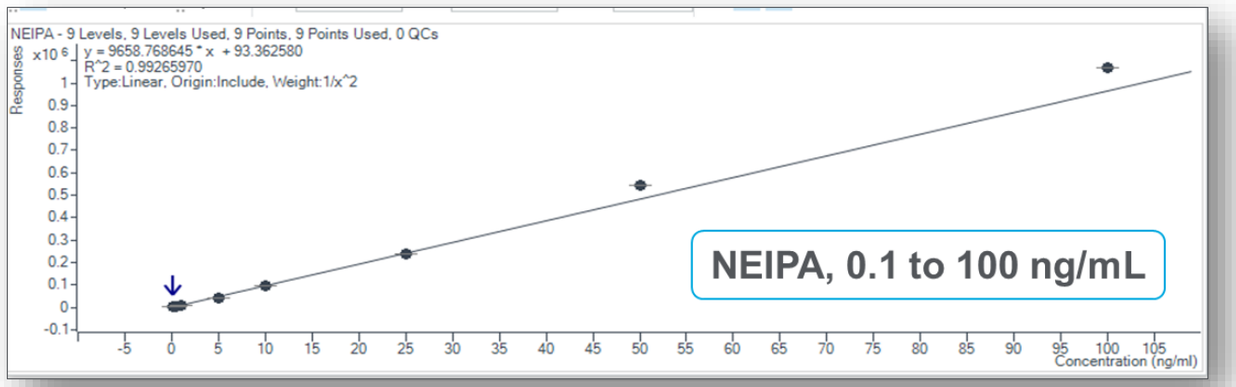
- NDMA (RT 0.97 min)
- NDEA (RT 3.49 min)
- NEIPA (RT 4,23min)
- NDIPA (RT 5.15 min)
- NDBA (RT 7.26min)
- NMBA (RT 1.70 min)
- Candesartan (RT 9.623 min)

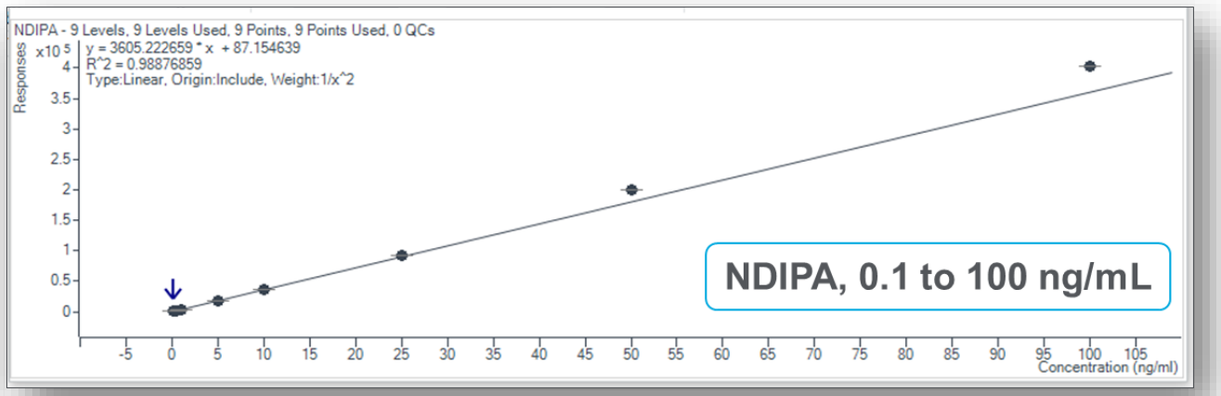
Candesartan

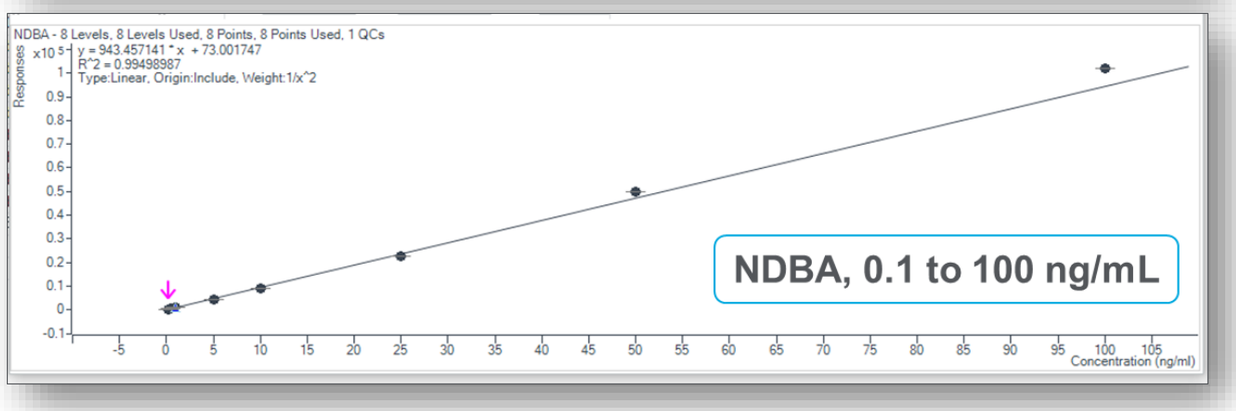
Calibration Curves

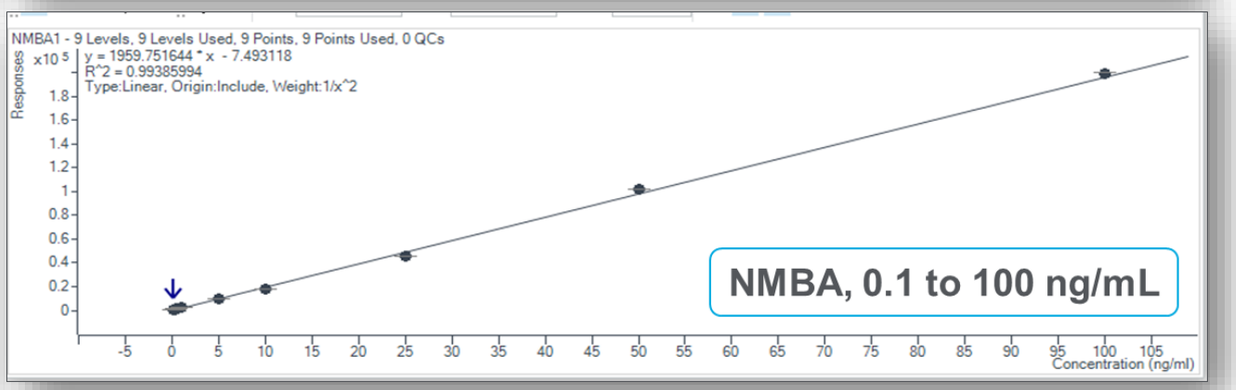












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Candesartan

Representative Recovery % of Nitrosamine Impurities

using 20mg/mL sample size

Concentration (ng/mL)	Recovery %					
	NDMA	NDEA	NMBA	NEIPA	NDIPA	NDBA
0.5	106	102	97	102	96	118
1	97	104	101	107	103	108
5	101	99	95	97	95	90

Note: Use of corresponding internal standards for each nitrosamines may further help in any recovery issue.

Benefits of Agilent LC/TQ	
Optimized methods	• Optimized method for candesartan drug substance • Compatible with stringent FDA regulations
Scalable application	• Best precision = best ion ratios = best quant results; Rugged ion source design
Sample prep	• Sample preparation as per EDQM guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument • Efficient Quant review with MassHunter • Data Integrity

GC/MS Method for Analysis

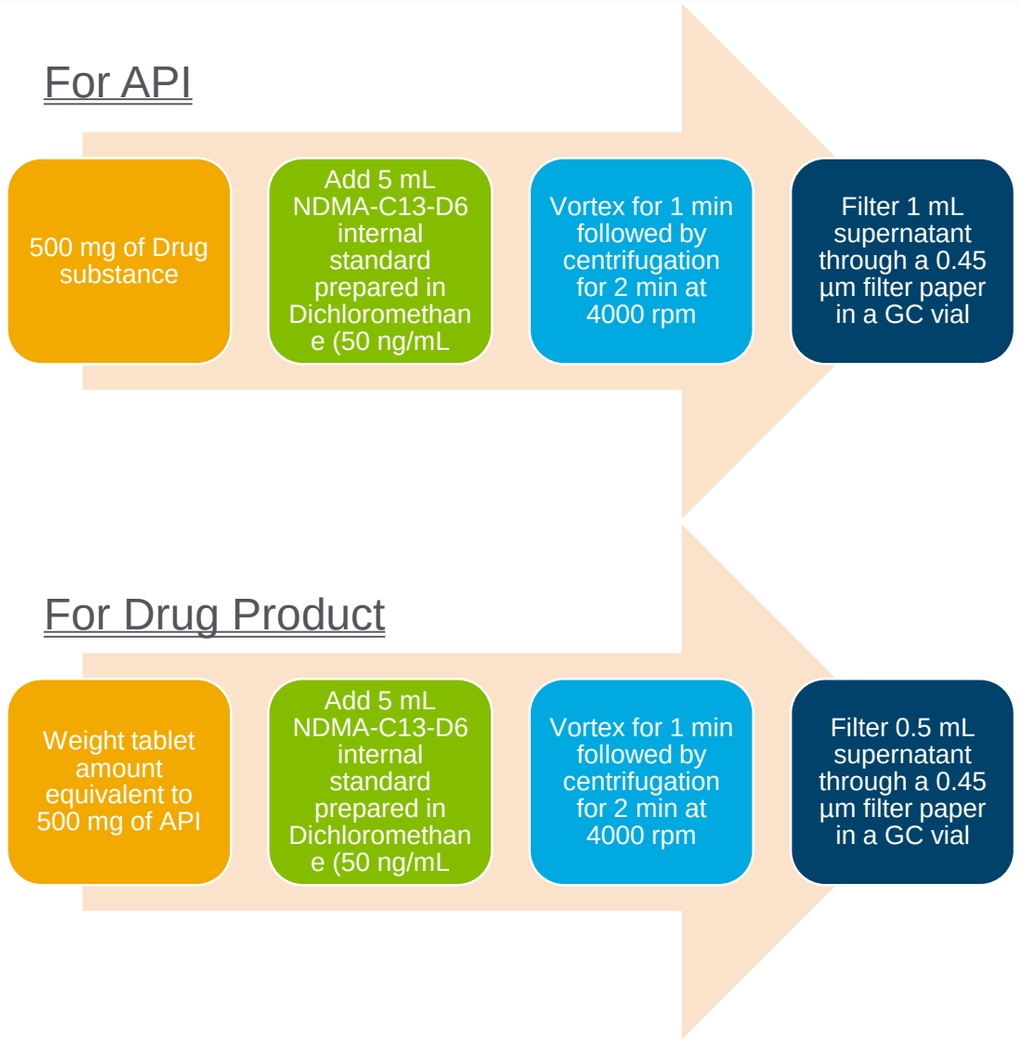
Instrument Method

ALS	GC	MS
Injection Volume: 2µL	Carrier Gas: He 1mL/min	EI Mode

Parameter	Value
MMI injection mode	Pulsed splitless: 12.285 psi until 0.5 min
Inlet temperature	250 °C
Oven temperature program	40 °C (0.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)
Total run time	12.33 min
MS transfer line temperature	250 °C

Parameter	Value	
Source temperature	250 °C	
Quadrupole temperature	Q1 and Q2 = 150 °C	
MS1 and MS2 resolution	All compounds Unit	
Collision gas flow	Nitrogen at 1.5 mL/min,	
Quenching gas flow	Helium at 4 mL/min	
Quant./qual. transitions (FDA method)	Start time: 6.5 min NDMA	74 → 44, CE 15, dwell 150 ms 74 → 42, CE 20, dwell 50 ms NDMA:C13-d_6 82 →48, CE 20, dwell 100 ms
	Start time: 7.60 min NDEA	102 →85, CE 10 V, dwell 150 ms 102 →56, CE 18 V, dwell 150 ms
	Start time: 8.03 min NEIPA	116 →99, CE 10 V, dwell 150 ms 71 →56, CE 10 V, dwell 150 ms
	Start time: 8.25 min NDIPA	130 →88, CE 10 V, dwell 150 ms 130 →42, CE 10 V, dwell 150 ms
	Start time: 8.70 min NDBA	158 →99, CE 10 V, dwell 150 ms 84 →56, CE 22 V, dwell 150 ms

Sample Preparation



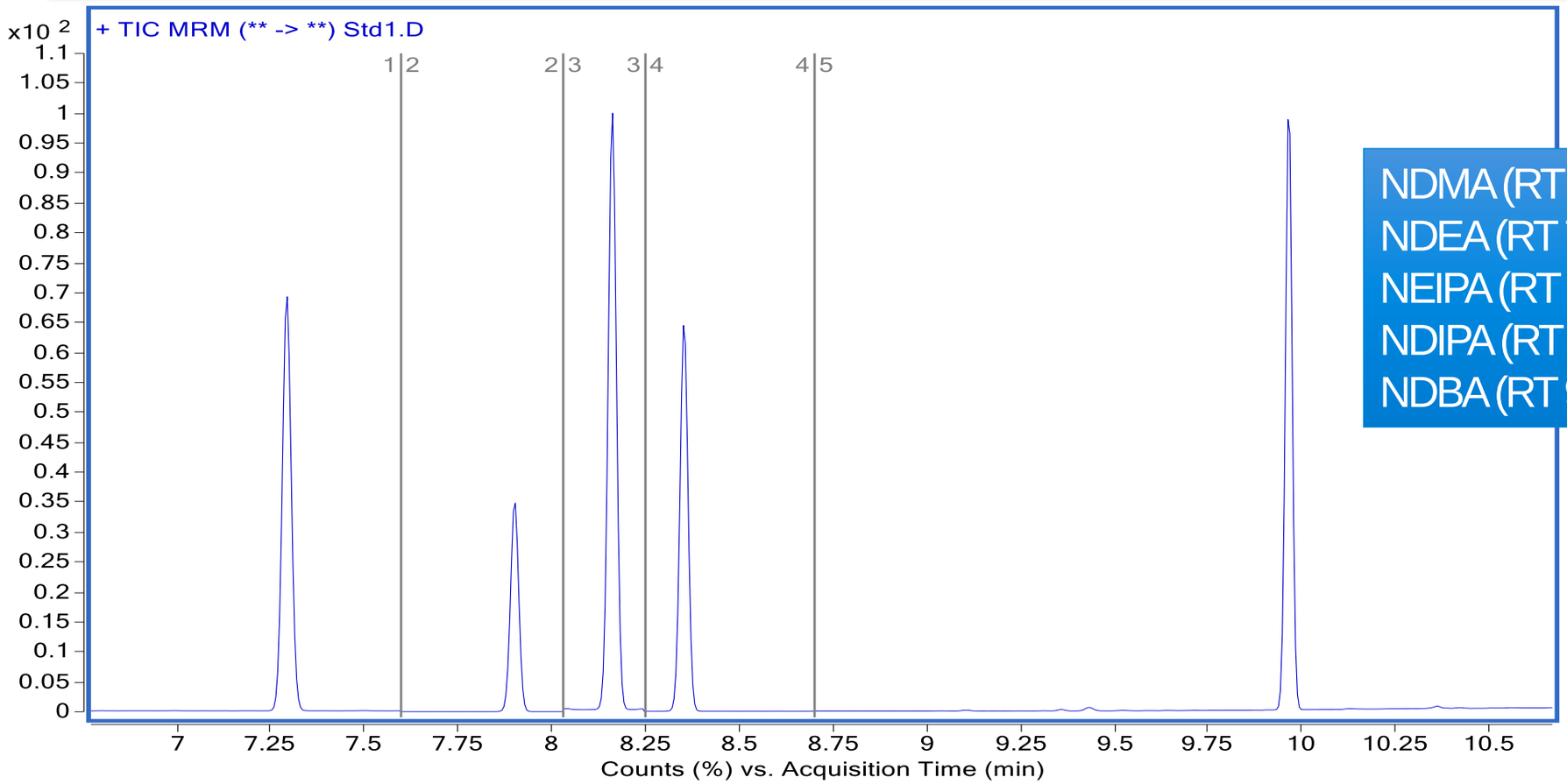
Calibrations

2.5 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 40 ng/ml, 80 ng/ml and 100 ng/ml each prepared in Dichloromethane containing 50 ng/mL of NDMA –C13-D6

System Suitability

The coefficient of determination (R2) of the linear calibration curve should be ≥ 0.998.
The S/N ratio of the 5 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 40 ng/mL standard should be ≤ 5

Results for 5 nitrosamine impurities at 100 ng/mL in Irbesartan API



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NDEA (RT 7.903 min)
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NDBA (RT 9.777 min)

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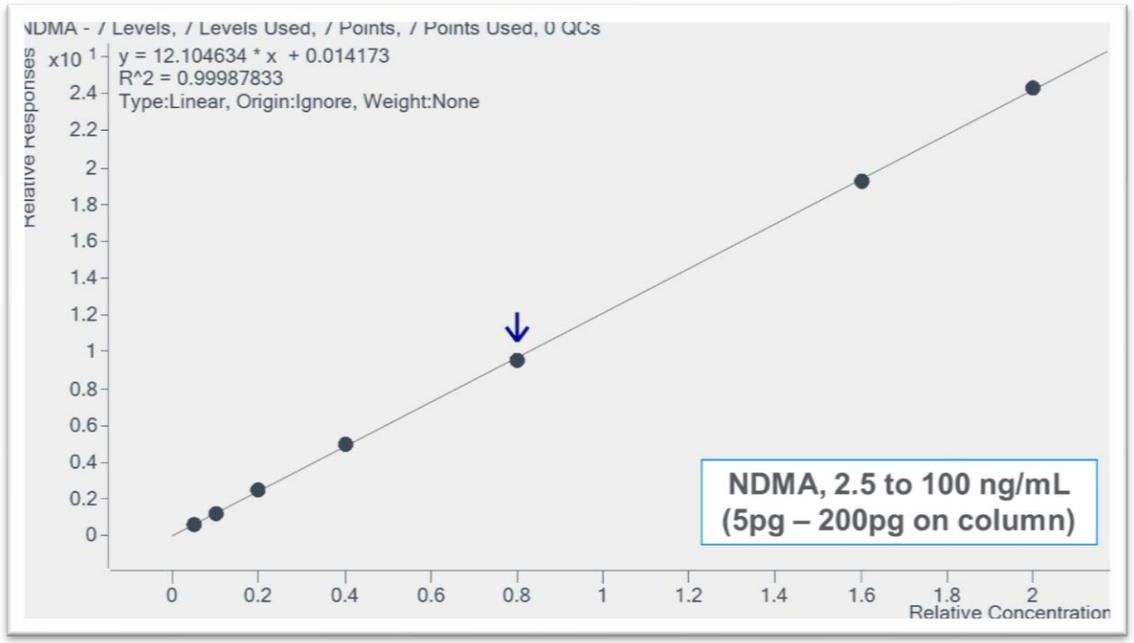
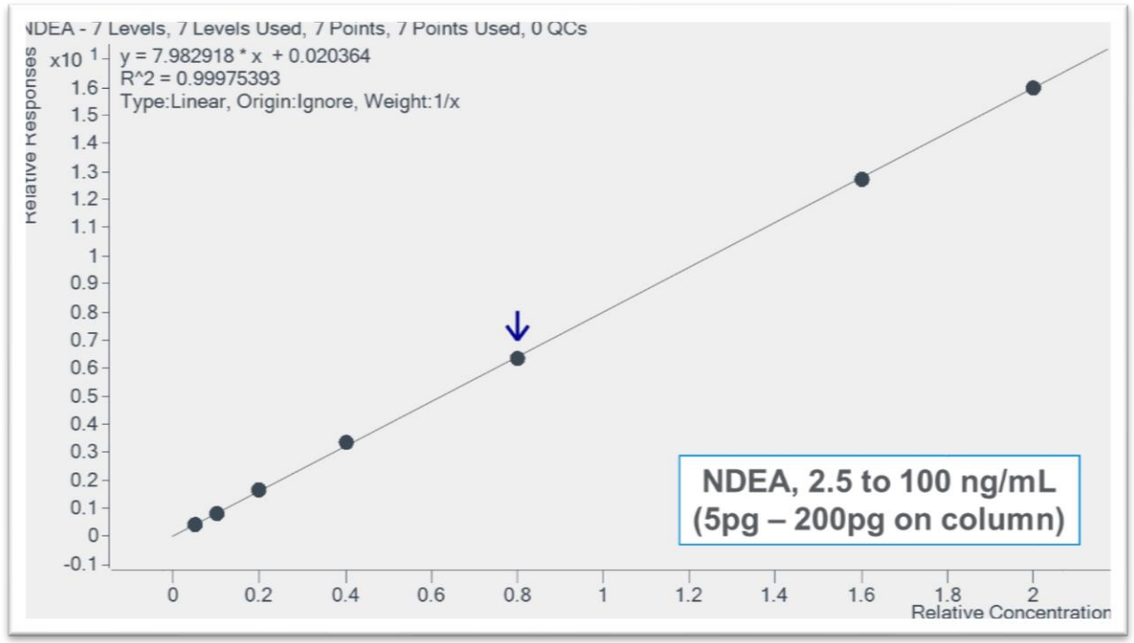
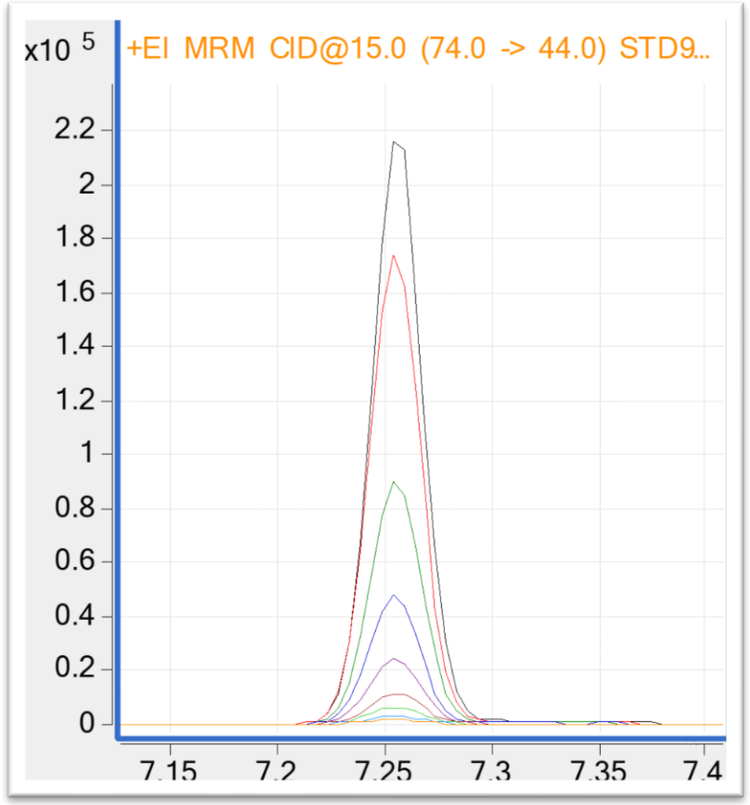
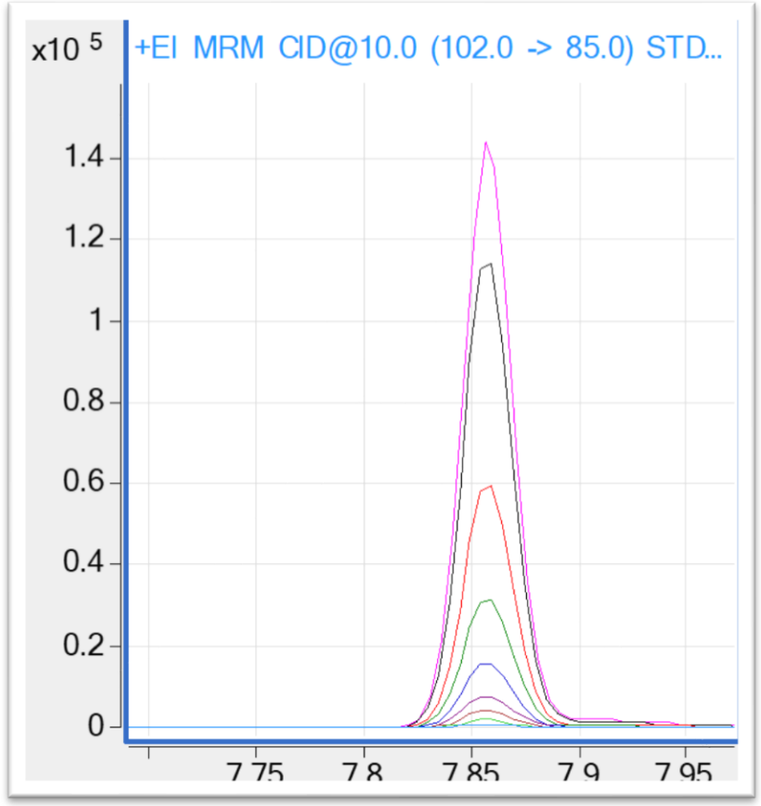
Calibration Curves









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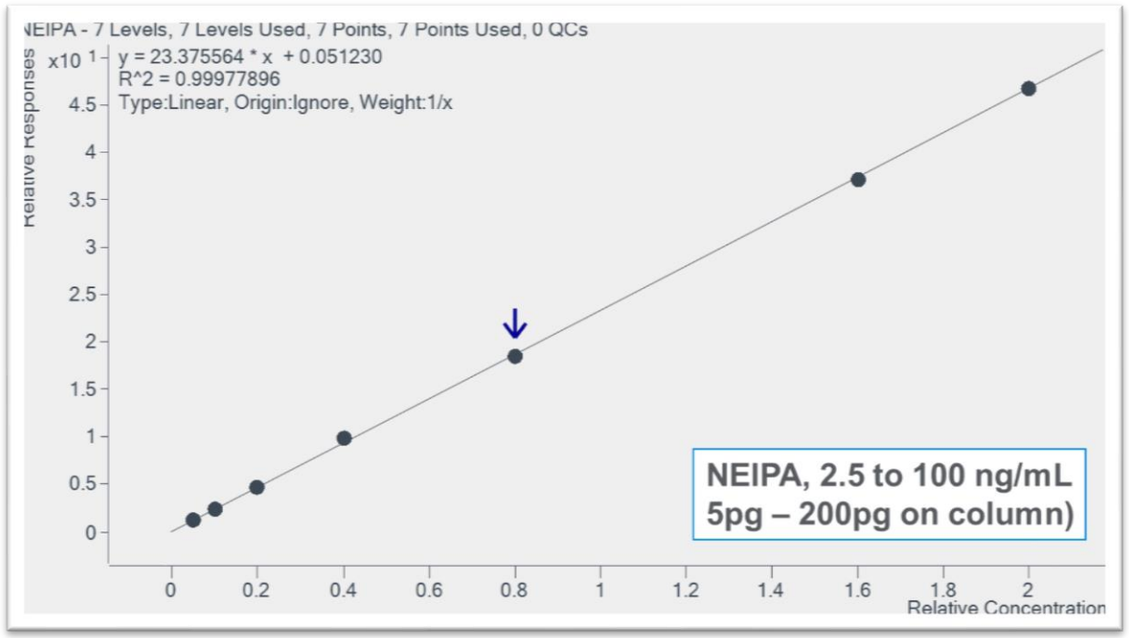

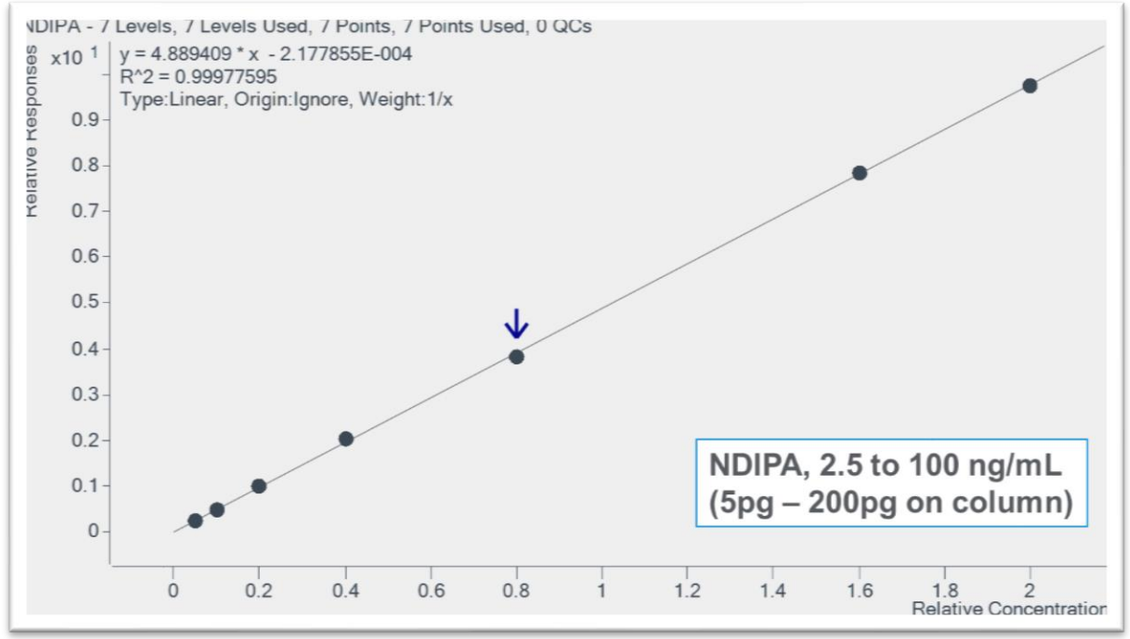

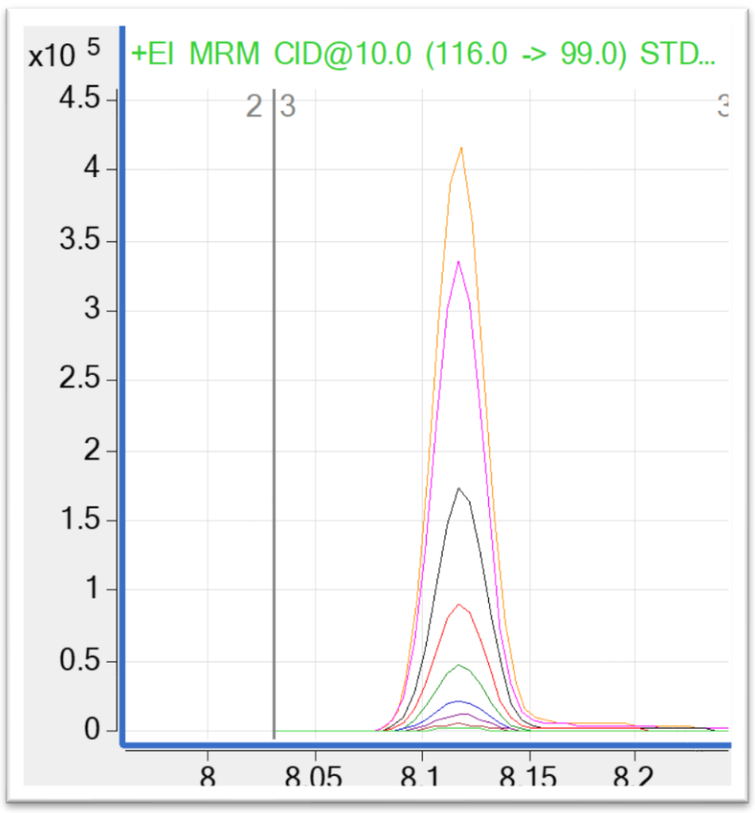

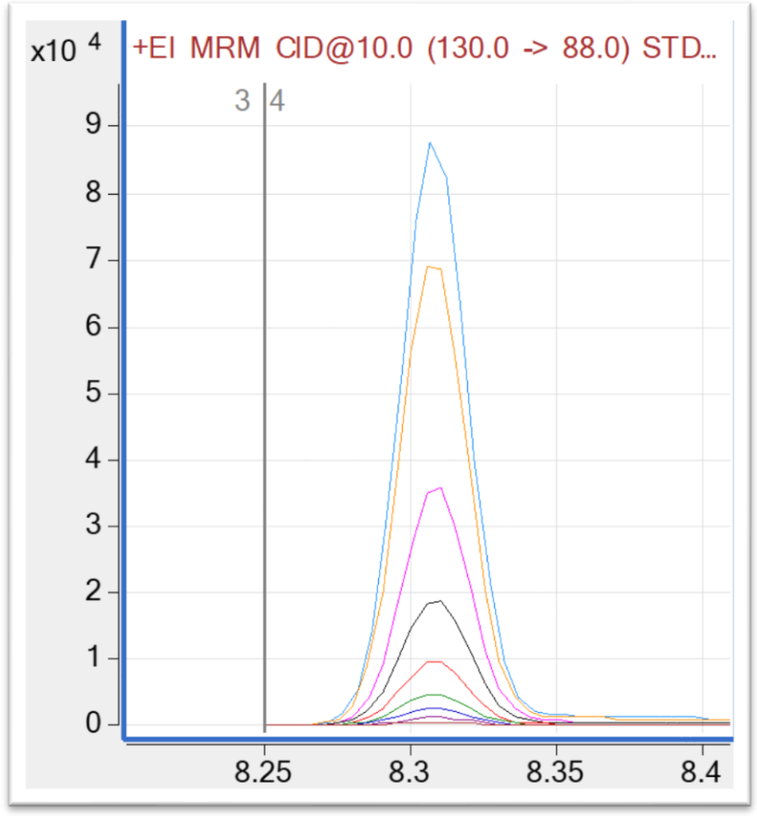

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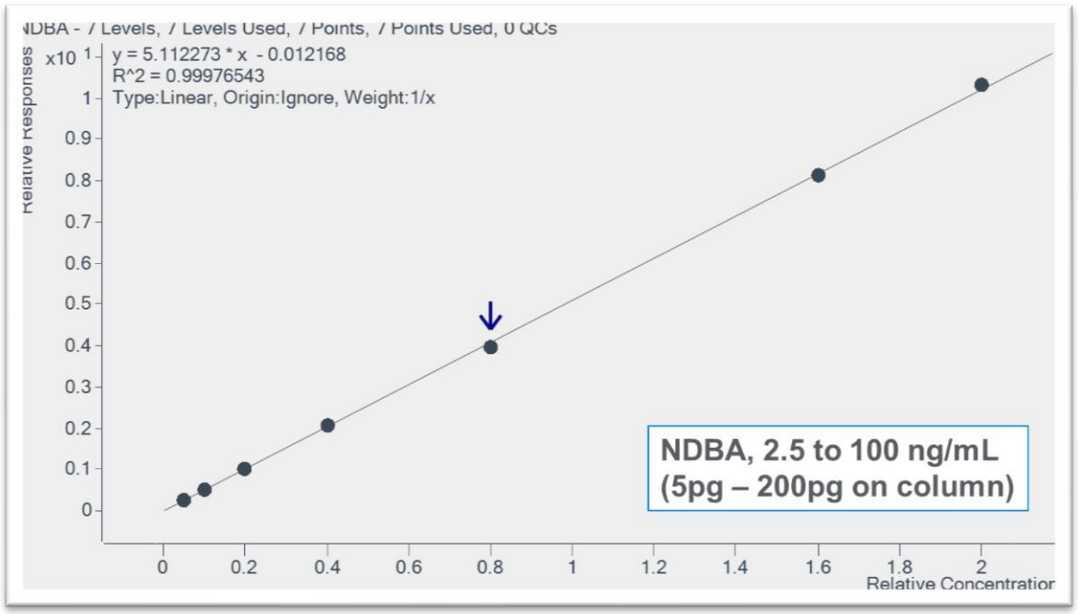
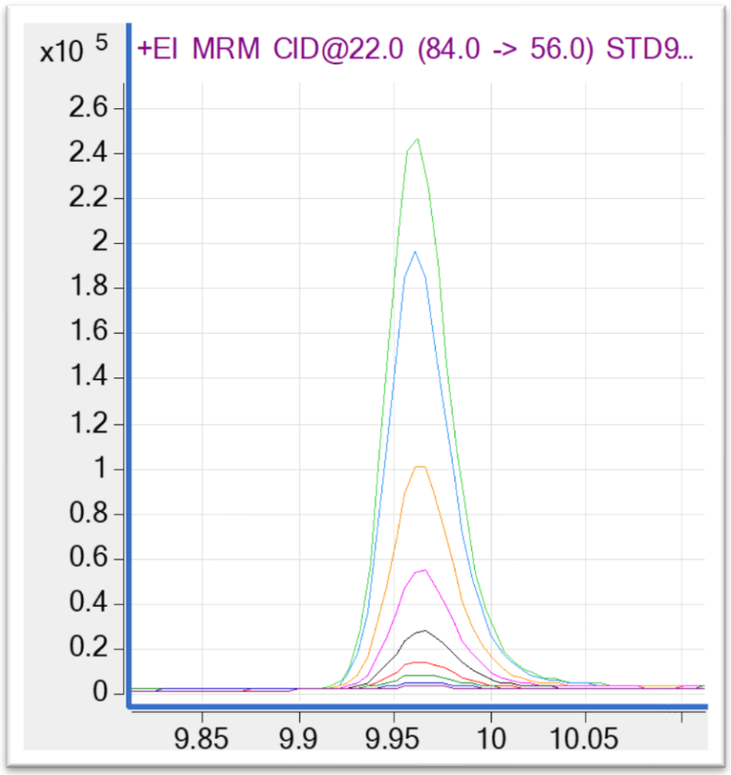
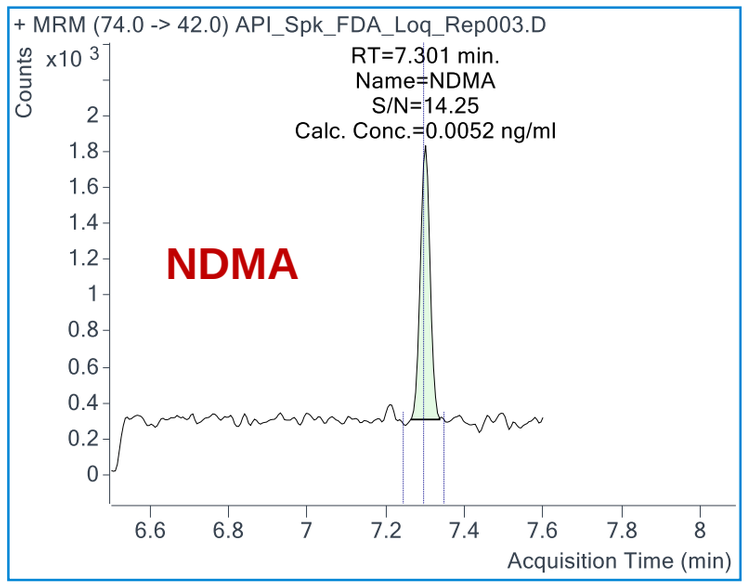
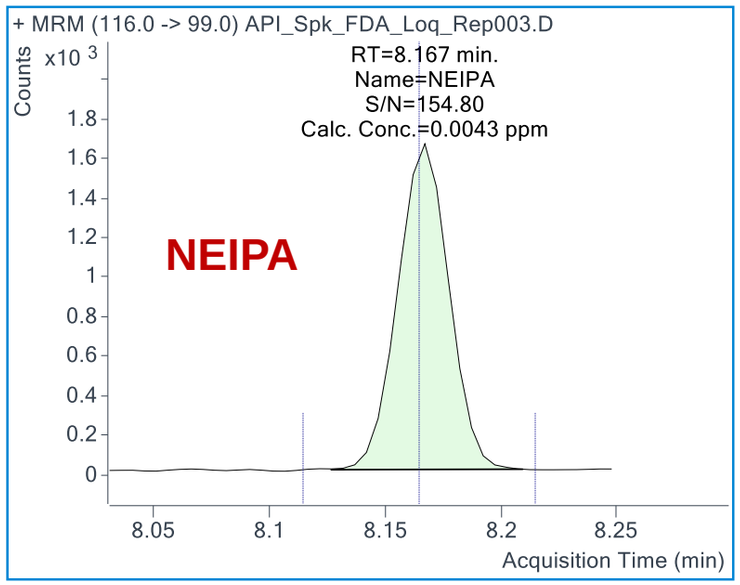
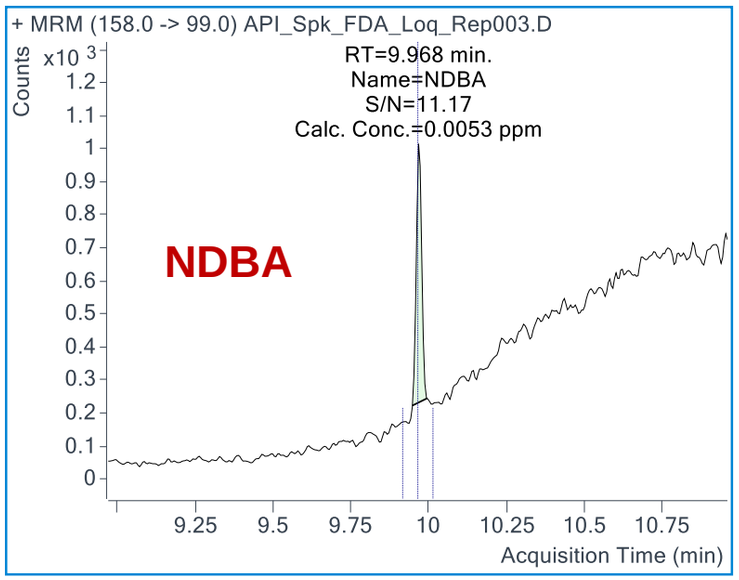


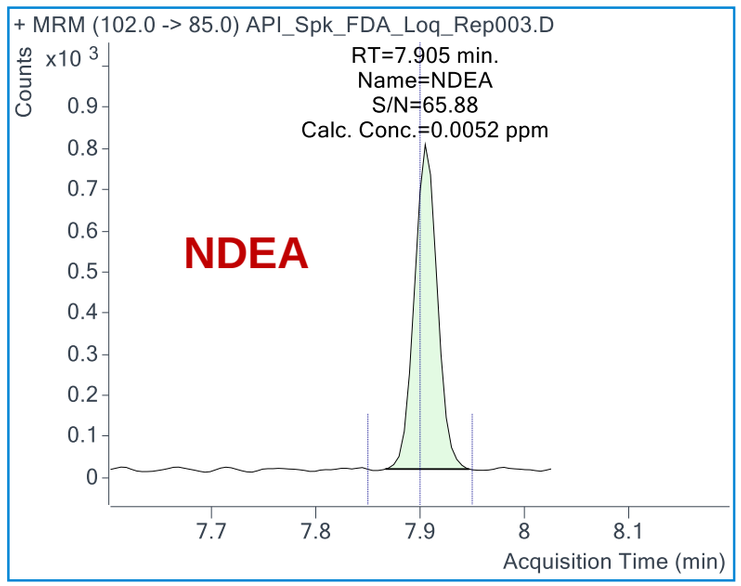


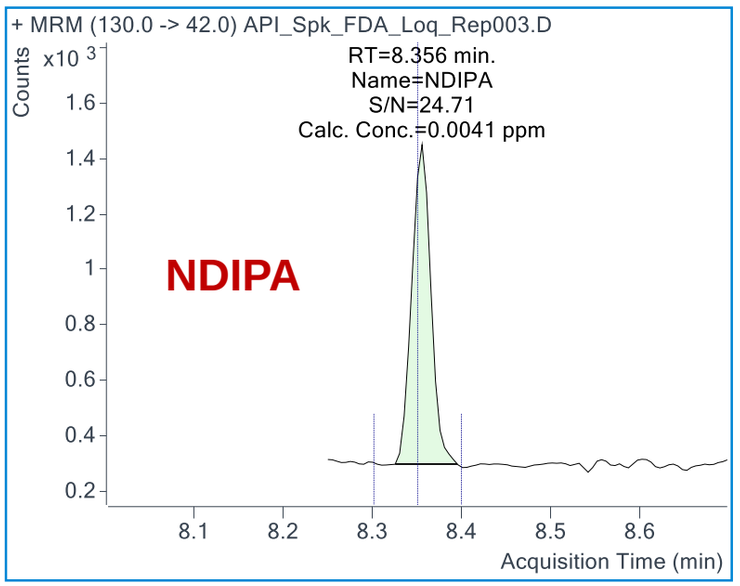
Representative Recovery % of Nitrosamine Impurities in Irbesartan at 0.005 ppm











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NDEA	0.005	0.0052	104
NEIPA	0.005	0.0043	86
NDIPA	0.005	0.0041	82
NDBA	0.005	0.0053	106

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GC/MS Method for Analysis

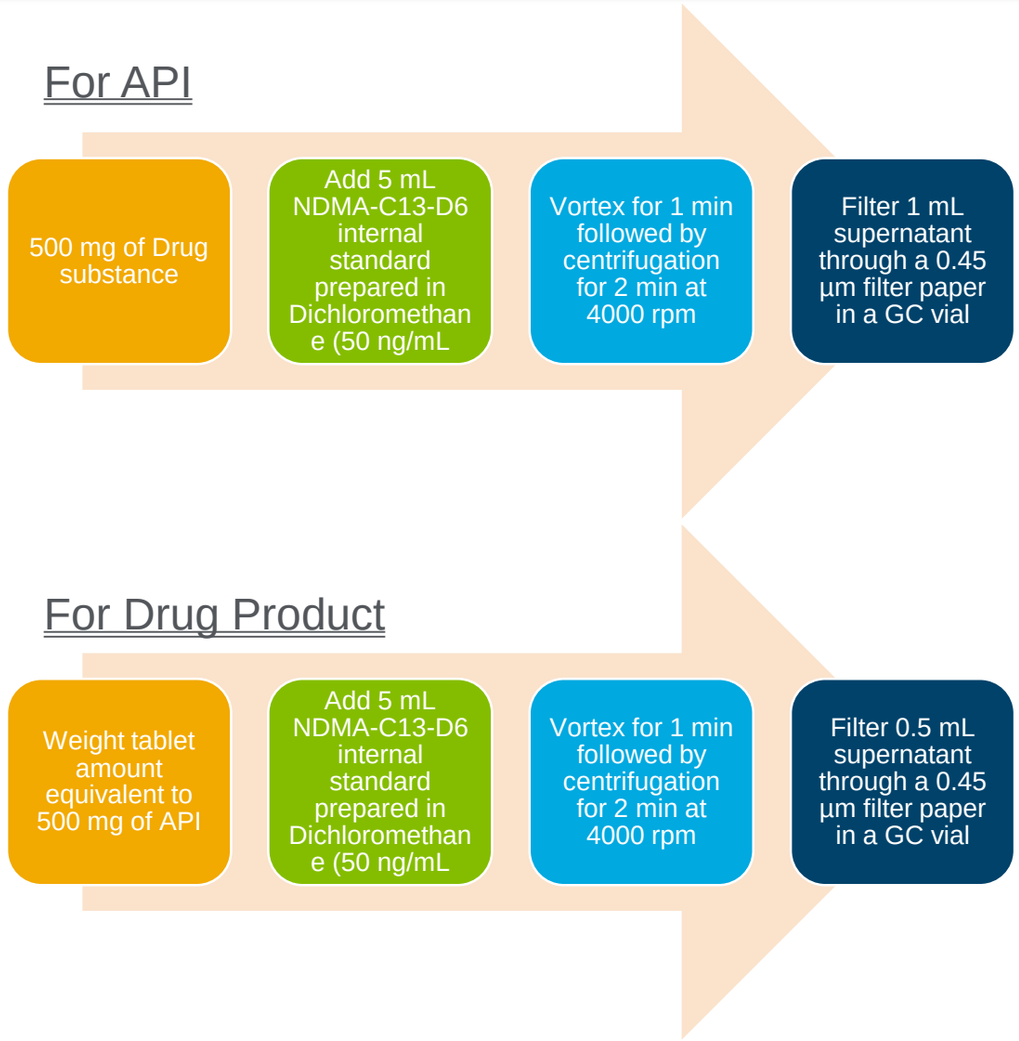
Instrument Method

ALS	GC	MS
Injection Volume: 2µL	Carrier Gas: He 1mL/min	EI Mode

Parameter	Value
MMI injection mode	Pulsed splitless: 12.285 psi until 0.5 min
Inlet temperature	250 °C
Oven temperature program	40 °C (0.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)
Total run time	12.33 min
MS transfer line temperature	250 °C

Parameter	Value	
Source temperature	250 °C	
Quadrupole temperature	Q1 and Q2 = 150 °C	
MS1 and MS2 resolution	All compounds Unit	
Collision gas flow	Nitrogen at 1.5 mL/min,	
Quenching gas flow	Helium at 4 mL/min	
Quant./qual. transitions (FDA method)	Start time: 6.5 min NDMA	74 → 44, CE 15, dwell 150 ms 74 → 42, CE 20, dwell 50 ms NDMA:C13-d_6 82 →48, CE 20, dwell 100 ms
	Start time: 7.60 min NDEA	102 →85, CE 10 V, dwell 150 ms 102 →56, CE 18 V, dwell 150 ms
	Start time: 8.03 min NEIPA	116 →99, CE 10 V, dwell 150 ms 71 →56, CE 10 V, dwell 150 ms
	Start time: 8.25 min NDIPA	130 →88, CE 10 V, dwell 150 ms 130 →42, CE 10 V, dwell 150 ms
	Start time: 8.70 min NDBA	158 →99, CE 10 V, dwell 150 ms 84 →56, CE 22 V, dwell 150 ms

Sample Preparation



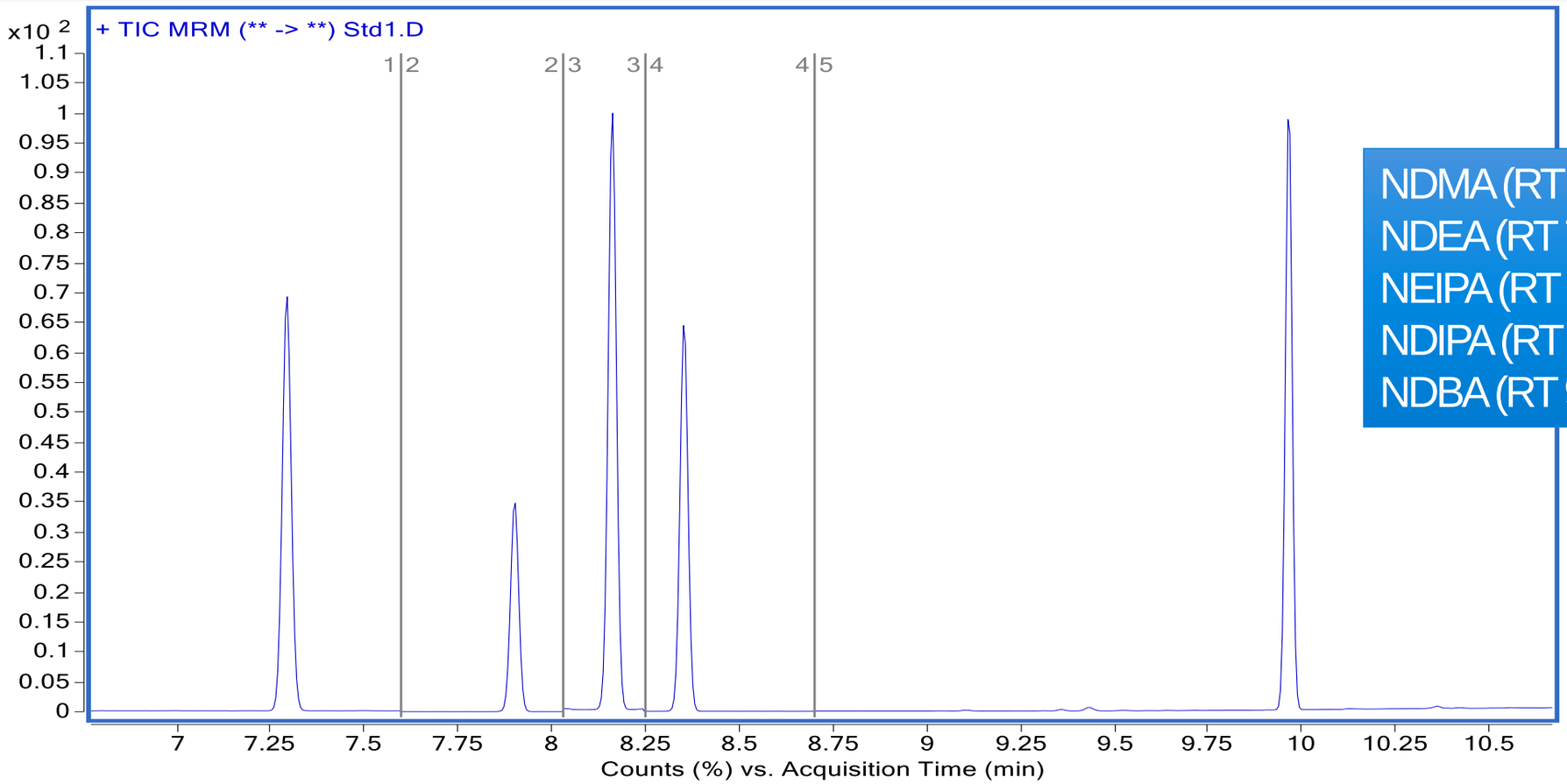
Calibrations

2.5 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 40 ng/ml, 80 ng/ml and 100 ng/ml each prepared in Dichloromethane containing 50 ng/mL of NDMA –C13-D6

System Suitability

The coefficient of determination (R2) of the linear calibration curve should be ≥ 0.998.
The S/N ratio of the 5 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 40 ng/mL standard should be ≤ 5

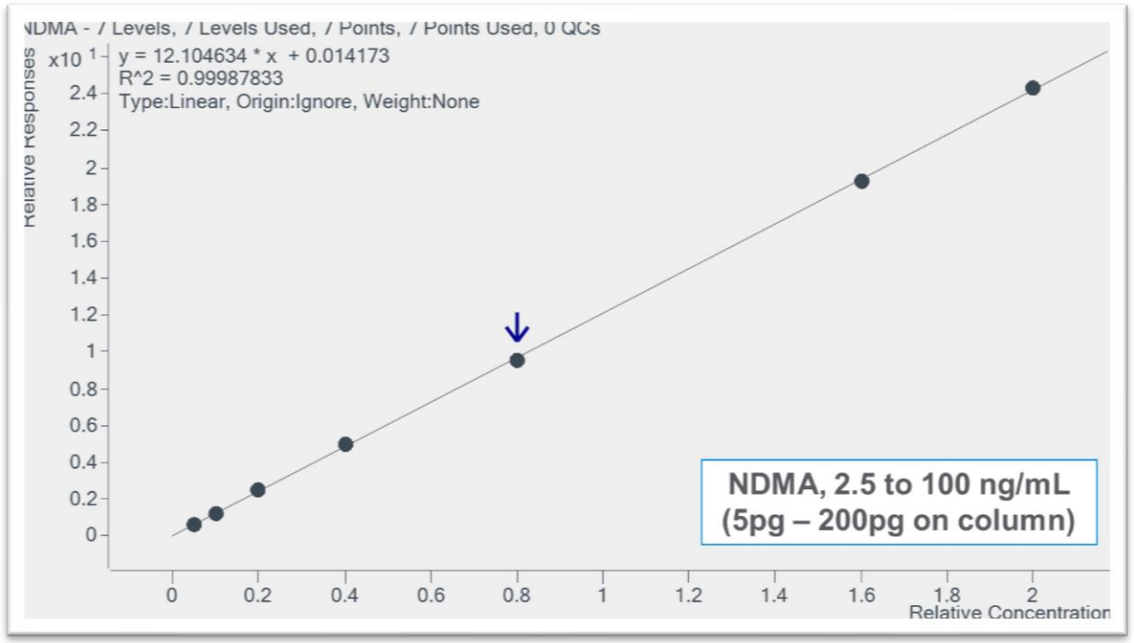
Results for 5 nitrosamine impurities at 100 ng/mL in Olmesartan API

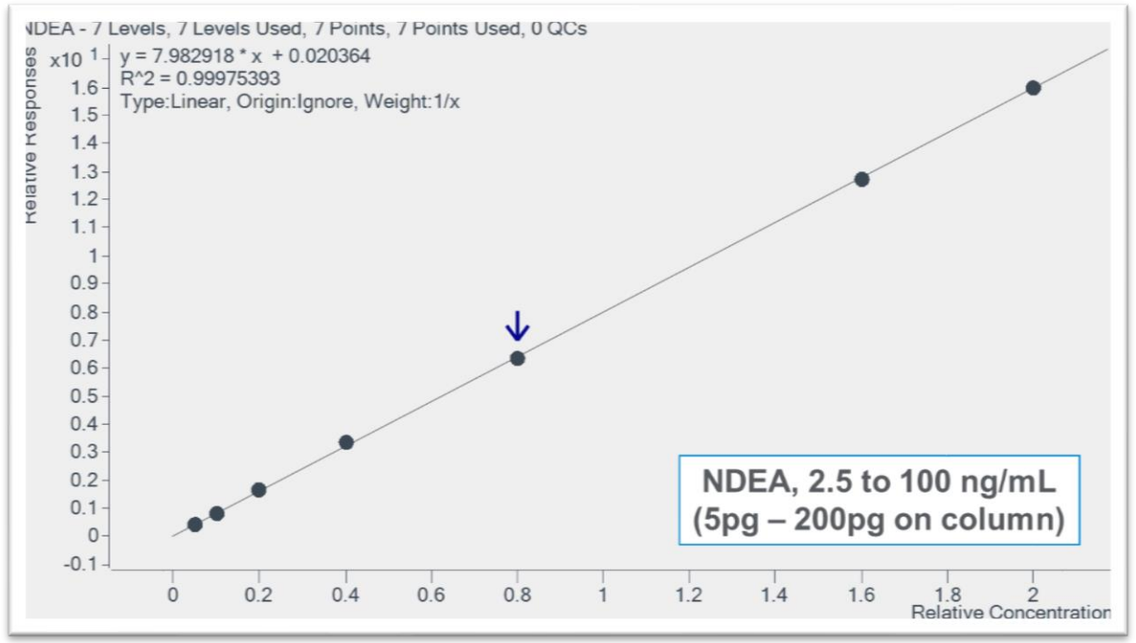


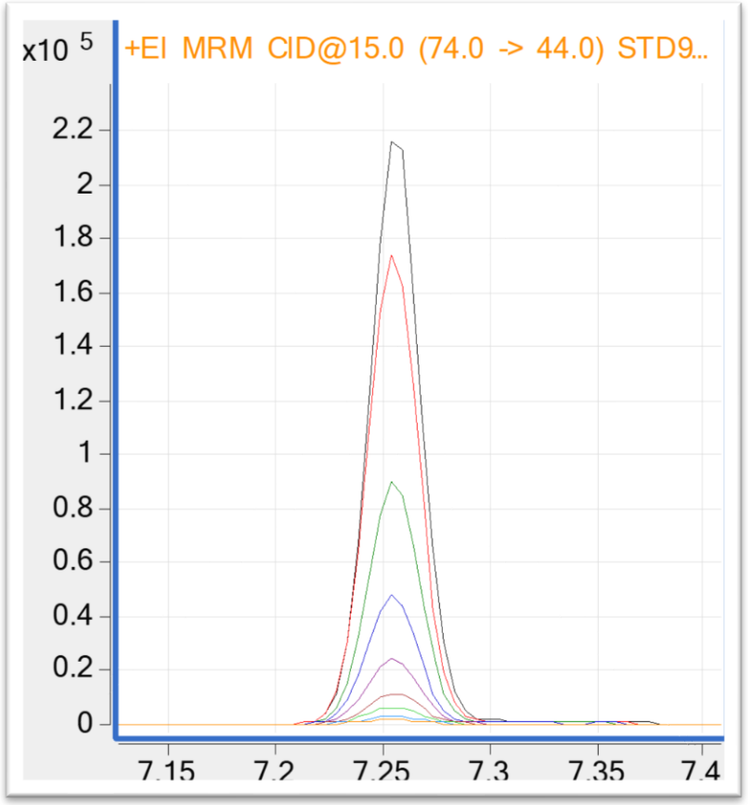
NDMA (RT 7.294 min)
NDEA (RT 7.903 min)
NEIPA (RT 8.157 min)
NDIPA (RT 8.346 min)
NDBA (RT 9.777 min)

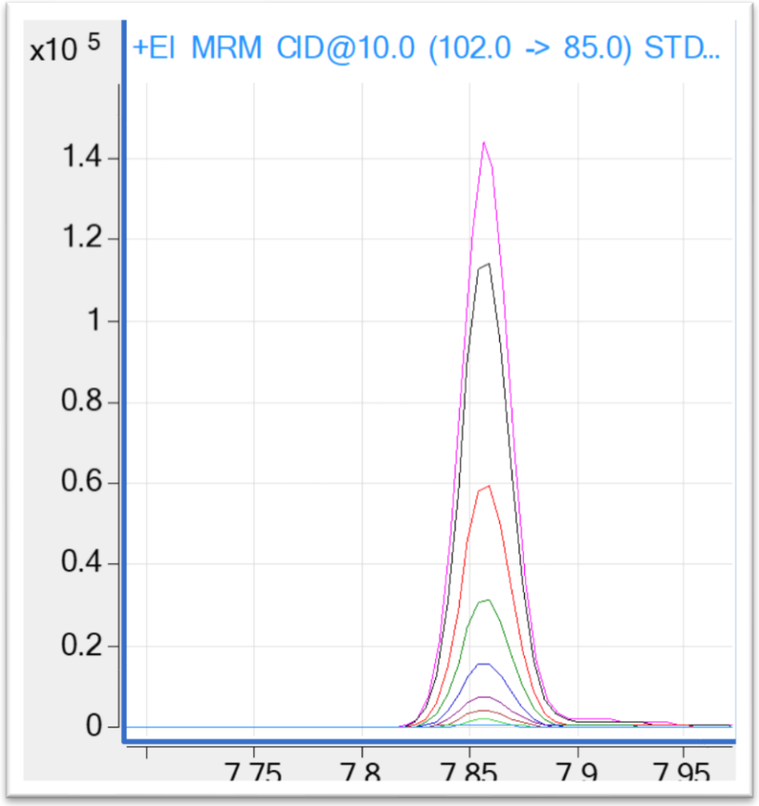
Benefits Agilent GC/TQ	
Optimized methods	• Optimized method for both API and Formulation • Compatible with stringent FDA regulations
Scalable application	• Best precision = best ion ratios = best quant results Rugged ion source design • Retention Time Locking for reproducible methods over time and between labs
Sample prep	• Sample preparation as per FDA guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument. • Efficient Quant review with MassHunter • Data Integrity

Calibration Curves

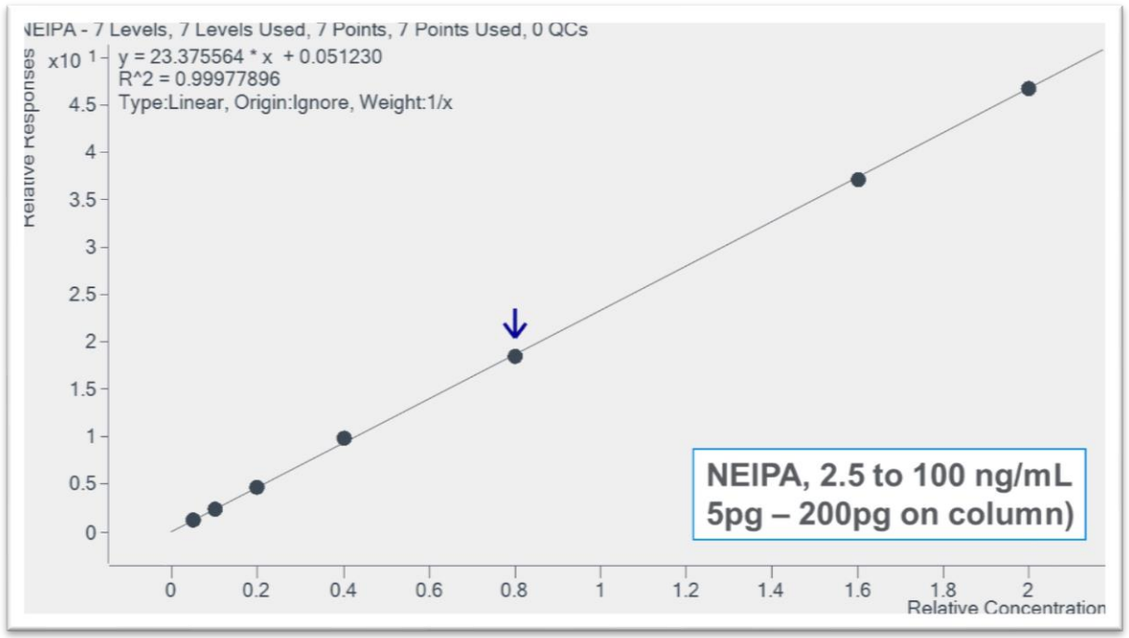


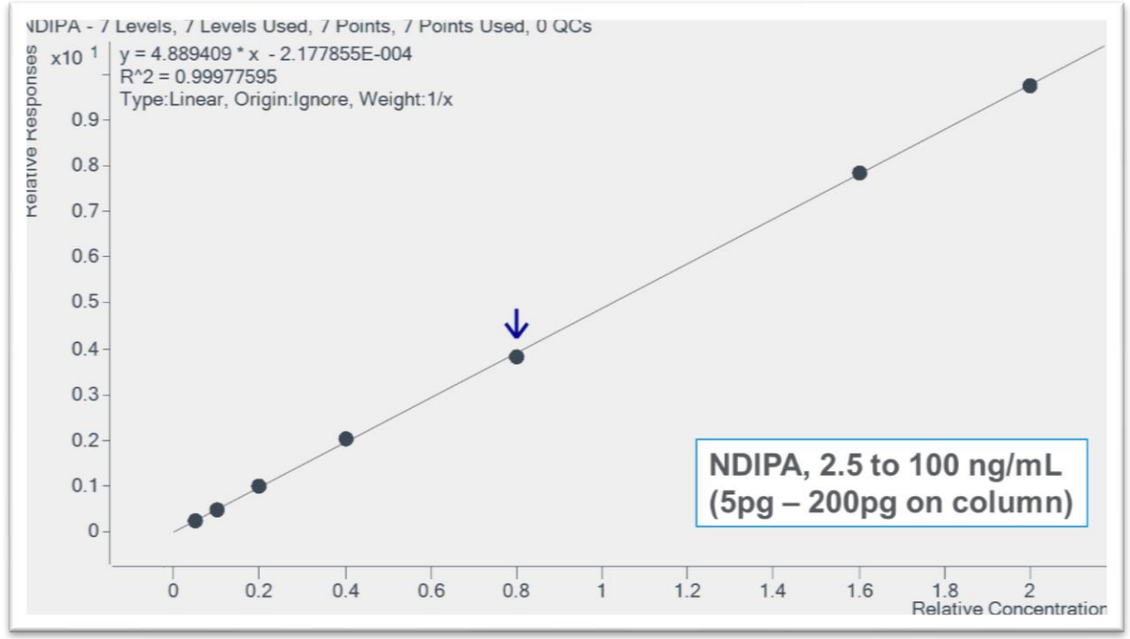


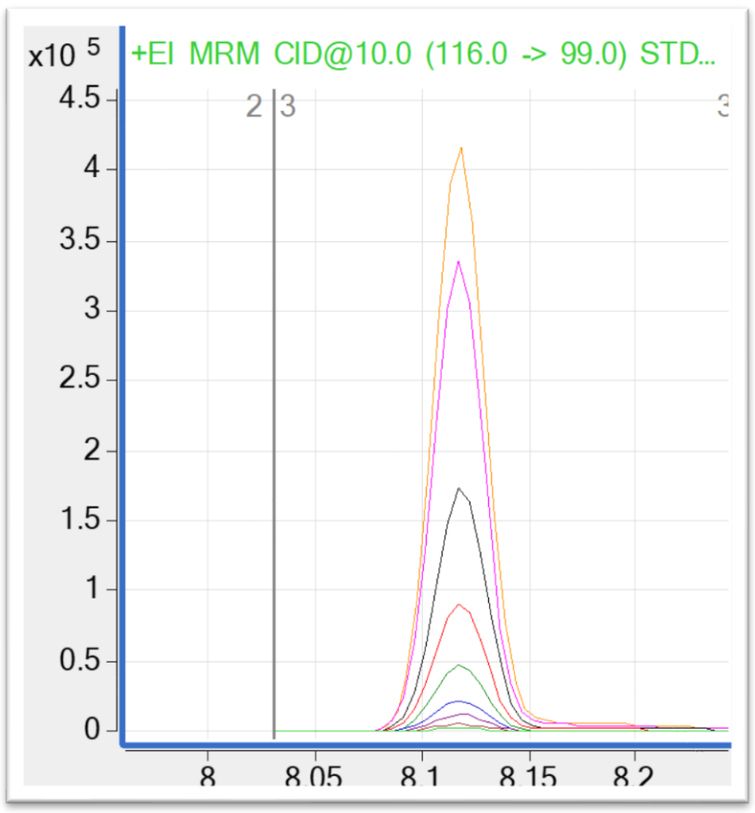


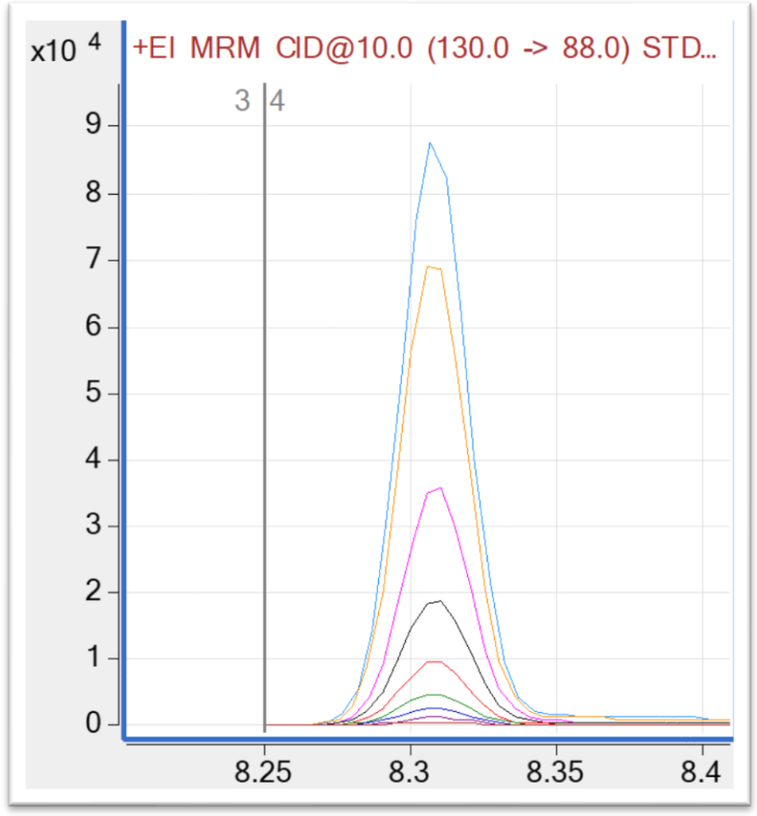


Calibration Curves

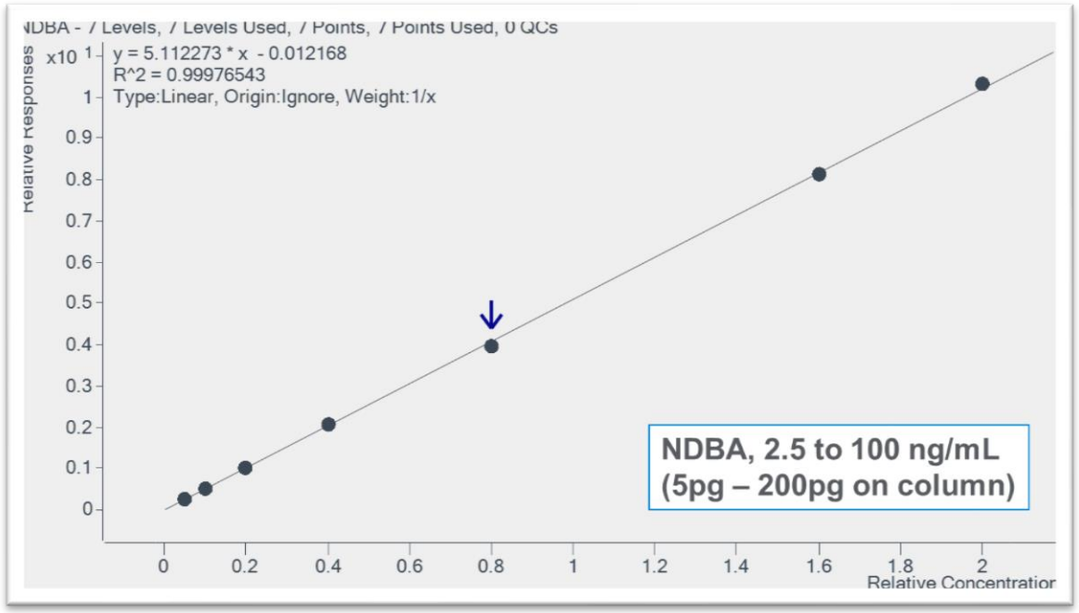


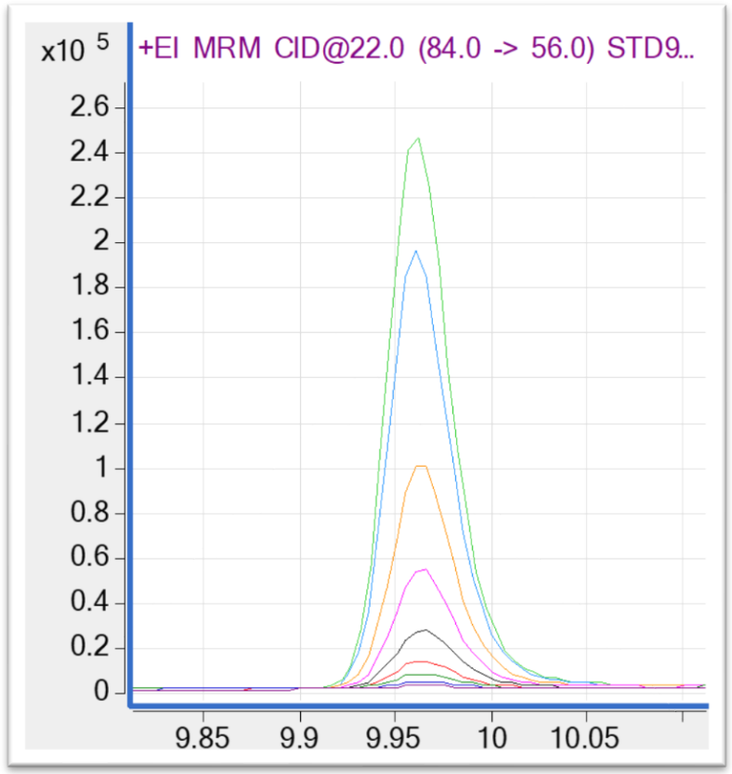




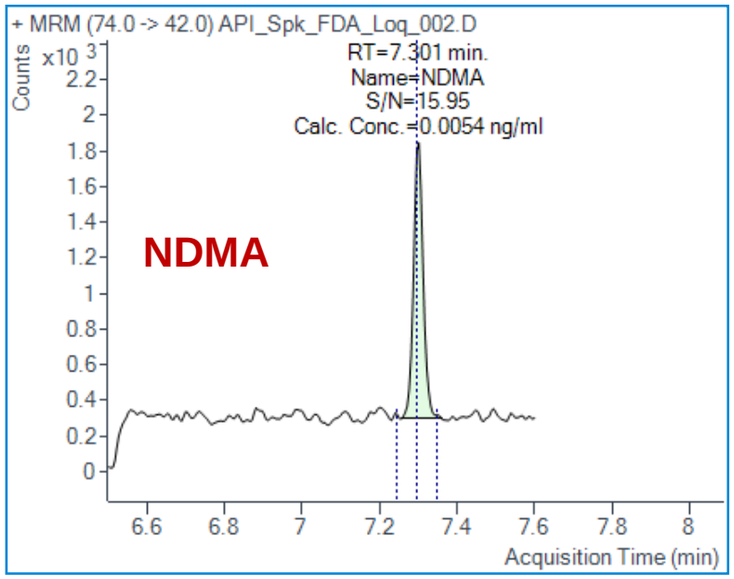


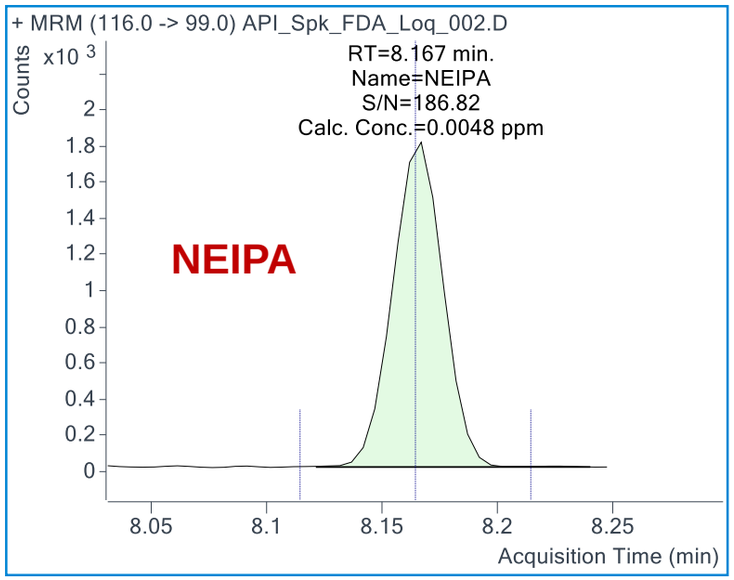
Calibration Curves

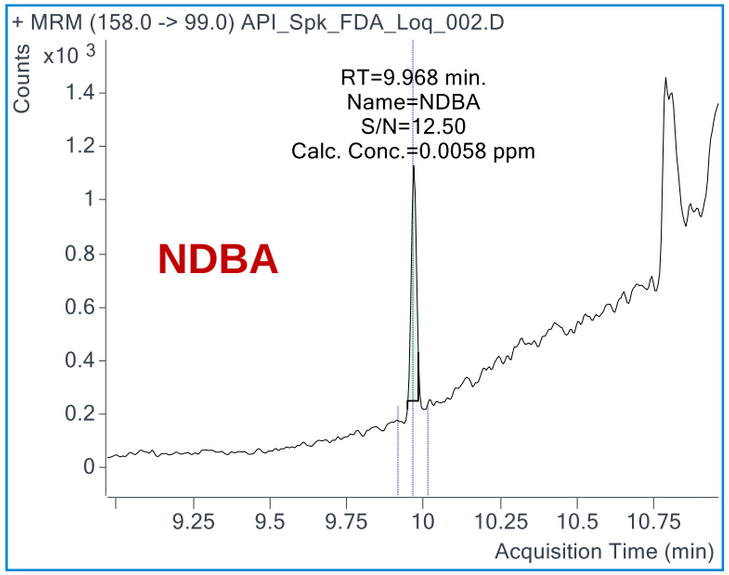


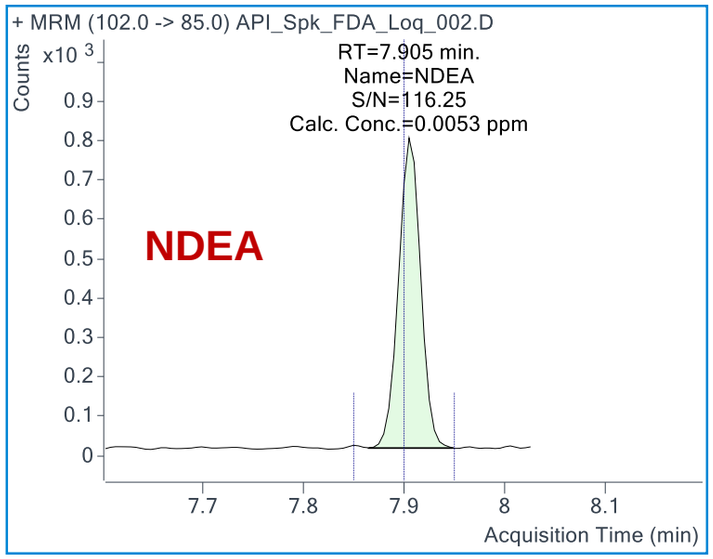


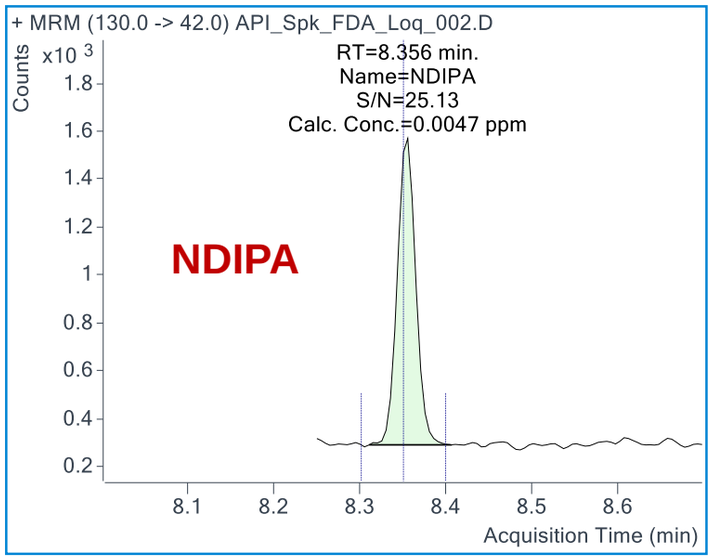
Representative Recovery % of Nitrosamine Impurities in Olmesartan at 0.005 ppm











Compound	Spiking Level (ppm)	Sample Results (ppm)	Recovery (%)
NDMA	0.005	0.0054	108
NDEA	0.005	0.0053	106
NEIPA	0.005	0.0048	96
NDIPA	0.005	0.0047	94
NDBA	0.005	0.0058	116

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Pregabalin

Method for Analysis

Instrument Method

Chromatographic Condition:

Mobile Phase A: 0.2 % Formic Acid in Water

Mobile Phase B: Methanol

Sample Diluent: Water

Flow Rate: 0.5mL/min

Injection Volume: 20µL

Column Used: Infinity Lab Poroshell HPH C18 3 x 150mm 4µm (P/N 693970-502T)

Column Temperature: 40°C

Gradient Program:

Time (Min)	Mobile Phase A	Mobile Phase B
0	95	5
5	70	30
6.2	66.5	33.5
8	5	95
11	5	95
11.1	95	5
14	95	5

Post Run Time: 1 minutes

Instrument	Agilent 6470 /Ultivo Triple Quadrupole mass spectrometer
Ion source	Atmospheric Pressure Chemical Ionization (APCI)
MS/MS mode	MRM
Ion mode	Positive
Drying gas temperature	300 °C
Drying gas flow	6 L/min
Nebulizer pressure	55 psi
APCI heater	350 °C
APCI needle positive	4 µA
Capillary voltage, positive	3000 V
MS1/MS2 resolution	0.7/0.7 (unit/unit)

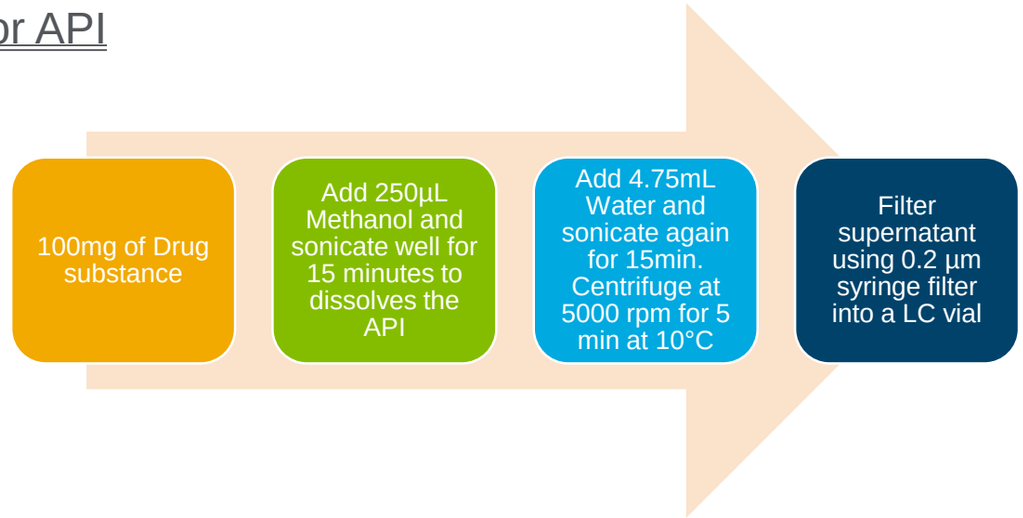
Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy(V)	CAV(V)	Polarity
NDEA	103.1	75.1	80	9	3	+
NDEA	103.1	47.1	80	17	3	+
NDMA	75.1	58	75	10	1	+
NDMA	75.1	43.1	75	18	1	+
NDBA	159.1	57.2	90	12	1	+
NDBA	159.1	41.1	90	22	3	+
NPIP	115.1	69.1	90	12	1	+
NPIP	115.1	41.2	90	24	1	+
N-nitrosomethylaminopyridine	138.1	108	60	6	5	+
N-nitrosomethylaminopyridine	138.1	79.2	60	42	5	+

Calibrations

0.1 ng/mL to 100 ng/mL

Sample Preparation

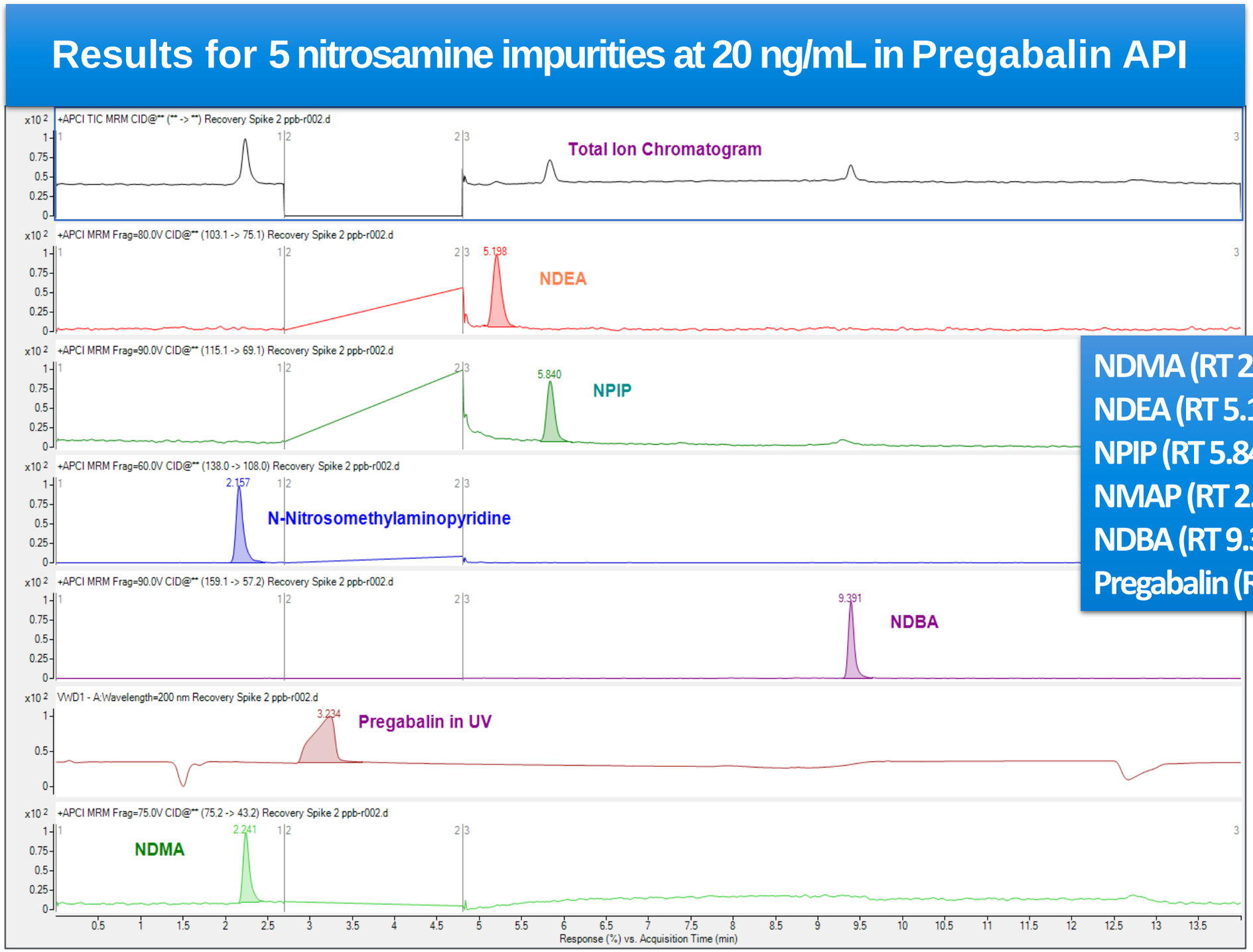
For API



System Suitability

The coefficient of determination (R^2) of the linear calibration curve should be ≥ 0.990.
The S/N ratio of the 1 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 1 ng/mL standard should be ≤ 10

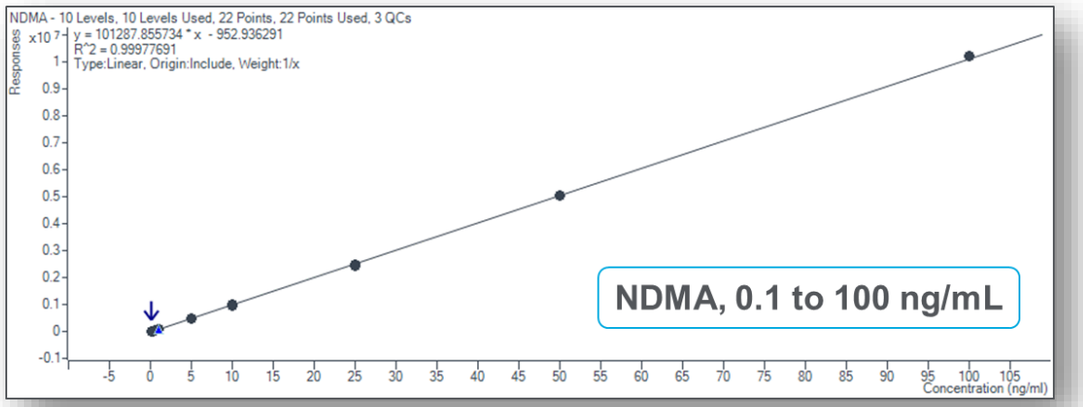
Results for 5 nitrosamine impurities at 20 ng/mL in Pregabalin API

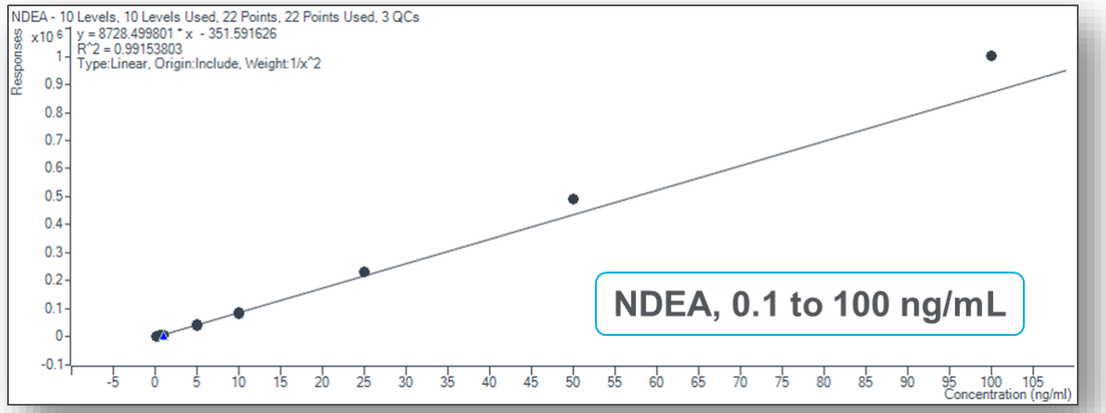


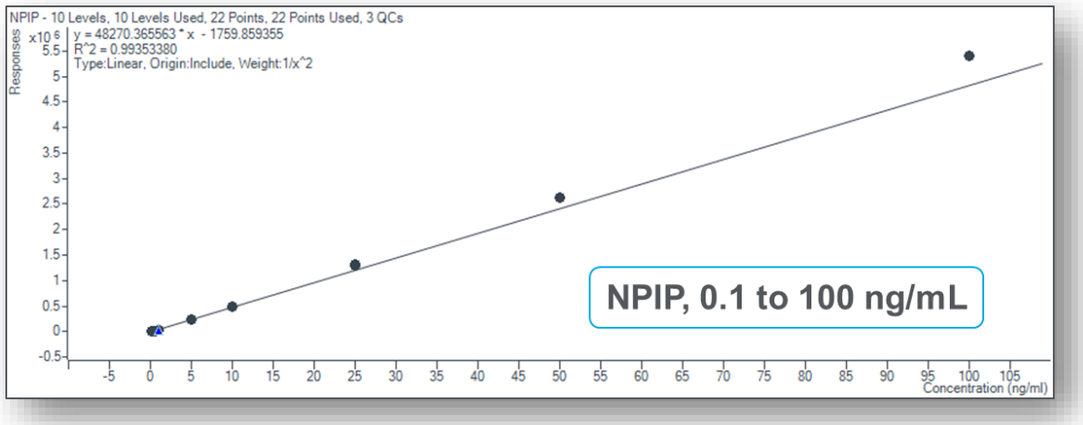
NDMA (RT 2.24 min)
NDEA (RT 5.19 min)
NPIP (RT 5.84min)
NMAP (RT 2.16min)
NDBA (RT 9.39 min)
Pregabalin (RT 2.95min)

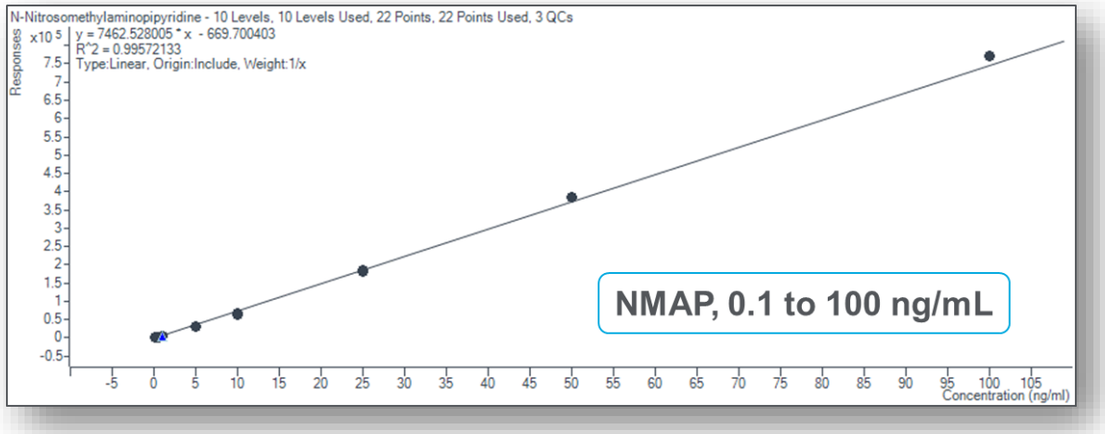
Pregabalin

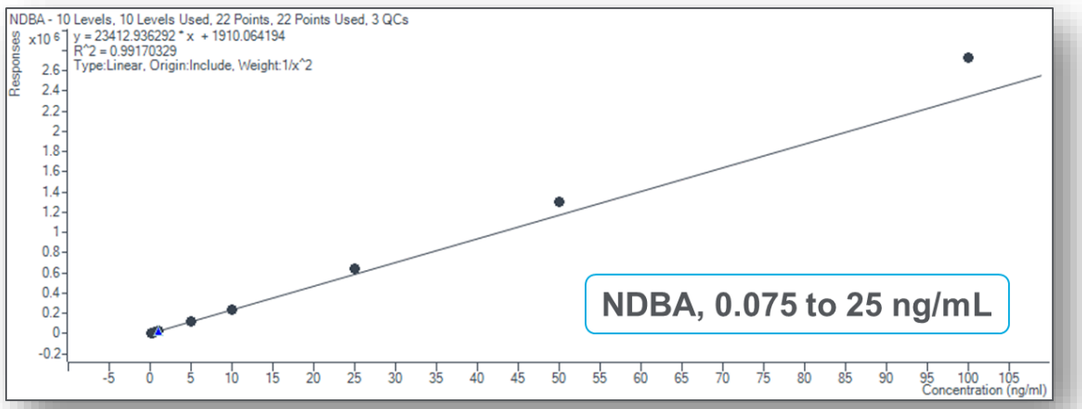
Calibration Curves











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Pregabalin

Representative recovery % of Nitrosamine Impurities

@ different concentrations using 20mg/mL sample size

Spike Conc. (ng/mL)	Recovery %				
	NDEA	NPIP	NMAP	NDBA	NDMA
0.5	102.2	91.1	94.99	102.96	102.7
1	98.86	93.3	115	107.45	94.7
2	88.7	96.9	100.5	94.62	105.8
5	93.11	95.89	100.3	104.12	103
10	86.1	96.11	105.4	97.99	97.6

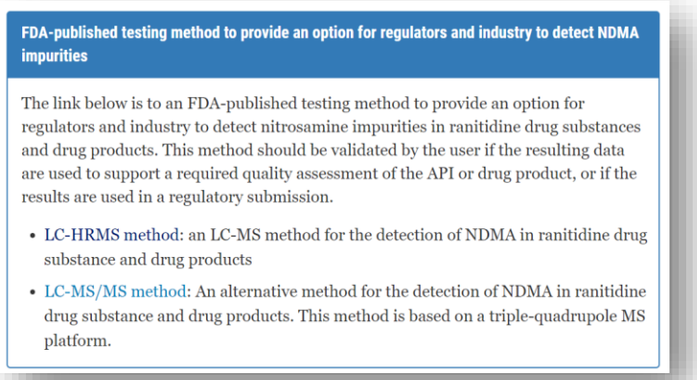
Note: Use of corresponding internal standards for each nitrosamines may further help in any recovery issue.

Benefits of Agilent LC/TQ	
Optimized methods	• Optimized method for sartan drug substance • Detect and quantify nitrosamine impurities limits per published FDA regulatory testing method guidance
Scalable application	• Best precision = best ion ratios = best quant results; Rugged ion source design
Sample prep	• Sample preparation as per EDQM guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument • Efficient Quant review with MassHunter • Data Integrity

Ranitidine Based Drugs

- Ranitidine is a histamine-2 receptor antagonist (acid inhibitor or H2 blocker) and is available as both prescription and over-the-counter drug to treat acid reflux. Examples of H2 receptor blockers include: Ranitidine (Zantac), Nizatidine (Axid), Famotidine (Pepcid, Pepcid AC) and Cimetidine (Tagamet, Tagamet HB).
- N-nitrosodimethylamine (NDMA) impurity was detected in some ranitidine products and the levels were found to increases with time and temperature, and thus ranitidine drugs were recently recalled from the U.S. market
- Regulatory agencies (for e.g. including US Food and Drug administration (US FDA)) provided guidance on the detection and quantification of NDMA impurity in ranitidine based drugs

US FDA



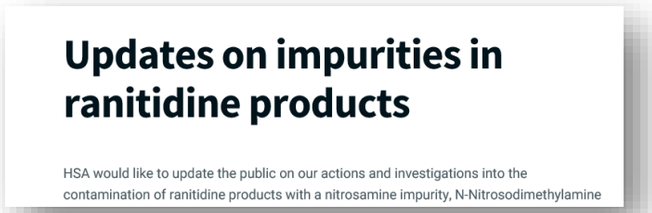
FDA-published testing method to provide an option for regulators and industry to detect NDMA impurities

The link below is to an FDA-published testing method to provide an option for regulators and industry to detect nitrosamine impurities in ranitidine drug substances and drug products. This method should be validated by the user if the resulting data are used to support a required quality assessment of the API or drug product, or if the results are used in a regulatory submission.

- LC-HRMS method: an LC-MS method for the detection of NDMA in ranitidine drug substance and drug products
- LC-MS/MS method: An alternative method for the detection of NDMA in ranitidine drug substance and drug products. This method is based on a triple-quadrupole MS platform.

https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-zantac-ranitidine

HAS, Singapore

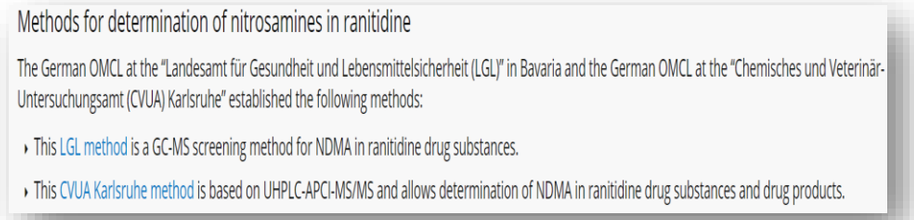


Updates on impurities in ranitidine products

HSA would like to update the public on our actions and investigations into the contamination of ranitidine products with a nitrosamine impurity, N-Nitrosodimethylamine

https://www.hsa.gov.sg/announcements/safety-alert/updates-on-impurities-in-ranitidine-products

Council of Europe



Methods for determination of nitrosamines in ranitidine

The German OMCL at the "Landesamt für Gesundheit und Lebensmittelsicherheit (LGL)" in Bavaria and the German OMCL at the "Chemisches und Veterinär-Untersuchungsamt (CVUA) Karlsruhe" established the following methods:

- This LGL method is a GC-MS screening method for NDMA in ranitidine drug substances.
- This CVUA Karlsruhe method is based on UHPLC-APCI-MS/MS and allows determination of NDMA in ranitidine drug substances and drug products.

https://www.edqm.eu/en/ad-hoc-projects-omcl-network

Ranitidine

Mutagenic Impurity Analysis LC/MS Workflow Solution

Separate

InfinityLab Poroshell Column

1290 Infinity II LC System

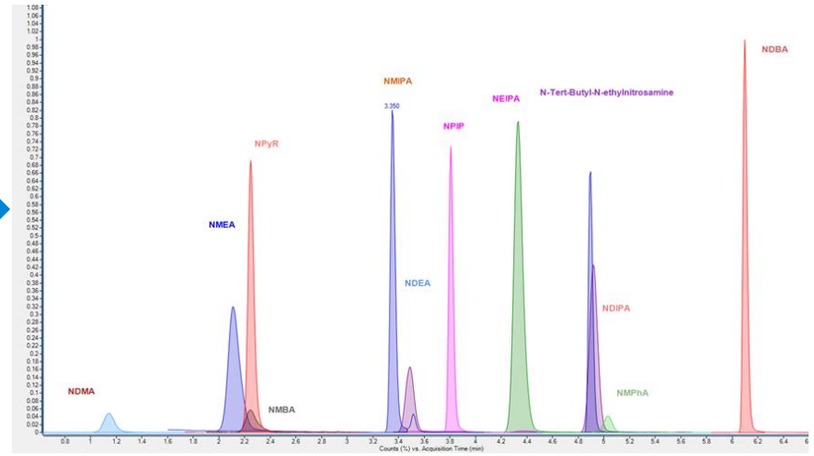
Acquire

6470 LC/TQ

Ultivo LC/TQ

6546 LC/Q-TOF

Analyze



MassHunter Software

Agilent LC/MS Solution for NDMA Analysis in Ranitidine Based Drugs

Typical LC Configuration

Agilent 1290 Infinity II High-Speed Pump (G7120A)
Agilent 1290 Infinity II Multisampler (G7167B)
Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
Agilent 1290 Infinity II Variable Wavelength Detector (G7114B)

HPLC

1290 Infinity II LC System

TQ

6470LC/TQ

Ultivo LC/TQ

Application Area

Analyte	NDMA
Matrices	Ranitidine drug substances
Customers	Pharmaceuticals and contract labs

Columns and supplies

Columns: Infinity Lab Poroshell HPH C18 3 x 150mm 4µm (P/N 693970-502T)

HPLC Vials and Caps: Vial, screw 2mL Amber p/n 5182-0716 and Cap p/n 5183-2077

Syringe Filter Paper: 5190-5261 (PVDF, 13mm 0.2 µm)

Highlights – LC/MS/MS approaches

- ❑Easy to operate
- ❑Quick implementation in labs
- ❑Optimized methods
- ❑Sample size used as per US FDA recommendations
- ❑Easy sample preparation
- ❑Ranitidine API elutes after NDMA so diverter valve programmed accordingly

Ranitidine

Method for Analysis

Instrument Method

Parameter	Value
Instruments	Agilent 1290 Infinity II high speed pump (G7120A) Agilent 1290 Infinity II multisampler (G7167B) Agilent 1290 Infinity II multicolumn thermostat (G7116B) Agilent 1290 Infinity II variable wavelength detector (G7114B)
Needle wash	80:20, Methanol: Water
Sample diluent	Water
Multisampler temperature	6 ± 2 °C
Injection volume	20 µL
Analytical column	Agilent InfinityLab Poroshell HPH-C18, 4.6 × 150 mm, 2.7 µm (p/n 693975-702)
Column temperature	40 °C
Mobile phase A	0.1 % formic acid in water
Mobile phase B	0.1 % formic acid in Methanol
Flow rate	0.3 mL/min
Gradient	Time (min) / % A / % B / Flow (mL/min) 0 / 95 / 5 / 0.3 6 / 92 / 8 / 0.3 6.1 / 92 / 8 / 0.5 11 / 5 / 95 / 0.5 11.1 / 5 / 95 / 0.3 11.2 / 95 / 5 / 0.3 14 / 95 / 5 / 0.3
Stop time	14 minutes
Post time	1 minutes
UV Wavelengths	230 nm, 300 nm

Instrument	Agilent 6470A triple quadrupole LC/MS
Ion source	Atmospheric Pressure Chemical Ionization (APCI)
MS/MS mode	MRM
Ion mode	Positive
Drying gas temperature	300 °C
Drying gas flow	5 L/min
Nebulizer pressure	35 psi
APCI heater	350 °C
APCI needle positive	4 µA
Capillary voltage, positive	4000 V
MS1/MS2 resolution	0.7/0.7 (unit/unit)
Dwell time	200 ms

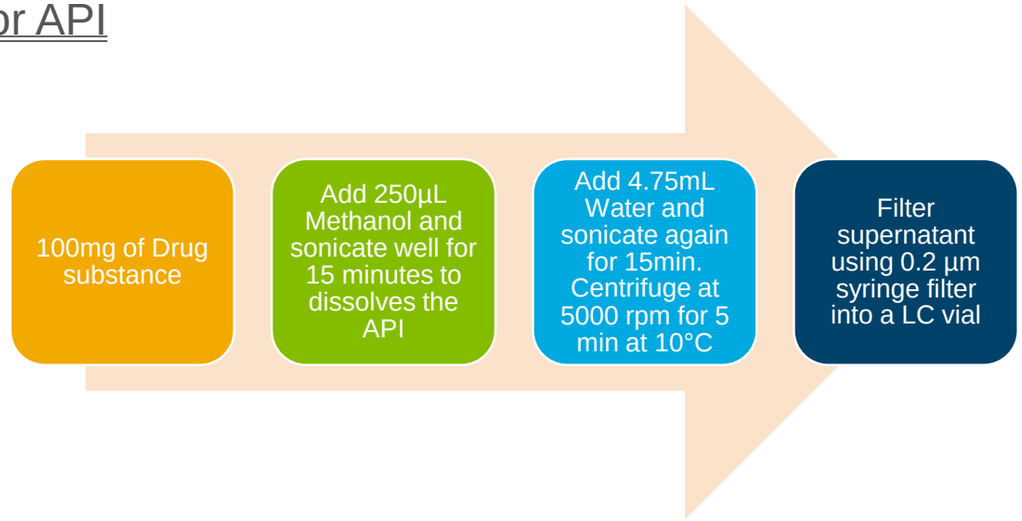
Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy(V)	CAV(V)	Polarity
NDMA(Quantifier)	75.1	43.1	75	18	1	+
NDMA(Qualifier)	75.1	58.1	75	10	1	+

Calibrations

0.1 ng/mL to 100 ng/mL

Sample Preparation

For API



System Suitability

The coefficient of determination (R^2) of the linear calibration curve should be ≥ 0.990.
The S/N ratio of the 1 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 1 ng/mL standard should be ≤ 10

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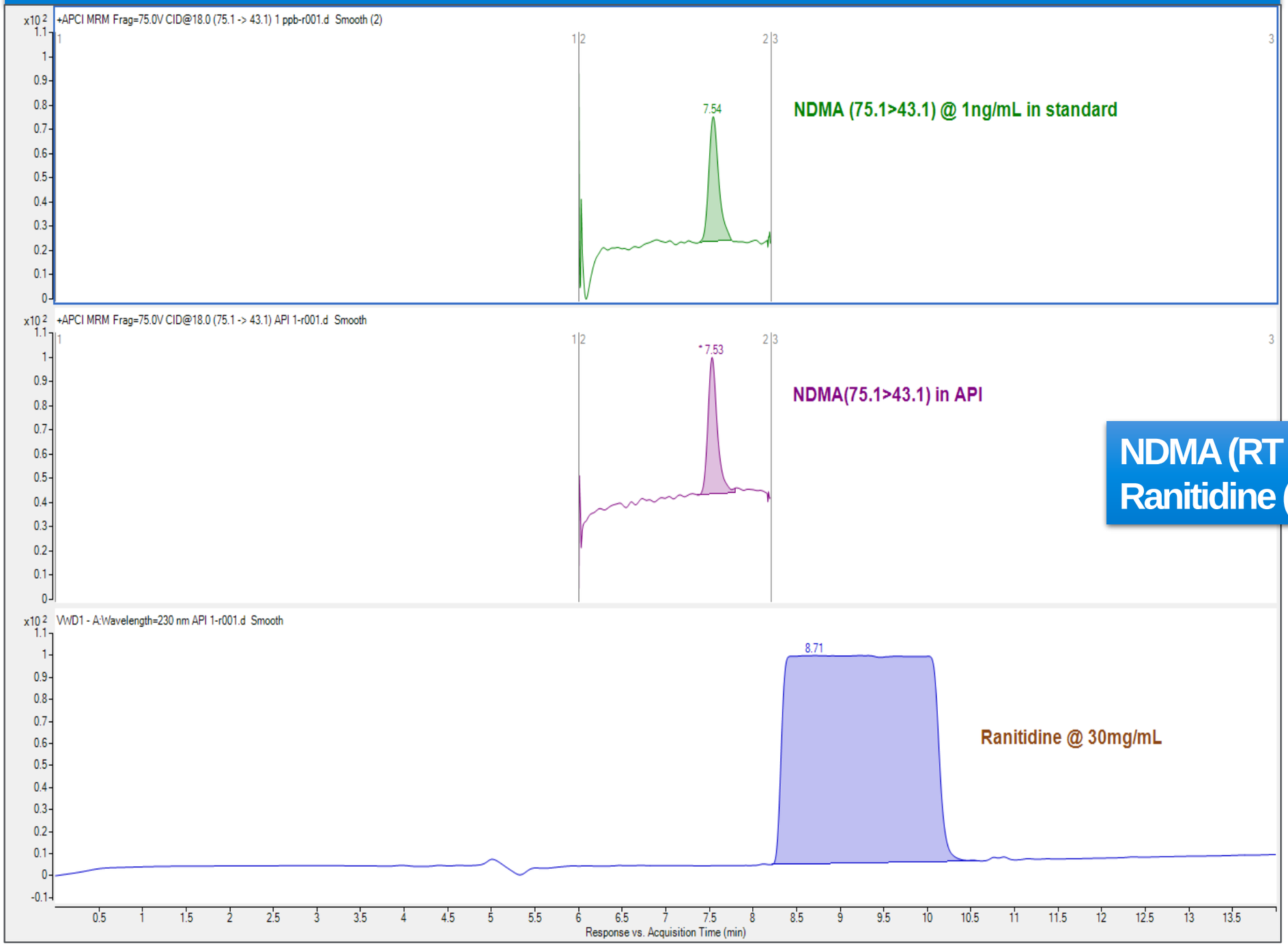
Ranitidine

Results for NDMA impurity at 30 ng/mL in Ranitidine API



NDMA (RT 7.54 min)
Ranitidine (RT 8.2 min)

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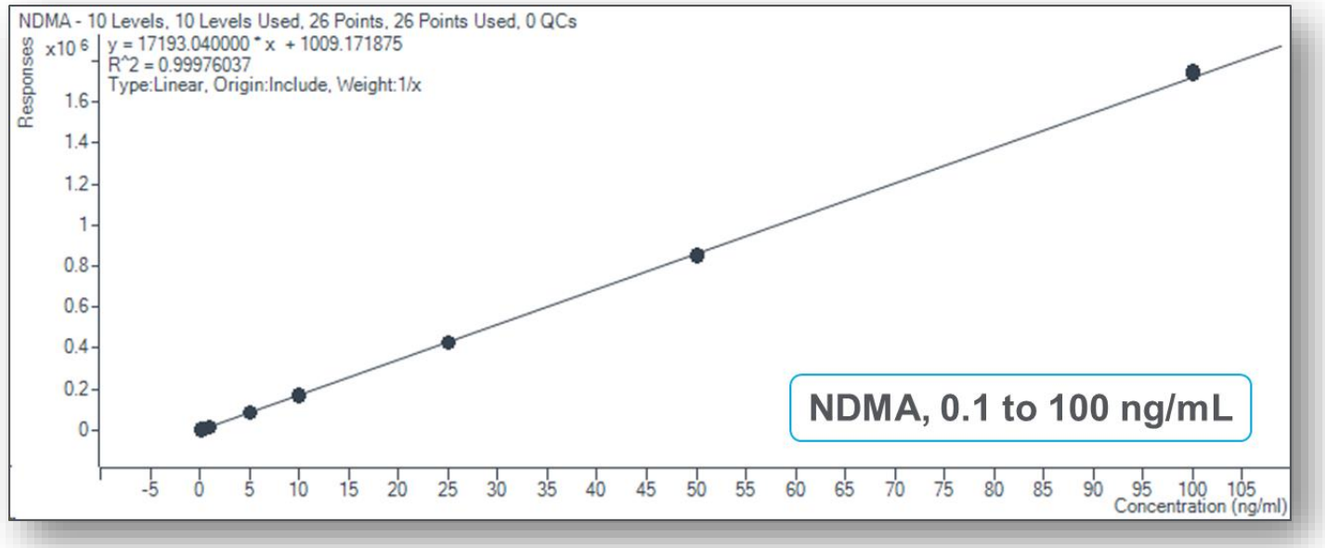
Calibration Curve



Representative recovery % of NDMA impurity @ different concentrations using 30mg/mL sample size

Nitrosamine Impurity	Spiked Concentration (ng/mL) mixed with Ranitidine API (30 mg/mL)	Recovery %
NDMA	1.2	86.4
	3	93.3
	6	86.5

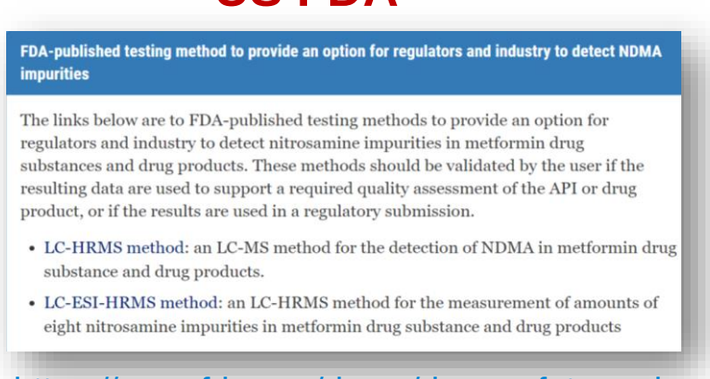
Nitrosamine Impurity	Spiked Concentration (ng/mL) in 30 mg/mL of Ranitidine Tablet	Recovery %
NDMA	24	94.9
	48	87.4

Note:
- Recovery experiments were performed at higher concentrations, as both the drug substance and drug product already contained NDMA in reasonable amounts.
- Use of corresponding internal standards for each nitrosamines may further help in any recovery issue.

Metformin-Based Drugs

- Metformin is an oral diabetes medicine to help control blood sugar levels
- N-nitrosodimethylamine (NDMA) impurity was detected in some metformin drugs at an unacceptable intake limits, and thus certain metformin products were recently recalled from the U.S. market
- Regulatory agencies (for e.g. US Food and Drug administration (US FDA)) provided guidance on the detection and quantification of NDMA impurity in metformin drugs

US FDA



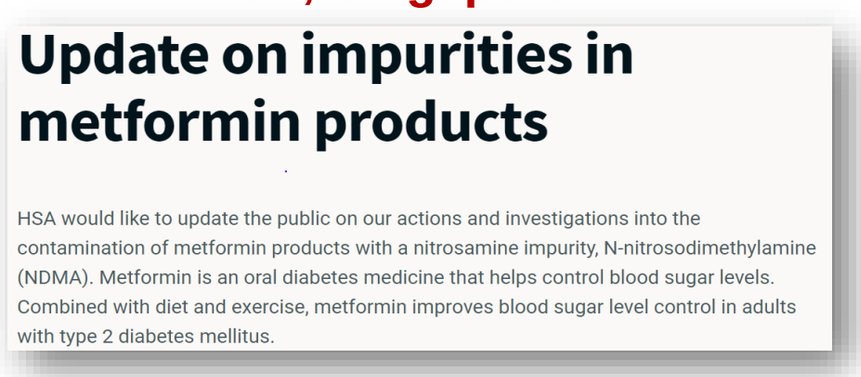
FDA-published testing method to provide an option for regulators and industry to detect NDMA impurities

The links below are to FDA-published testing methods to provide an option for regulators and industry to detect nitrosamine impurities in metformin drug substances and drug products. These methods should be validated by the user if the resulting data are used to support a required quality assessment of the API or drug product, or if the results are used in a regulatory submission.

- LC-HRMS method: an LC-MS method for the detection of NDMA in metformin drug substance and drug products.
- LC-ESI-HRMS method: an LC-HRMS method for the measurement of amounts of eight nitrosamine impurities in metformin drug substance and drug products

https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-metformin

HSA, Singapore

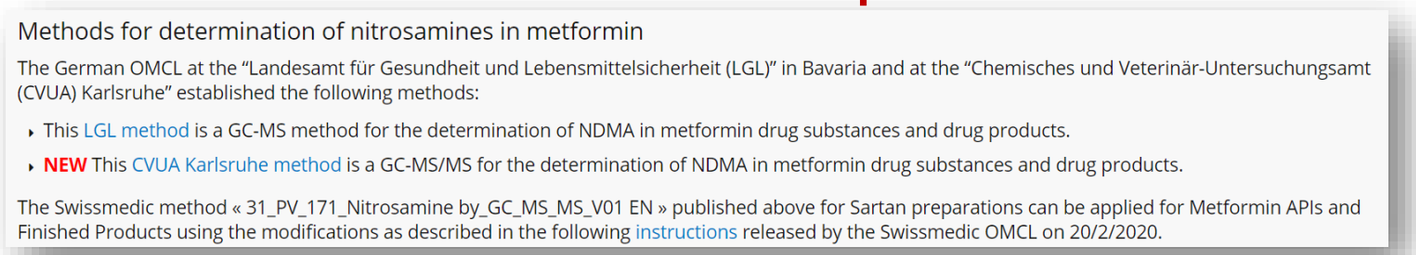


Update on impurities in metformin products

HSA would like to update the public on our actions and investigations into the contamination of metformin products with a nitrosamine impurity, N-nitrosodimethylamine (NDMA). Metformin is an oral diabetes medicine that helps control blood sugar levels. Combined with diet and exercise, metformin improves blood sugar level control in adults with type 2 diabetes mellitus.

https://www.hsa.gov.sg/announcements/safety-alert/update-on-impurities-in-metformin-products

Council of Europe



Methods for determination of nitrosamines in metformin

The German OMCL at the "Landesamt für Gesundheit und Lebensmittelsicherheit (LGL)" in Bavaria and at the "Chemisches und Veterinär-Untersuchungsamt (CVUA) Karlsruhe" established the following methods:

- This LGL method is a GC-MS method for the determination of NDMA in metformin drug substances and drug products.
- **NEW** This CVUA Karlsruhe method is a GC-MS/MS for the determination of NDMA in metformin drug substances and drug products.

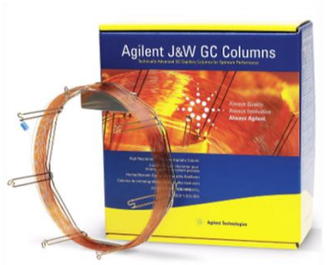
The Swissmedic method « 31_PV_171_Nitrosamine by_GC_MS_MS_V01 EN » published above for Sartan preparations can be applied for Metformin APIs and Finished Products using the modifications as described in the following instructions released by the Swissmedic OMCL on 20/2/2020.

https://www.edqm.eu/en/ad-hoc-projects-omcl-network#Methods%20for%20determination%20of%20nitrosamines%20in%20metformin

Metformin

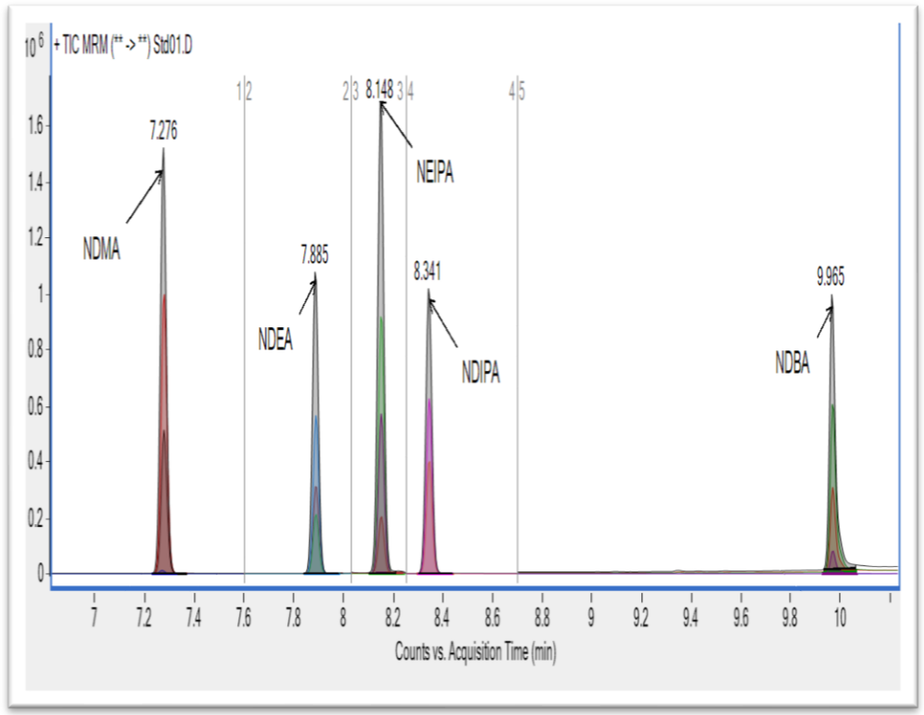
Mutagenic Impurity Analysis GC/MS Workflow Solution

Separate | **Acquire** | **Analyze**



[WAX GC Columns]

[8890 GC/7693 LS/ 7010B GC/TQ]



[MassHunter Software]

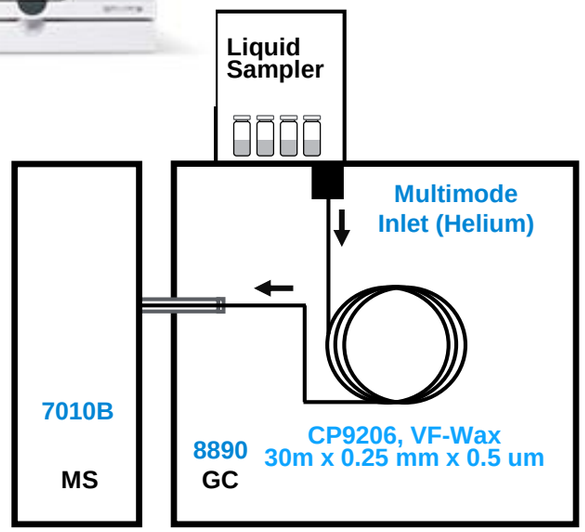
Metformin

Agilent GC/MS Solution for Analysis of Nitrosamines in

Typical Configuration

	Add 8890 GC and ALS with one of the MS Options		
GC	G3540A	Agilent 8890 GC System	112, 201, 313 (for TQ only)
GC	G4513A	7693A Autoinjector	NO OPT
GC	G4514A	7693A Tray, 150 Vial	NO OPT
	MS Option 1		
TQ	G7012BA	7010B Quadrupole MS/MS EI Bundle	#010 (optional),245

[8890 GC/7693 LS/ 7010B GC/TQ]



Application Area

Analytes	NDMA, NDEA, NEIPA, NDIPA, NDBA
Matrices	Metformin drug substances and drug products
Customers	Pharmaceuticals and contract labs

Columns and supplies

Columns J&W DB-WAX GC Column, 30 m, 0.25 mm, 0.5 µm, 7inch cage (CP9206)

GC Vials and Caps: Screw top MS analyzed vial kit (5190-2277)

Syringe Filter Paper: Nylon, 0.45 µm (5190-5091)

GC Inlet Liner: Ultra Inert, splitless, single taper, glass wool (5190-2293)

Highlights – GC/MS/MS approaches

- ❑ Cost effective, easy to use
- ❑ Quick implementation in labs
- ❑ Optimized methods and RTL based MRMs
- ❑ More API can be used (100 mg/mL or more) for Sample prep.
- ❑ APIs is insoluble in Dichloromethane, so doesn't overload column
- ❑ Easy sample preparation

GC/MS Method for Analysis

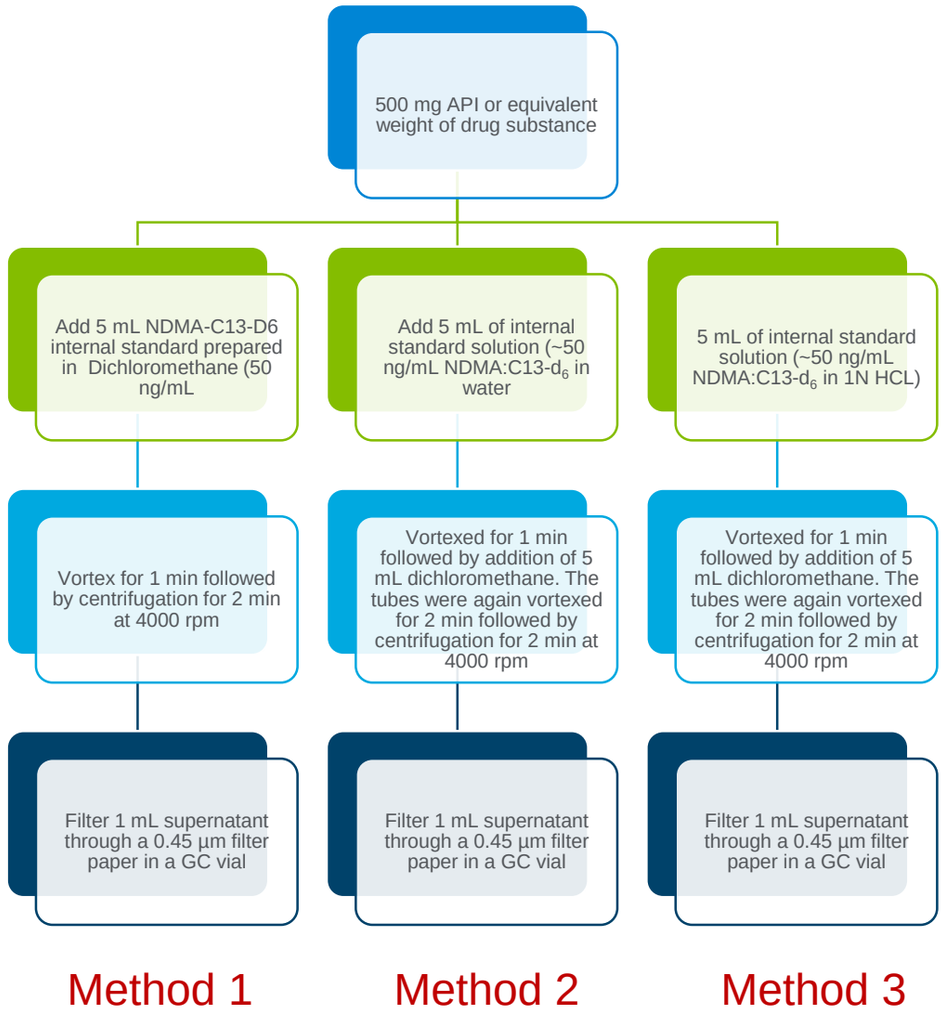
Instrument Method

ALS	GC	MS
Injection Volume: 2µL	Carrier Gas: He 1mL/min	EI Mode

Parameter	Value
MMI injection mode	Pulsed splitless: 12.285 psi until 0.5 min
Inlet temperature	250 °C
Oven temperature program	40 °C (1.5 min)
	20 °C/min to 200 °C (0 min)
	60 °C/min to 250 °C (3 min)
Total run time	12.33 min
MS transfer line temperature	250 °C

Parameter		Value
Source temperature	250 °C	
Quadrupole temperature	Q1 and Q2 = 150 °C	
MS1 and MS2 resolution	All compounds Unit	
Collision gas flow	Nitrogen at 1.5 mL/min,	
Quenching gas flow	Helium at 4 mL/min	
Quant./qual. transitions (FDA method)	Start time: 6.5 min NDMA	74 → 44.1, CE 6, dwell 150 ms 74 → 42.1, CE 22, dwell 50 ms NDMA:C13-d6 82 →48, CE 20, dwell 100 ms
	Start time: 7.60 min NDEA	102 →85, CE 4 V, dwell 80 ms 102 →56.1, CE 18 V, dwell 80 ms 102 →44.1, CE 14 V, dwell 80 ms
	Start time: 8.03 min NEIPA	116 →99.1, CE 4 V, dwell 80 ms 71 →56.1, CE 4 V, dwell 80 ms 116 →44.1, CE 14V, dwell 80 ms
	Start time: 8.25 min NDIPA	130 →88, CE 4 V, dwell 150 ms 130 →42, CE 10 V, dwell 150 ms
	Start time: 8.70 min NDBA	158 →99.1, CE 2 V, dwell 75 ms 84 →56.1, CE 20 V, dwell 75 ms 84 →42.1, CE 14 V, dwell 75 ms 158 →141.2, CE 2 V, dwell 75 ms

Sample Preparation



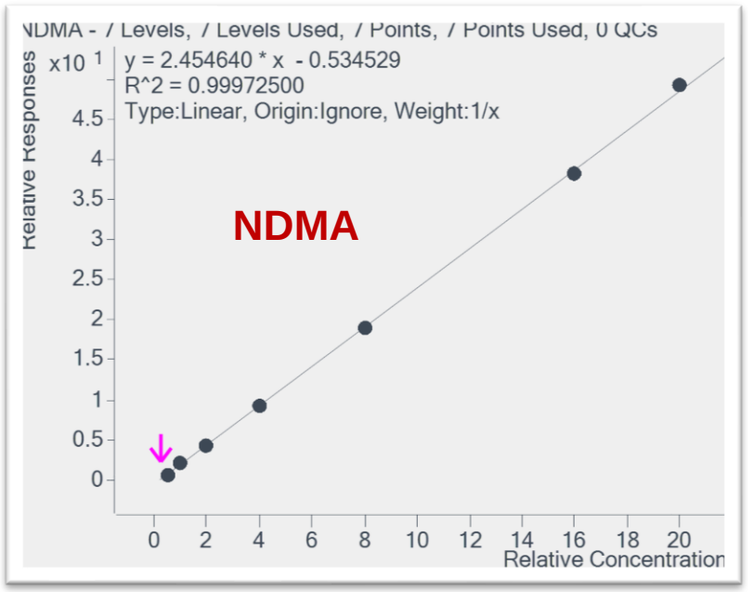
Calibrations

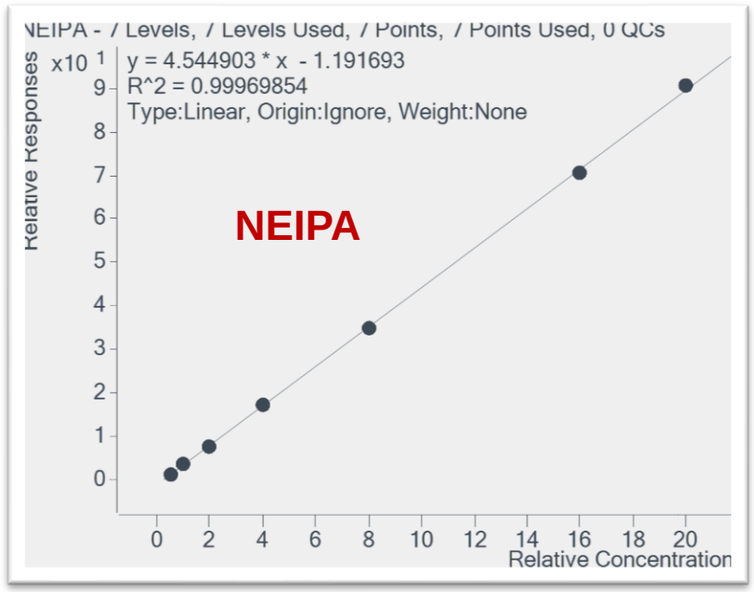
2.5 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 40 ng/ml, 80 ng/ml and 100 ng/ml each prepared in Dichloromethane containing 50 ng/mL of NDMA –C13-D6

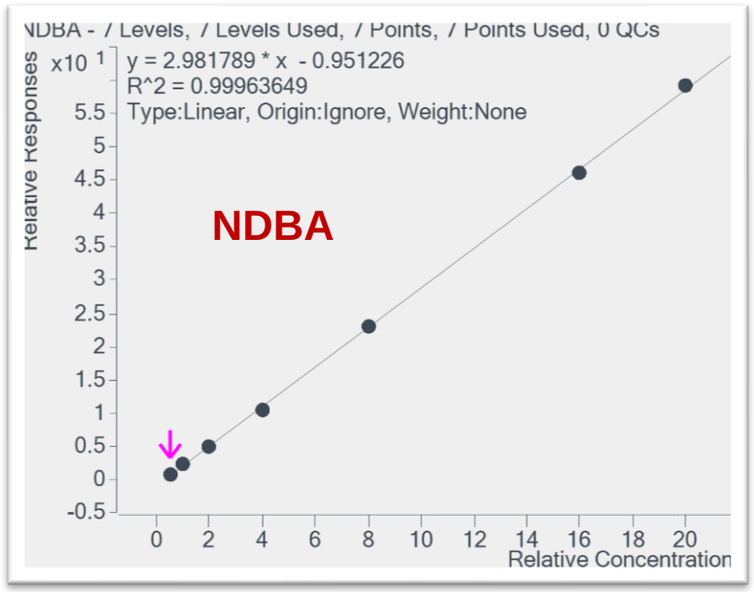
System Suitability

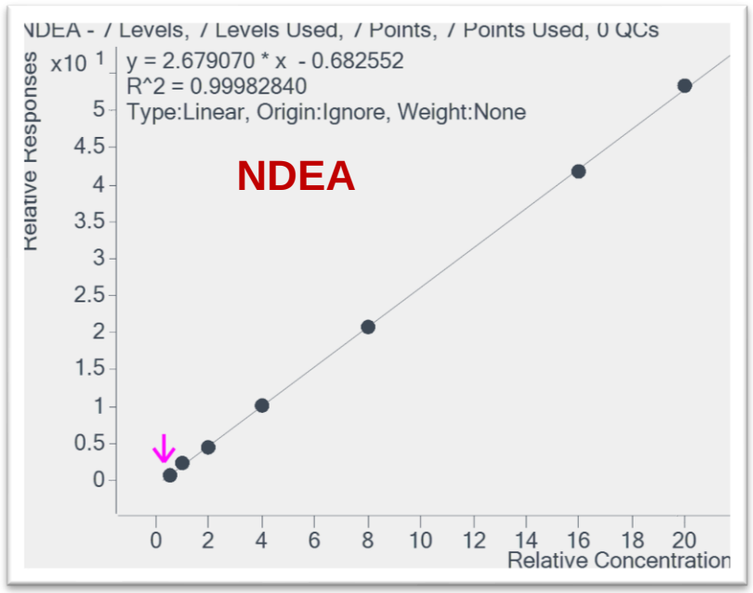
The coefficient of determination (R2) of the linear calibration curve should be ≥ 0.998.
The S/N ratio of the 5 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 40 ng/mL standard should be ≤ 5

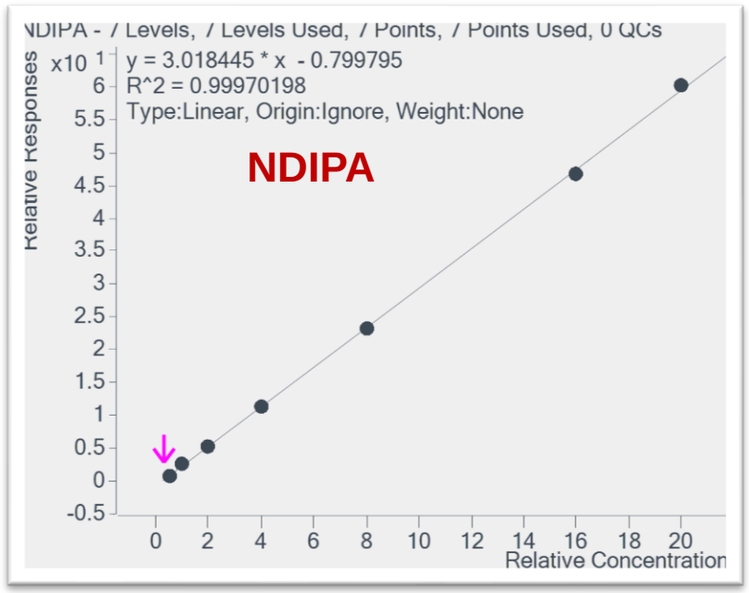
Calibration Curves











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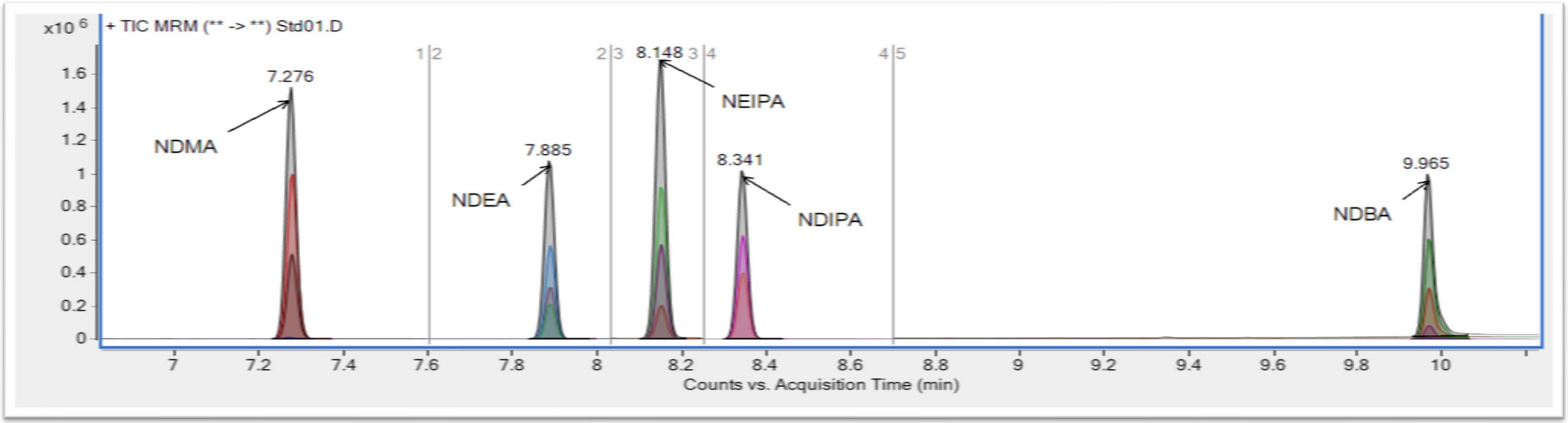
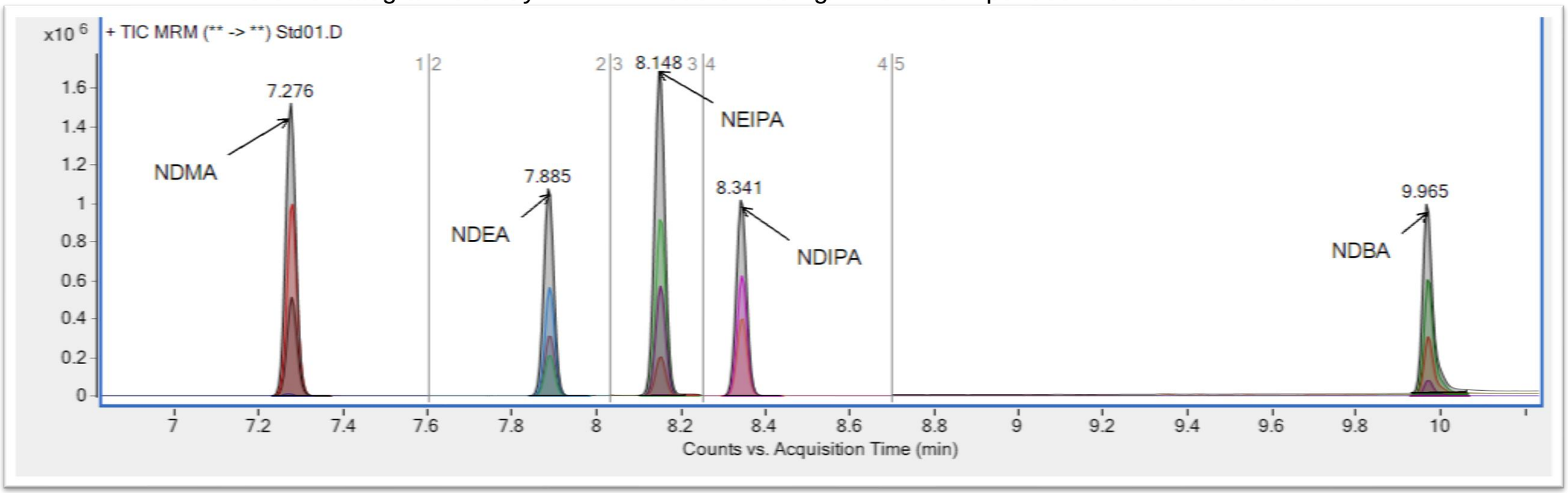
MRM Chromatogram of 5 Nitrosamine Impurities

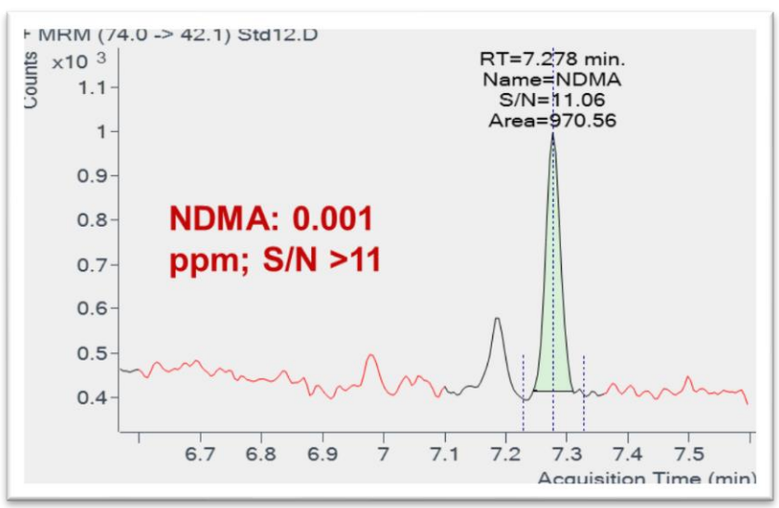
Extracted MRM chromatogram (quant and qual transition) of lowest calibration standard at 2.5 ng/mL mix of five impurities in dichloromethane.

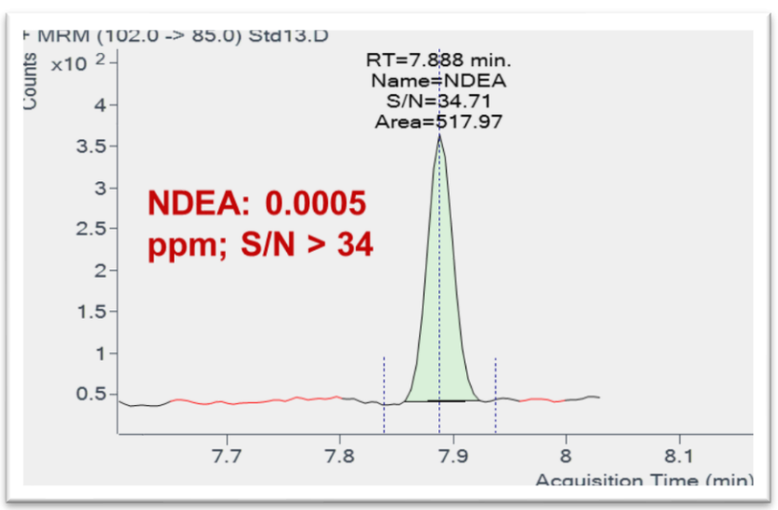


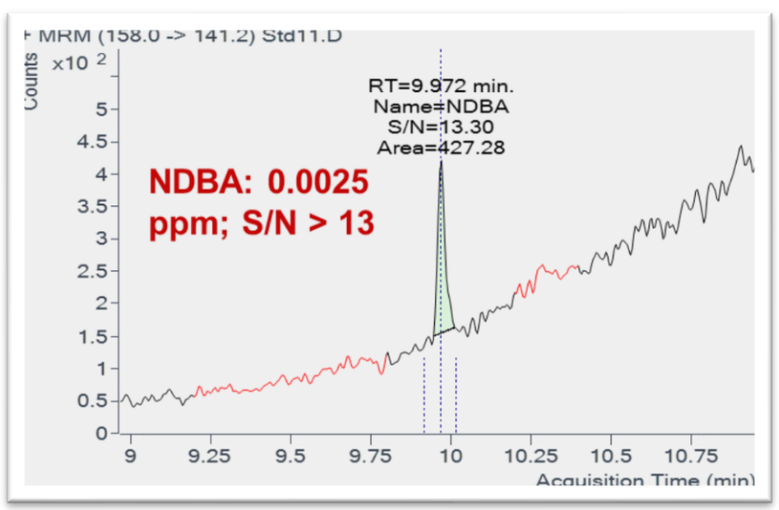
TIC chromatogram overlay in MRM mode of 100 ng/mL of five impurities in dichloromethane.

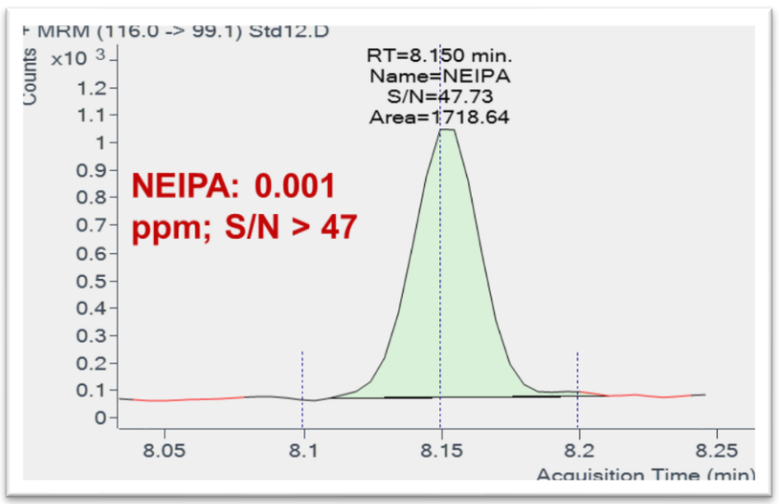


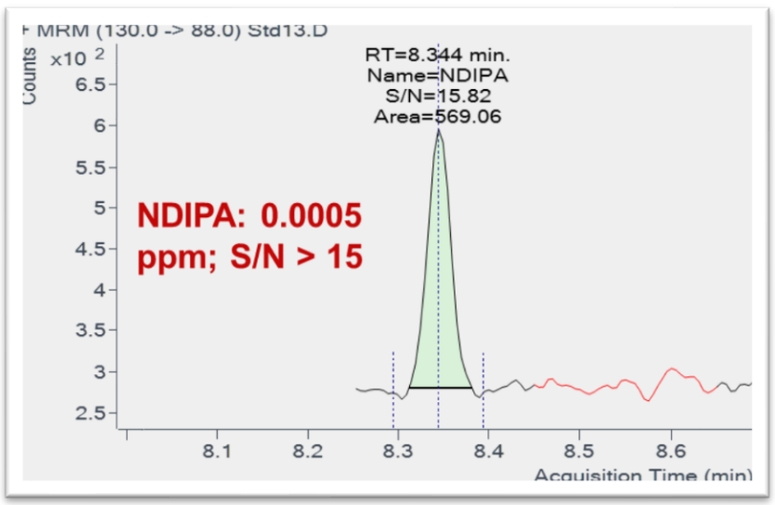
LOQ of Nitrosamine Impurities in Metformin











NDMA and NDEA in Metformin Samples

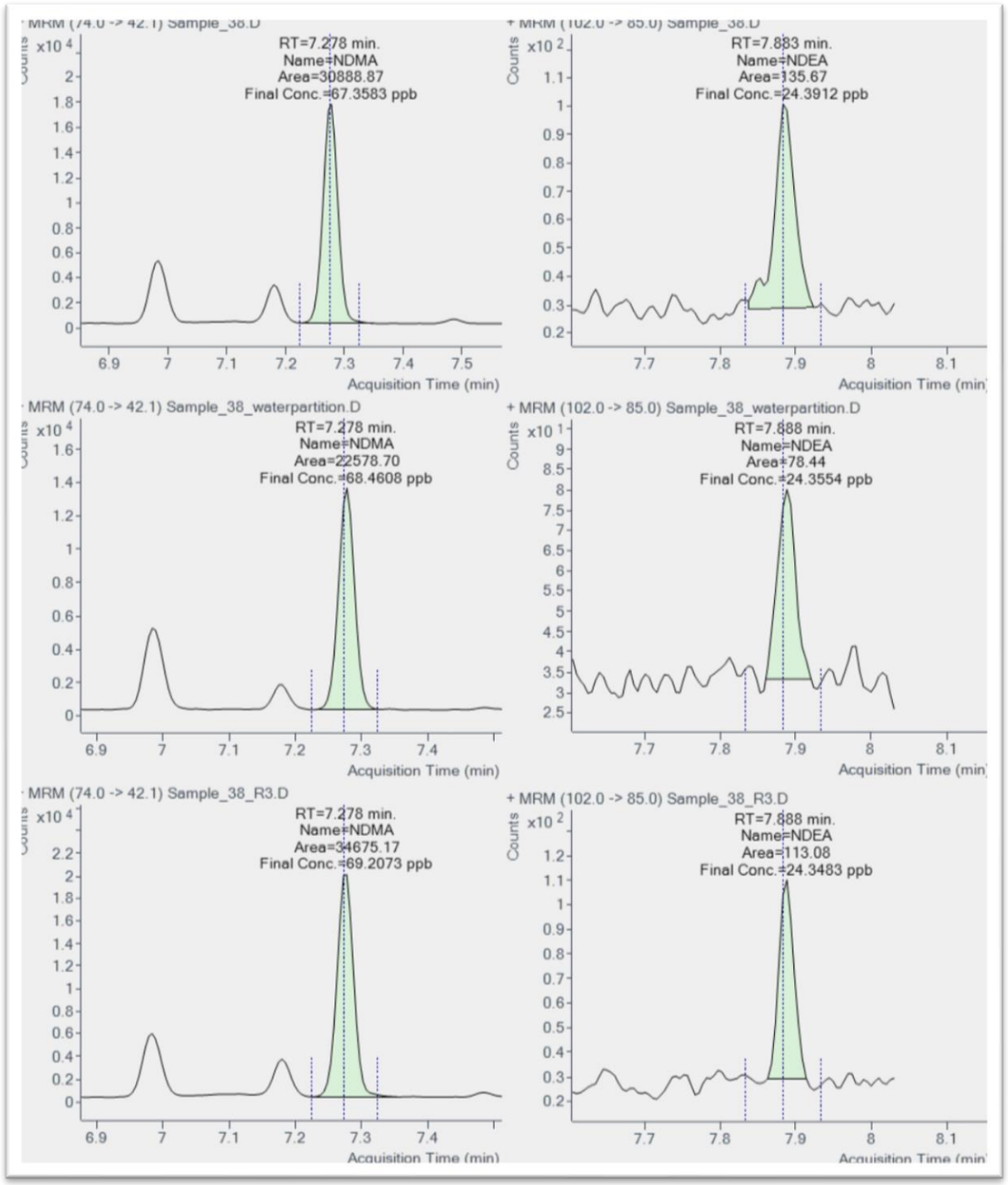
Drug Substance

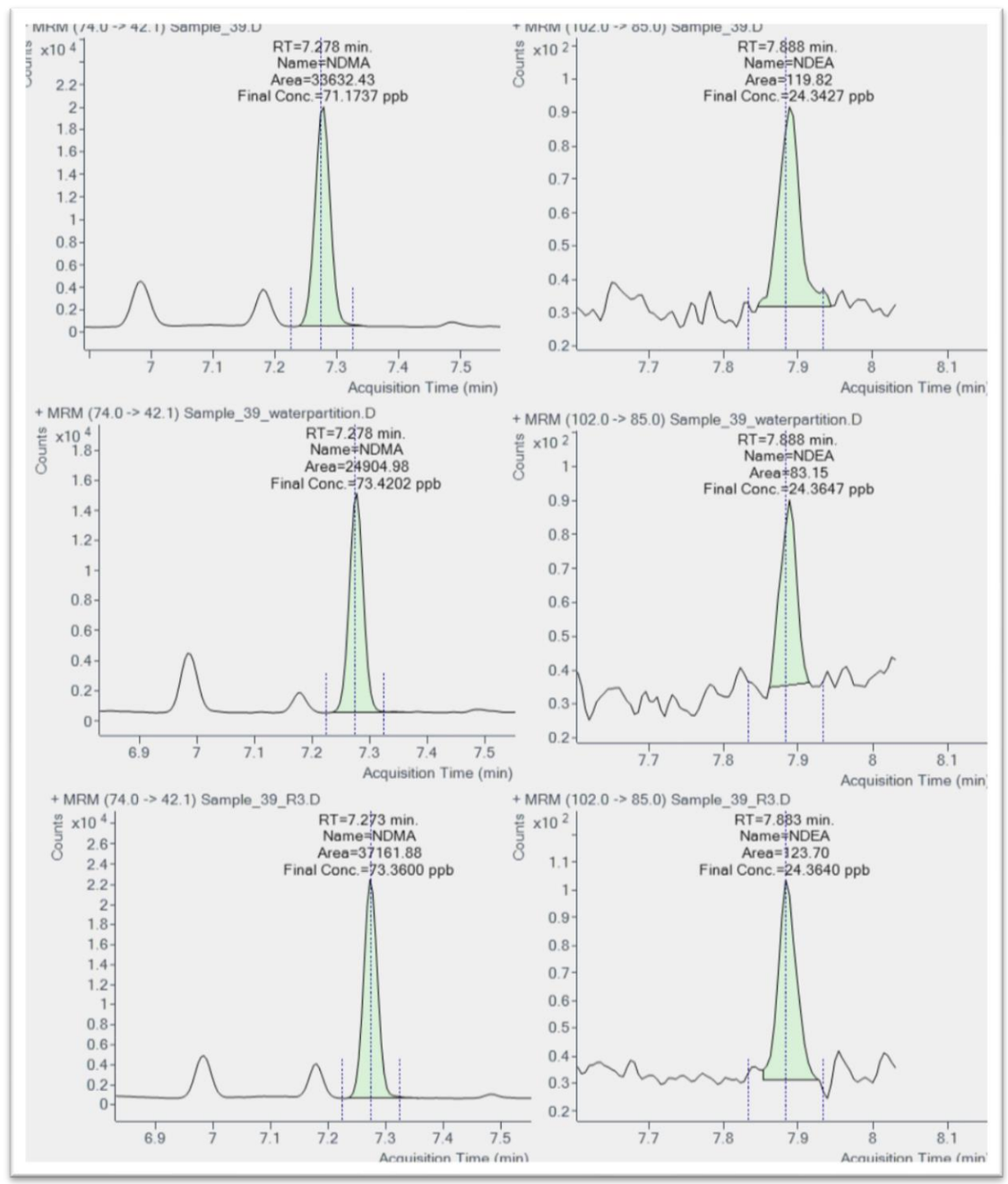
Drug Product

Method 1

Method 2

Method 3





Metformin

Mutagenic Impurity Analysis LC/MS Workflow Solution

Separate

InfinityLab Poroshell Column

1290 Infinity II LC System

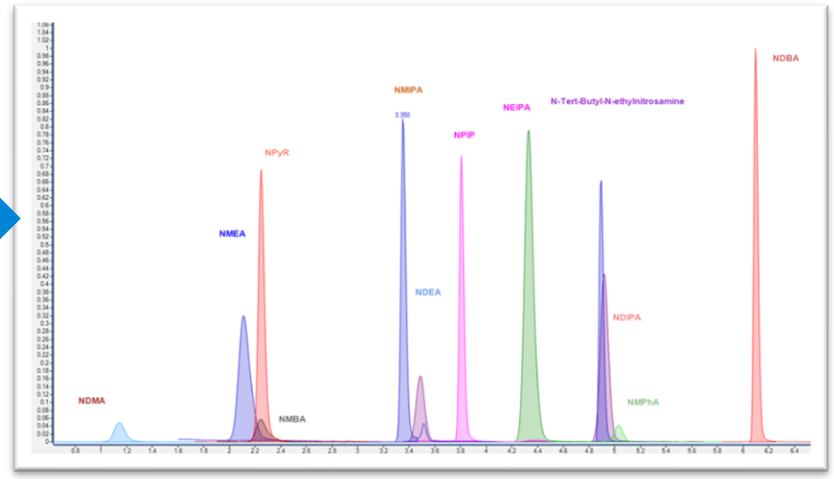
Acquire

6470 LC/TQ

Ultivo LC/TQ

6546 LC/Q-TOF

Analyze



MassHunter Software

Agilent LC/MSMS Solution for Nitrosamines Analyses in Metformin Based Drugs

Typical LC Configuration

Agilent 1290 Infinity II High-Speed Pump (G7120A)
Agilent 1290 Infinity II Multisampler (G7167B)
Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
Agilent 1290 Infinity II Variable Wavelength Detector (G7114B)

HPLC

1290 Infinity II LC System

TQ

6470LC/TQ

Ultivo LC/TQ

Application Area

Analytes	NDMA, NDEA, NEIPA, NDIPA, NDPA,NMPA NDBA and NMBA
Matrices	Metformin drug substances
Customers	Pharmaceuticals and contract labs

Columns and supplies

Columns: InfinityLab Poroshell HPH-C18, 4.6 × 150 mm, 2.7 µm (p/n 693975-702)
HPLC Vials and Caps: Vial, screw 2mL Amber p/n 5182-0716 and Cap p/n 5183-2077
Syringe Filter Paper: 5190-5261 (PVDF, 13mm 0.2 µm)

Highlights – LC/MS/MS approaches

- ❑Easy to operate
- ❑Quick implementation in labs
- ❑Optimized methods
- ❑Sample size used as per US FDA recommendations
- ❑Easy sample preparation
- ❑Metformin API elutes before all nitrosamines, so diverter valve programmed accordingly

Metformin

Method for Analysis

Instrument Method

Mobile phase A: 0.1 % formic acid in water

Mobile phase B: 0.1 % formic acid in Methanol

Sample diluent: Water: Methanol (95:5)

Multisampler temperature: 10°C

Injection volume: 20 µl

Column temperature: 40 °C

Analytical column: Agilent Infinity Lab Poroshell HPH-C18, 4.6 × 150 mm, 2.7 µm (p/n:693975-702)

Flow rate: 0.5 mL/min

Time	%A	%B	Flow(0.5ml/min)
0	95	5	0.5
2	95	5	0.5
7	40	60	0.5
10	25	75	0.5
11	10	90	0.5
16.5	10	90	0.5
16.6	95	5	0.5
20	95	5	0.5

Instrument	Agilent 6470 Triple Quadrupole mass spectrometer
Ion source	Atmospheric Pressure Chemical Ionization (APCI)
MS/MS mode	MRM
Ion mode	Positive
Drying gas temperature	300 °C
Drying gas flow	7 L/min
Nebulizer pressure	25 psi
APCI heater	350 °C
APCI needle positive	4 µA
Capillary voltage, positive	4000 V
MS1/MS2 resolution	0.7/0.7 (unit/unit)
Dwell time	50 ms

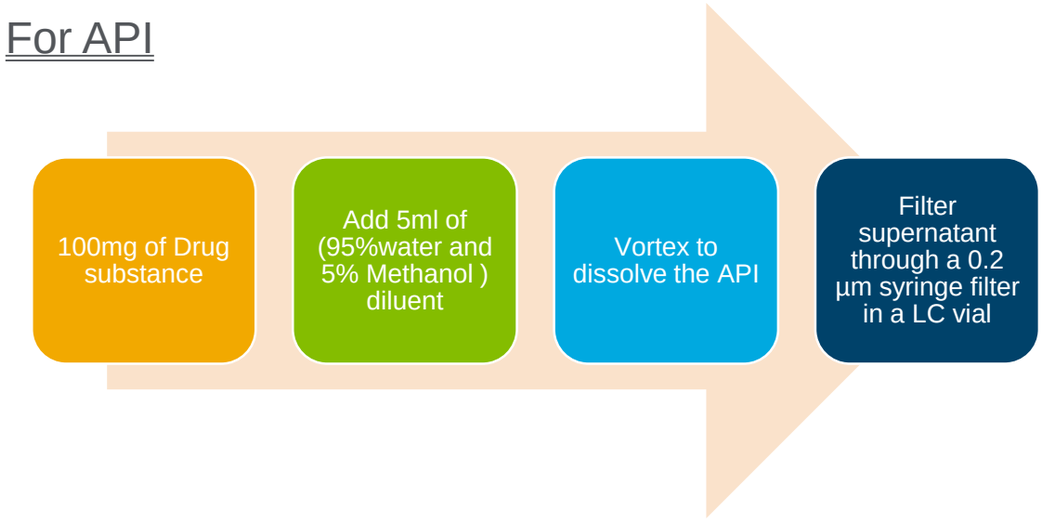
Compound	Precursor Ion (m/z)	Product Ion (m/z)	Dwell time (ms)	Fragmentor (V)	Collision Energy (V)	CAV (V)	Polarity
NDMA(Quantifier)	75	43.1	50	110	16	3	+
NDMA (Qualifier)	75	58	50	80	10	2	+
NMBA(Quantifier)	147	117	50	60	4	2	+
NMBA(Qualifier)	147	44	50	60	12	2	+
NDEA(Quantifier)	103	75	50	78	12	4	+
NDEA(Qualifier)	103	47	50	78	20	4	+
NEIPA(Quantifier)	117	74.9	50	82	8	8	+
NEIPA(Qualifier)	117.1	47.1	50	82	15	8	+
NDIPA(Quantifier)	131	89.1	50	80	5	4	+
NDIPA(Qualifier)	131	43	50	80	20	4	+
NDPA(Quantifier)	131	89.1	50	80	5	4	+
NDPA(Qualifier)	131	43	50	80	20	4	+
NMPA(Quantifier)	137	66	50	70	20	5	+
NMPA(Qualifier)	137	107	50	70	10	5	+
NDBA(Quantifier)	159.1	57	50	86	12	4	+
NDBA(Qualifier)	159.1	41.3	50	86	20	4	+

Calibrations

0.1 ng/mL to 50 ng/mL

Sample Preparation

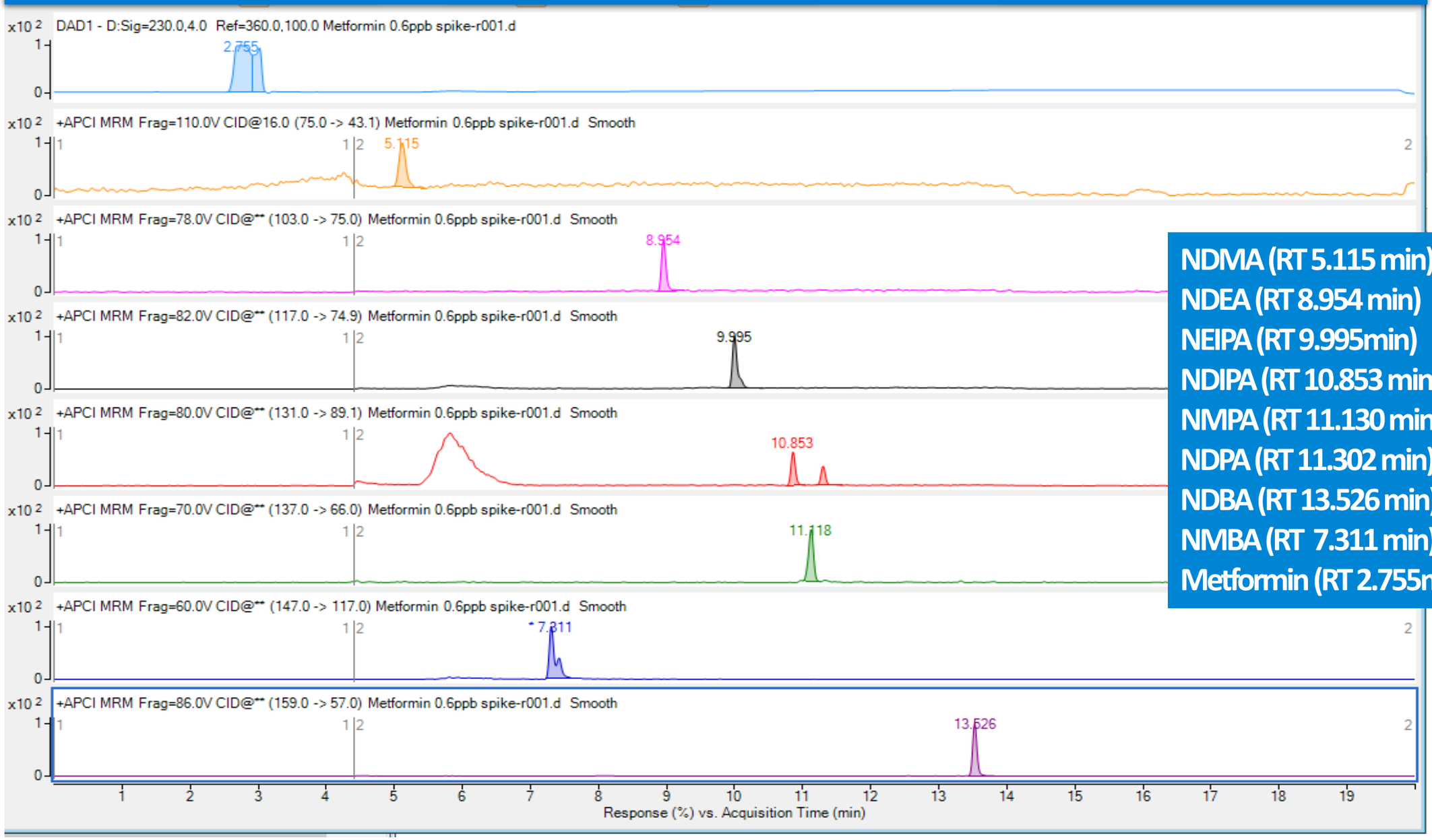
For API



System Suitability

The coefficient of determination (R2) of the linear calibration curve should be ≥ 0.990.
The S/N ratio of the 1 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 1 ng/mL standard should be ≤ 10

Results for 8 nitrosamine impurities at 20 ng/mL in Metformin API



NDMA (RT 5.115 min)
NDEA (RT 8.954 min)
NEIPA (RT 9.995min)
NDIPA (RT 10.853 min)
NMPA (RT 11.130 min)
NDPA (RT 11.302 min)
NDBA (RT 13.526 min)
NMBA (RT 7.311 min)
Metformin (RT 2.755min)

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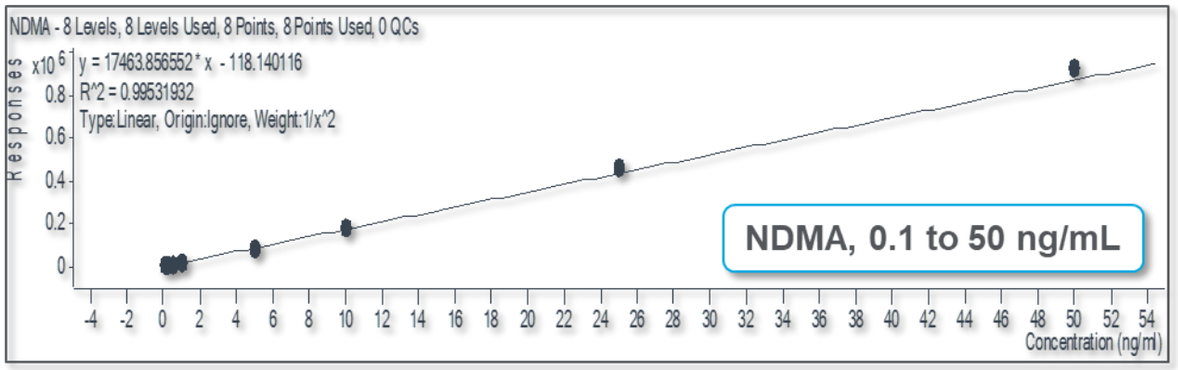
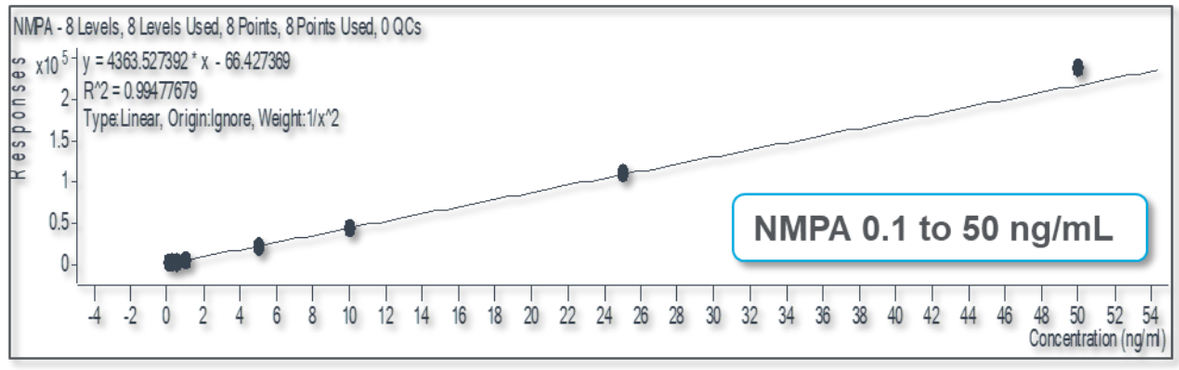
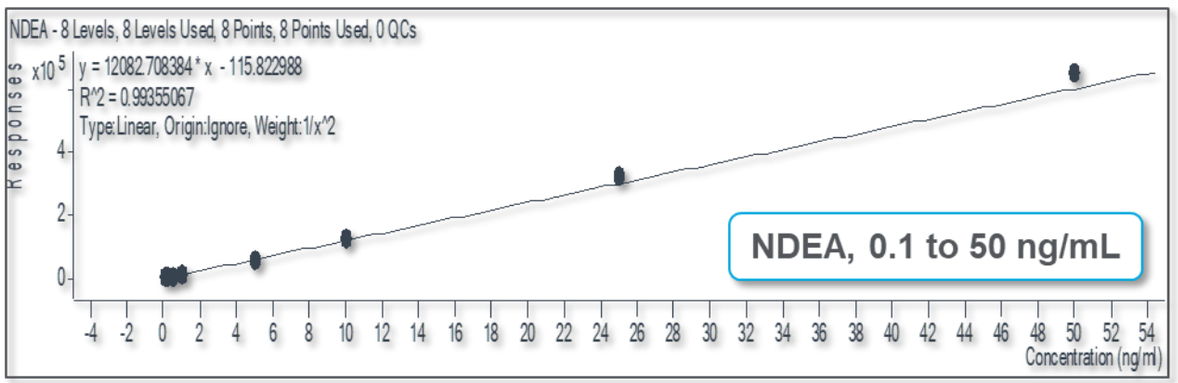
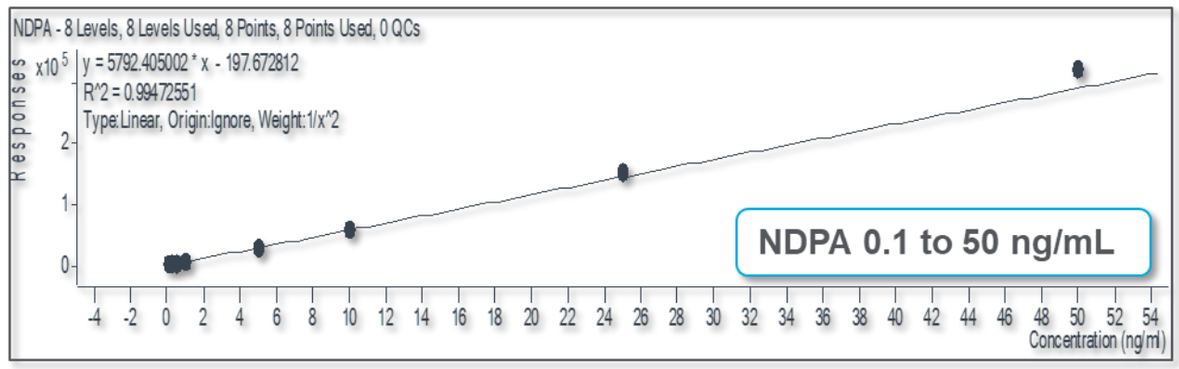
Metformin

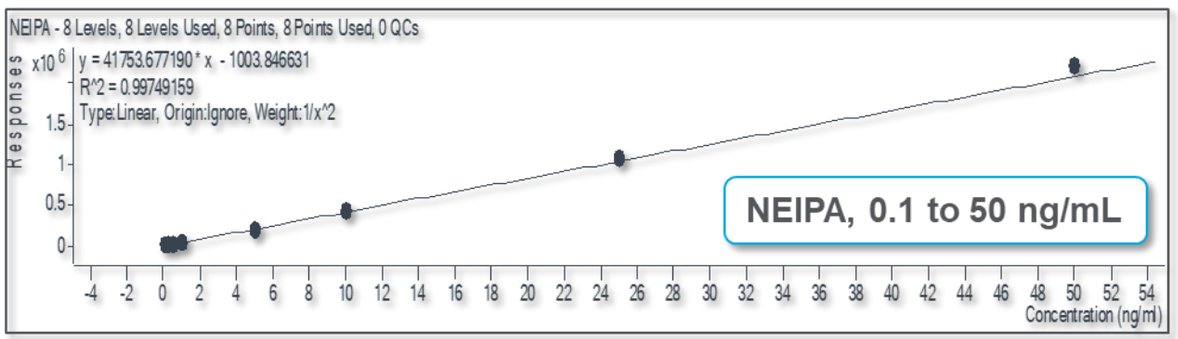
Calibration Curves

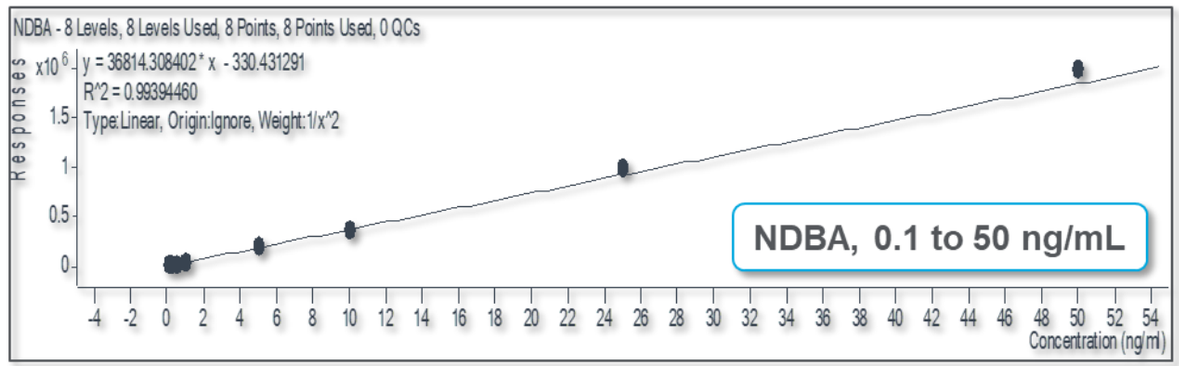


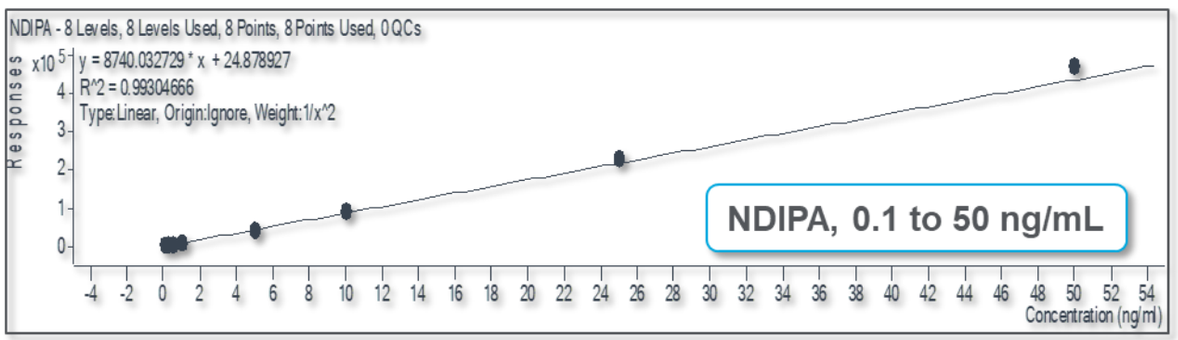


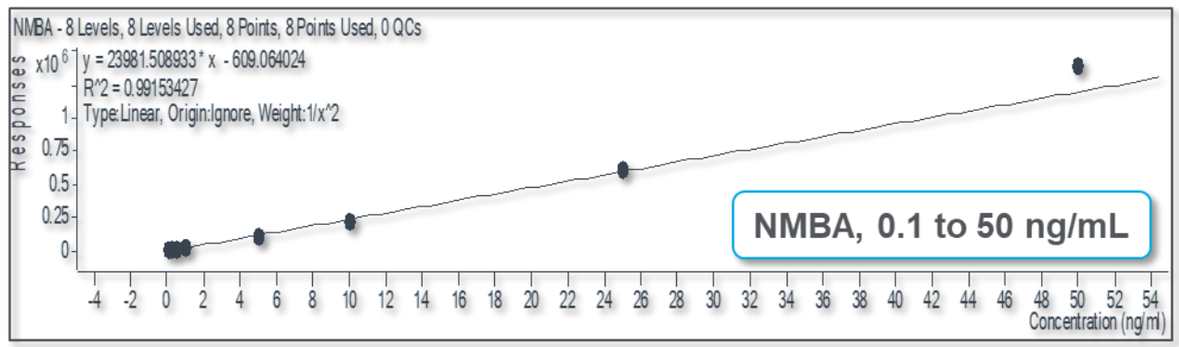












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Representative Recovery % of Nitrosamine Impurities

@ 0.5ng/mL (0.025ppm) concentration using 20mg/mL sample size

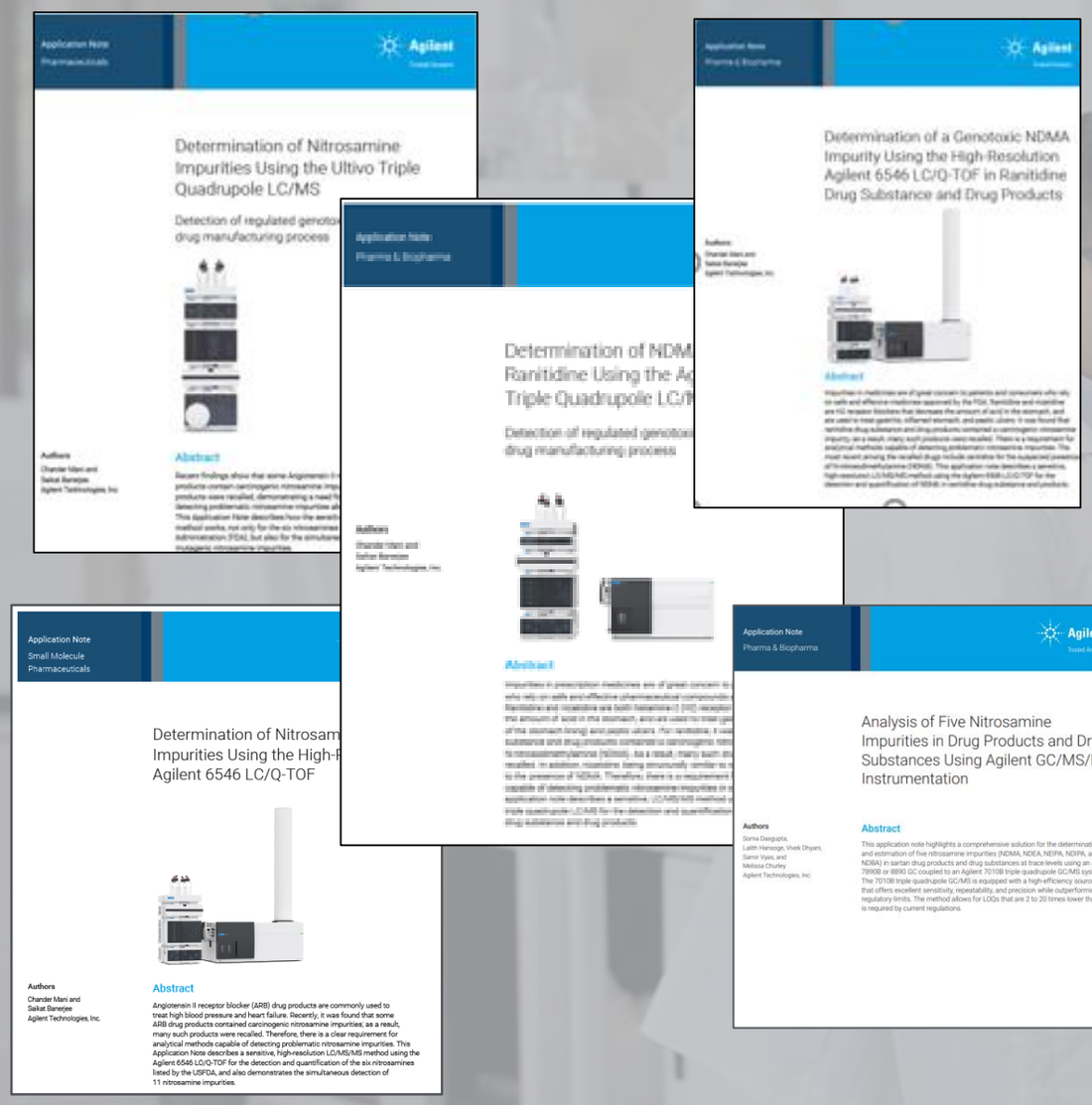
Entry	Nitrosamine Impurities	Average Recovery %
1	NDMA	101.2
2	NMBA	97.1
3	NDEA	98.4
4	NEIPA	94.9
5	NDIPA	102.6
6	NMPA	101.5
7	NDPA	95.8
8	NDBA	102

Note: Use of corresponding internal standards for each nitrosamines may further help in any recovery issue.

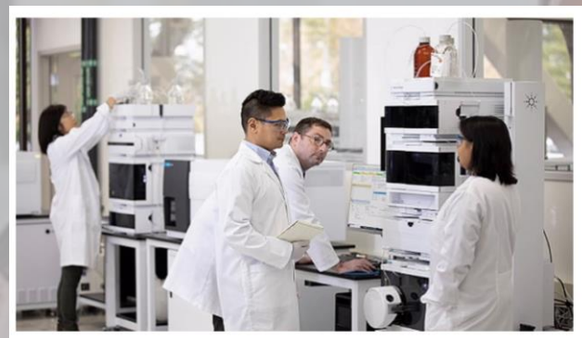
Benefits of Agilent LC/TQ	
Optimized methods	• Optimized method for metformin drug substance • Detect and quantify nitrosamine impurities limits per published FDA regulatory testing method guidance
Scalable application	• Best precision = best ion ratios = best quant results Rugged ion source design
Sample prep	• Sample preparation as per EDQM guidelines • Easy sample preparation
Time and costs	• Automated tuning, easy to use instrument. • Efficient Quant review with MassHunter • Data Integrity

Learn More

Application Notes



On-Demand Webinars



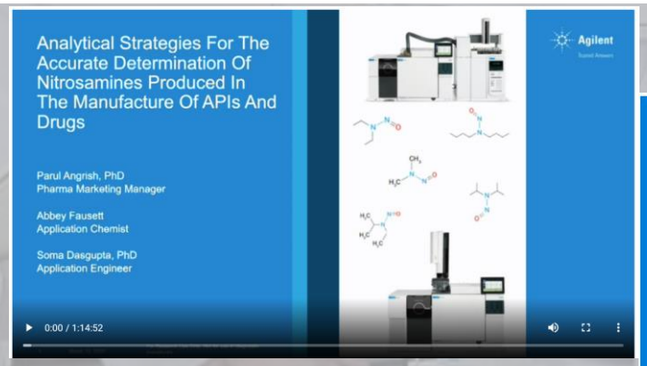
On-Demand Webinar

LC/MS Based Approaches for the Analysis of Nitrosamines in APIs and drugs

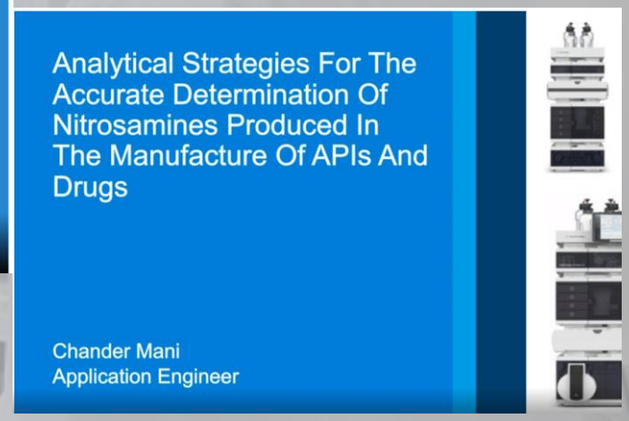
The U.S. FDA published analytical testing methods to provide options for regulators and industry to detect nitrosamine impurities in ranitidine drug substances and products. In this webinar, you will learn about the latest regulations for the evaluation of nitrosamines and the relevant LC/MS based analytical strategies for the accurate identification and quantification of nitrosamines in APIs and drug products.

Watch Video

[GC/MS methods for determination of nitrosamines produced in the manufacture of APIs and drugs]



[LC/MS methods for determination of nitrosamines produced in the manufacture of APIs and drugs]



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