Nitrosamine impurities analysis using Thermo Scientific™ Orbitrap Exploris™ 120 MS

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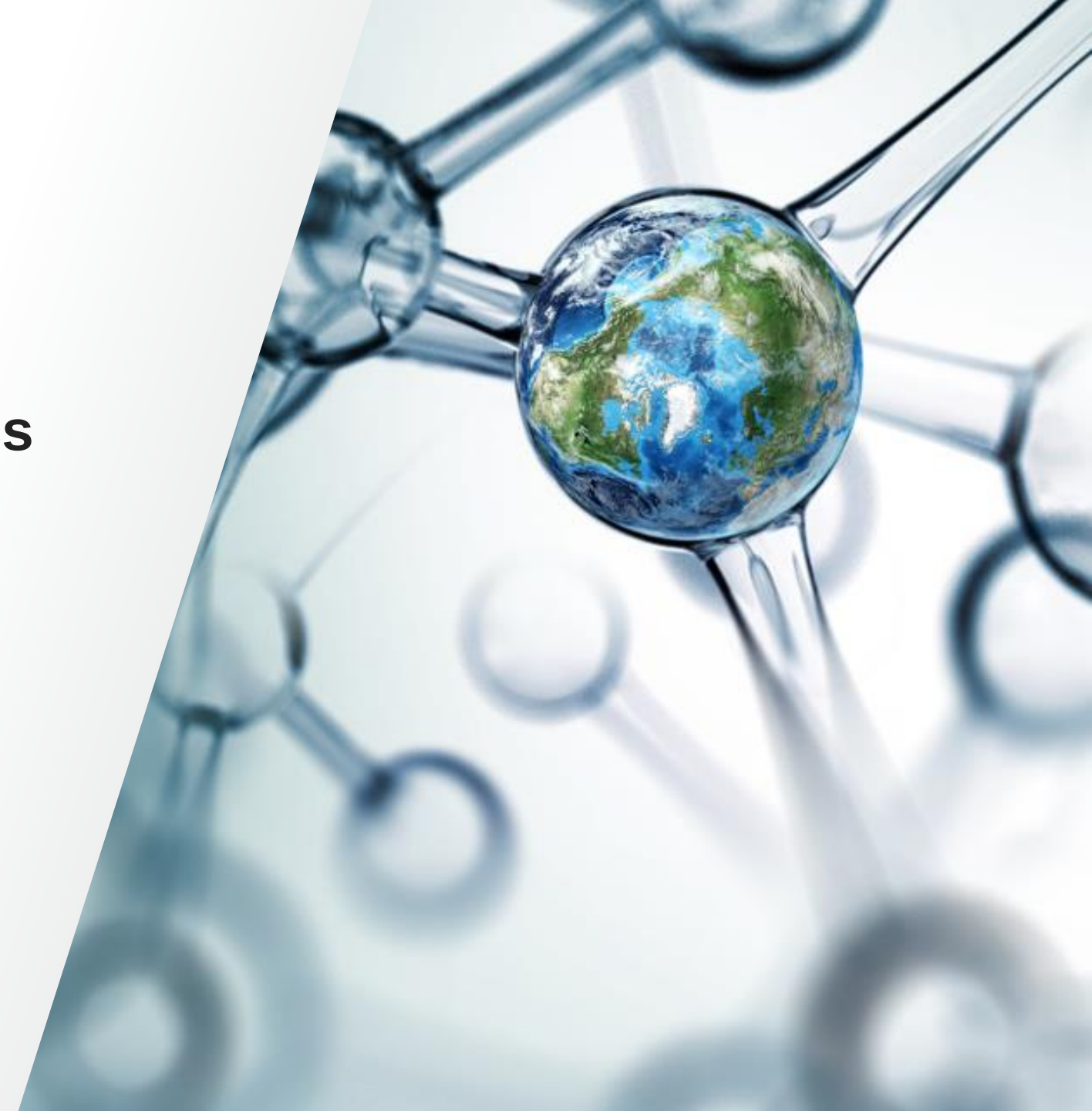

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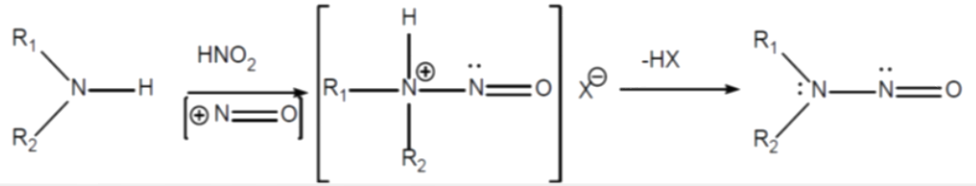
Application: Nitrosamine impurities analysis

Liquid chromatography high resolution mass spectrometry (LC-HRMS) method for NAs detection and quantitation

- Nitrosamines (NAs) in APIs and drug products
  - *Classified as genotoxic impurities, proven as probable human carcinogen*
  - *Detected elevated levels of NDMA and other impurities in several drug products, announced several recalls*
  - *US FDA published validated GC/LC-MS/MS and GC/LC-HRAM methods, and interim acceptable limits for several NAs*
  - *US FDA recently released guidance for industry to 1) conduct risk assessments on approved or marketed drugs and pending applications, and 2) implement control strategy to reduce and prevent formation of NAs in* ***all API and drug products***

- Goals
  - 1 single LC-HRMS method for detection and quantitation of 9 NAs
  - High selectivity and sensitivity
  - Robustness and reproducibility
  - Compliant software for data collection and processing

Figure 1. Representative Reaction to Form Nitrosamines



Application: Nitrosamine impurities analysis

- **Thermo Scientific™ Vanquish™ LC and Acclaim Polar Advantage II column, 2.1 x 100mm, 2.2µm**
  - Excellent retention time and injection reproducibility
  - Good peak shape for target NAs
  - No carryover for target NAs, except NDBA

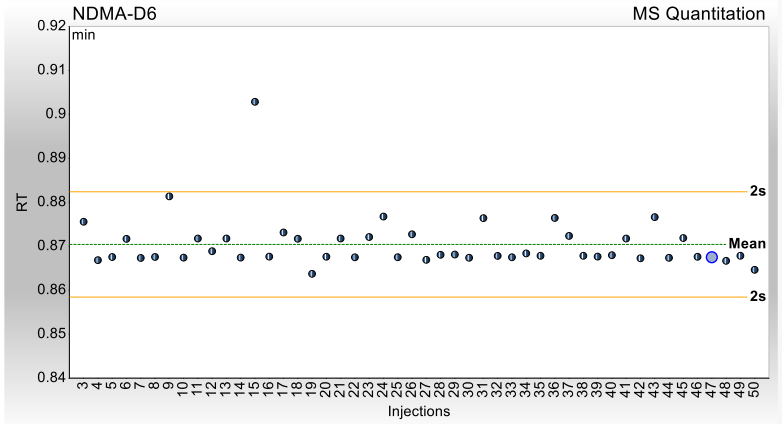
- **Thermo Scientific™ Orbitrap Exploris™ 120 MS**
  - Max 120K resolution at *m/z* 200
  - Sub ppm mass accuracy with EASY-IC
  - LLOQ ≤ 0.017 ppm for target NAs, in APCI mode for both neat and excipient standards

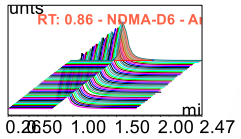
- **Thermo Scientific™ Chromeleon™ CDS 7.2.10**
  - Single platform for MS and chromatography
  - Fully integrated with instrument control, data collection, processing and reporting
  - 21 CFR Part 11 compliant ready with full instrument and data audit trail

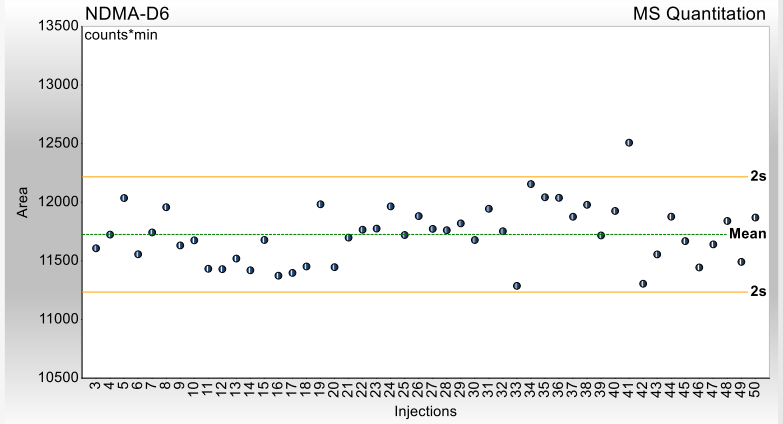
Application: Nitrosamine impurities analysis

Robust and reproducible injections

- **Vanquish LC and Acclaim Polar Advantage II column, 2.1 x 100mm, 2.2µm**
  - Excellent retention time and injection reproducibility
  - Good peak shape across all NAs
  - No carryover for target NAs, except NDBA







N = 50, Neat, APCI

Average RT: 0.87 min

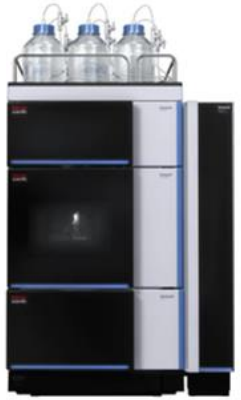
RT reproducibility: 0.69%

Average Peak area: 11700

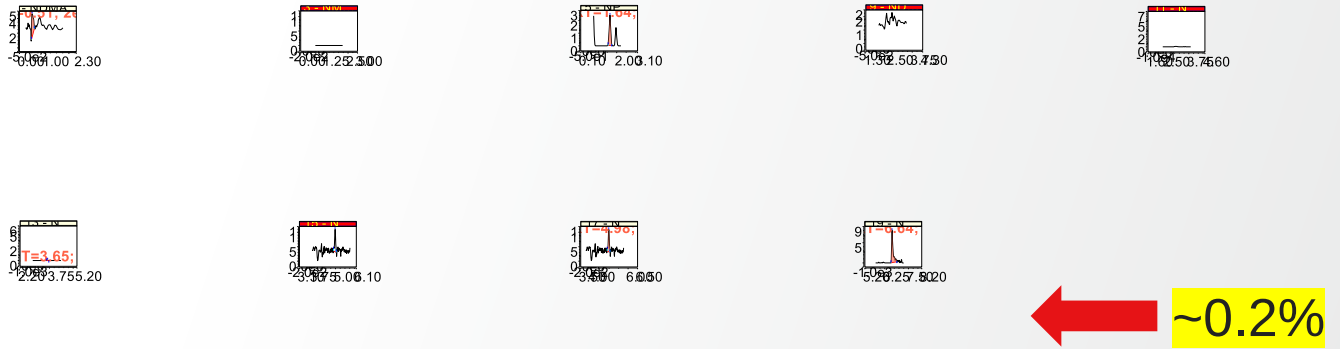
Peak area reproducibility: 2.1%

Application: Nitrosamine impurities analysis

Good separation with minimal carryover for excipient standards



- **Vanquish LC and Acclaim Polar Advantage II column, 2.1 x 100mm, 2.2µm**
  - Excellent retention time and injection reproducibility
  - Good peak shape across all NAs
  - Minimum carryover for target NAs, except NDBA



~0.2%

Carryover, Excipient, APCI

XIC of NAs at 50ng/ml, Excipient, APCI

Application: Nitrosamine impurities analysis

High sensitivity, could achieve LLOQ ≤ 0.017 ppm for target NAs in both neat and excipient matrix

- **Orbitrap Exploris 120 MS**
  - Max 120K resolution at *m/z* 200
  - Sub ppm mass accuracy with Thermo Scientific™ EASY-IC™ ion source
  - LLOQ ≤ 0.017 ppm for target NAs, in APCI mode for both neat and excipient standards

	Matrix	LOD (ng/ml)	LLOQ (ng/ml)	PPM*	Linearity
NDMA	Neat	0.2	0.2	0.0068	0.2 – 50
	Excipient	0.2	0.2	0.0068	0.2 – 50
NMEA	Neat	0.2	0.2	0.0068	0.2 – 50
	Excipient	0.2	0.2	0.0068	0.2 – 50
NPYR	Neat	0.1	0.2	0.0068	0.2 – 50
	Excipient	0.2	0.2	0.0068	0.2 – 50
NDEA	Neat	0.1	0.1	0.0034	0.1 – 50
	Excipient	0.1	0.1	0.0034	0.1 – 50
NPIP	Neat	0.1	0.1	0.0034	0.1 – 50
	Excipient	0.2	0.2	0.0034	0.1 – 50
NEIPA	Neat	0.5	0.5	0.017	0.5 – 50
	Excipient	0.5	0.5	0.017	0.5 – 50
NDIPA	Neat	0.1	0.1	0.0034	0.1 – 50
	Excipient	0.1	0.1	0.0034	0.1 – 50
NDPA	Neat	0.1	0.1	0.0034	0.1 – 50
	Excipient	0.1	0.1	0.0034	0.1 – 50
NDBA	Neat	0.1	0.5	0.017	0.5 – 50
	Excipient	0.1	0.5	0.017	0.5 – 50

* PPM is calculated based on 30mg/ml of drug substance and product extract

Application: Nitrosamine impurities analysis

Accuracy and precision for excipient standards at 0.017 ppm

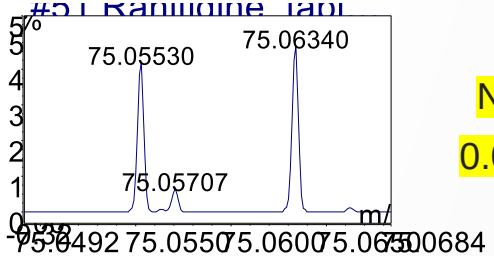
- **Orbitrap Exploris 120 MS**
  - Max 120K resolution at *m/z* 200
  - Sub ppm mass accuracy with EASY-IC ion source
  - LLOQ ≤ 0.017 ppm for target NAs, in APCI mode for both neat and excipient standards

	% Diff	Precision
NDMA	0.7	7.0
NMEA	-5.2	4.2
NPYR	-7.7	2.7
NDEA	-5.4	3.4
NPIP	-2.2	5.1
NEIPA	-6.7	7.8
NDIPA	-3.8	2.9
NDPA	-4.3	3.0
NDBA	-3.3	4.1

* Accuracy and precision based on 5 replicate injections, data were processed by linear with offset, 1/x weighting

Application: Nitrosamine impurities analysis

High selectivity can be achieved with maximum 120K resolution and sub ppm mass accuracy



NDMA

0.0 ppm

DMF, 13C isotope

0.0 ppm

DMF, 15N isotope

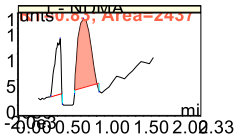
0.4 ppm

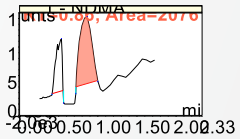
- **Orbitrap Exploris 120 MS**
  - Max 120K resolution at *m/z* 200
  - Sub ppm mass accuracy with EASY-IC ion source
  - LLOQ ≤ 0.017 ppm for target NAs, in APCI mode for both neat and excipient standards

N,N-dimethylformamide (DMF)15N isotope and NDMA are baseline resolved at 120K resolution

Application: Nitrosamine impurities analysis

High selectivity can be achieved with maximum 120K resolution and sub ppm mass accuracy





- **Orbitrap Exploris 120 MS**
  - Max 120K resolution at *m/z* 200
  - Sub ppm mass accuracy with EASY-IC ion source
  - LLOQ ≤ 0.017 ppm for target NAs, in APCI mode for both neat and excipient standards

NDMA with 25ppm mass tolerance

Measured: 2.8 ng/ml or 0.093ppm

NDMA with 3ppm mass tolerance

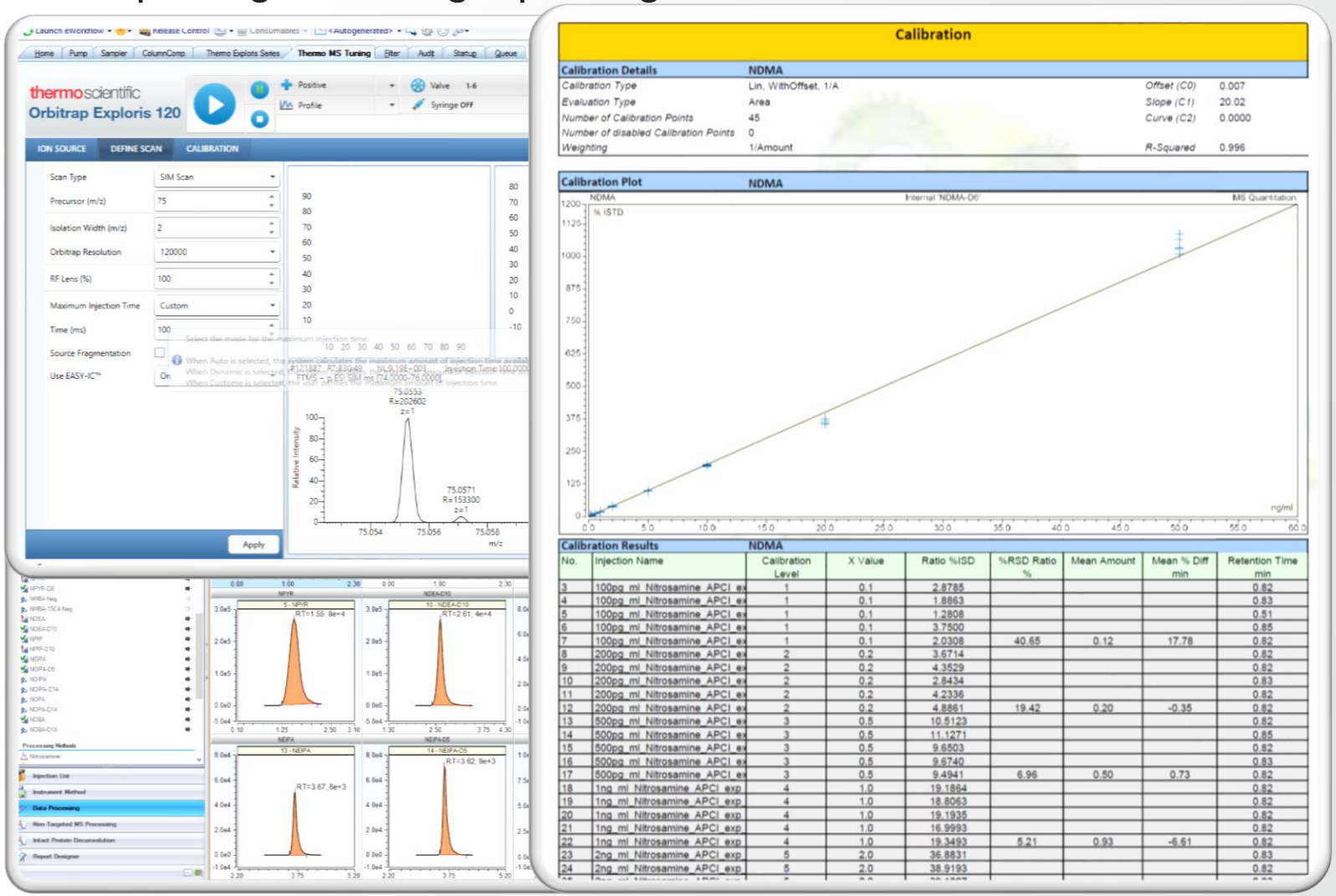
Measured: 2.4 ng/ml or 0.08ppm

With inadequate mass tolerance setting, the presence of DMF in Ranitidine tablet extract could cause overestimation of NDMA

Application: Nitrosamine impurities analysis

Instrument control, data processing, and reporting all in single package

- **Chromeleon CDS 7.2.10**
  - Single platform for MS and chromatography
  - Fully integrated with instrument control, data collection, processing and reporting
  - 21 CFR Part 11 compliant ready with full instrument and data audit trail



No.	Injection Name	Calibration Level	X Value	Ratio %ISD	%RSD Ratio %	Mean Amount	Mean % Diff min	Retention Time min
3	100pg_ml_Nitrosamine_APCI_ex	1	0.1	2.8785				0.82
4	100pg_ml_Nitrosamine_APCI_ex	1	0.1	1.8863				0.83
5	100pg_ml_Nitrosamine_APCI_ex	1	0.1	1.2808				0.51
6	100pg_ml_Nitrosamine_APCI_ex	1	0.1	3.7500				0.85
7	100pg_ml_Nitrosamine_APCI_ex	1	0.1	2.0308	40.65	0.12	17.78	0.82
8	200pg_ml_Nitrosamine_APCI_ex	2	0.2	3.6714				0.82
9	200pg_ml_Nitrosamine_APCI_ex	2	0.2	4.3529				0.82
10	200pg_ml_Nitrosamine_APCI_ex	2	0.2	2.8434				0.83
11	200pg_ml_Nitrosamine_APCI_ex	2	0.2	4.2336				0.82
12	200pg_ml_Nitrosamine_APCI_ex	2	0.2	4.8861	19.42	0.20	-0.35	0.82
13	500pg_ml_Nitrosamine_APCI_ex	3	0.5	10.5123				0.82
14	500pg_ml_Nitrosamine_APCI_ex	3	0.5	11.1271				0.85
15	500pg_ml_Nitrosamine_APCI_ex	3	0.5	9.6503				0.82
16	500pg_ml_Nitrosamine_APCI_ex	3	0.5	9.6740				0.83
17	500pg_ml_Nitrosamine_APCI_ex	3	0.5	9.4941	6.96	0.50	0.73	0.82
18	1ng_ml_Nitrosamine_APCI_exp	4	1.0	19.1864				0.82
19	1ng_ml_Nitrosamine_APCI_exp	4	1.0	18.8063				0.82
20	1ng_ml_Nitrosamine_APCI_exp	4	1.0	19.1935				0.82
21	1ng_ml_Nitrosamine_APCI_exp	4	1.0	16.9993				0.82
22	1ng_ml_Nitrosamine_APCI_exp	4	1.0	19.3493	5.21	0.93	-6.61	0.82
23	2ng_ml_Nitrosamine_APCI_exp	5	2.0	36.8831				0.83
24	2ng_ml_Nitrosamine_APCI_exp	5	2.0	38.9193				0.82

A complete Thermo Fisher Scientific solution

- A robust and reproducible LC-HRMS method for detection and quantitation of 9 NAs in Ranitidine tablet
- High resolution, minimum of 60K, is required to separate DMF isotope interferent peaks to avoid potential isobaric interferences
- Adequate mass accuracy and tolerance setting is essential for reliable and accurate quantitation of NDMA in presence of co-eluting DMF in tablet extract
- Achieved superior sensitivity with APCI, LLOQ ≤ 0.017 ppm for all NAs tested (FDA proposed limit is 0.03 ppm)
- Demonstrated the use of Chromeleon 7.2.10 compliant ready CDS software for instrument control, data acquisition, processing, and reporting

Backup slides

- Recovery and reproducibility of NAs from extraction process
- LLOQ of NAs using HESI mode for both neat and excipient standards
- LLOQ comparison between HESI vs APCI mode for target NAs

Application: Nitrosamine impurities analysis

NAs recovery and reproducibility from the extraction process

	2ng/ml		5ng/ml	
	% Recovery	% RSD	% Recovery	% RSD
NDMA	99	8.4	97	7.2
NMEA	93	9.0	97	4.5
NPYR	101	3.6	99	5.3
NMBA	94	13.3	94	8.8
NDEA	95	3.0	97	3.4
NPIP	97	1.6	96	3.8
NEIPA	116	10.5	98	7.0
NDIPA	95	3.1	100	2.9
NDPA	105	2.2	104	1.8
NDBA	101	2.8	100	4.9

Application: Nitrosamine impurities analysis

High sensitivity, LLOQ ≤ 0.068 ppm for target NAs for both neat and excipient standards, HESI mode

- **Orbitrap Exploris 120 MS**
  - Max 120K resolution at *m/z* 200
  - Sub ppm mass accuracy with EASY-IC ion source
  - LLOQ ≤ 0.068 ppm for target NAs in HESI mode for both neat and excipient standards

We can achieve less than 0.017 ppm for all NAs except NEIPA in HESI mode

	Matrix	LOD (ng/ml)	LLOQ (ng/ml)	PPM*	Linearity
NDMA	Neat	0.2	0.5	0.017	0.5 – 50
	Excipient	0.5	0.5	0.017	0.5 – 50
NMEA	Neat	0.5	0.5	0.017	0.5 – 50
	Excipient	0.5	0.5	0.017	0.5 – 50
NPYR	Neat	0.1	0.1	0.0034	0.1 – 50
	Excipient	0.1	0.2	0.0068	0.2 – 50
NDEA	Neat	0.2	0.5	0.017	0.5 – 50
	Excipient	0.5	0.5	0.017	0.5 – 50
NPIP	Neat	0.2	0.2	0.0068	0.2 – 50
	Excipient	0.2	0.2	0.0068	0.2 – 50
NEIPA	Neat	1	2	0.068	2 – 50
	Excipient	2	2	0.068	2 – 50
NDIPA	Neat	0.2	0.5	0.017	0.5 – 50
	Excipient	0.2	0.5	0.017	0.5 – 50
NDPA	Neat	0.2	0.2	0.0068	0.2 – 50
	Excipient	0.2	0.2	0.0068	0.2 – 50
NDBA	Neat	0.1	0.5	0.017	0.5 – 50
	Excipient	0.1	0.5	0.017	0.5 – 50

* PPM is calculated based on 30mg/ml of drug substance and product extract

Application: Nitrosamine impurities analysis

LLOQ comparison between HESI and APCI for all NAs (Neat only)

- **Orbitrap Exploris 120 MS**
  - Max 120K resolution at *m/z* 200
  - Sub ppm mass accuracy with EASY-IC ion source
  - Superior sensitivity can be achieved using both HESI and APCI mode

	Mode	LOD (ng/ml)	LLOQ (ng/ml)	PPM*	Factor
NDMA	HESI	0.2	0.5	0.017	-
	APCI	0.2	0.2	0.0068	**2.5x**
NMEA	HESI	0.5	0.5	0.017	-
	APCI	0.2	0.2	0.0068	**2.5x**
NPYR	HESI	0.1	0.1	0.0034	-
	APCI	0.1	0.2	0.0068	**1/2x**
NDEA	HESI	0.2	0.5	0.017	-
	APCI	0.1	0.1	0.0034	**5x**
NPIP	HESI	0.2	0.2	0.0068	-
	APCI	0.1	0.1	0.0034	**2x**
NEIPA	HESI	1	2	0.068	-
	APCI	0.5	0.5	0.017	**4x**
NDIPA	HESI	0.2	0.5	0.017	-
	APCI	0.1	0.1	0.0034	**5x**
NDPA	HESI	0.2	0.2	0.0068	-
	APCI	0.1	0.1	0.0034	**2x**
NDBA	HESI	0.1	0.5	0.017	-
	APCI	0.1	0.5	0.017	**1x**

* PPM is calculated based on 30mg/ml of drug substance and product extract