ANALYSIS OF IMPURITIES IN DRUG PRODUCTS

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1. Impurity Overview
2. Mutagenic Impurity: Regulatory Updates
3. Impurity Analysis: Solutions
4. Nitrosamines and Azido Impurity Case Study
5. Key Takeaways

- **Impurity -** Any component of the drug substance or drug product that is not the chemical entity defined as the *drug substance, an excipient, or other additives to the drug product*
- **Classification of impurities**
  - **Organic impurities** may arise during the manufacturing process and/or storage of drug substance or drug product
    - ✓Starting materials; By-products
    - ✓Intermediates; Degradation products
  - **Inorganic impurities** can result from the manufacturing process
    - ✓Reagents, ligands and catalysts
    - ✓Heavy metals or other residual metals
    - ✓Inorganic salts
  - **Residual solvents** are inorganic or organic liquids used as vehicles for the preparation of solutions or suspensions in the synthesis of drug substance
- **Impurities with 'unusual toxicity'**
  - Some impurities may cause adverse reactions to the patients at very low levels
  - Carcinogenic/mutagenic impurities have potential to react with DNA, causing a carcinogenic response & cancer

Development of drug product

Synthesis
Drug Substance/API
Starting materials & reagents

Formulation & Manufacturing
API + Inactive Ingredients

Drug Products
Storage & packaging

Impurities in Drug substance

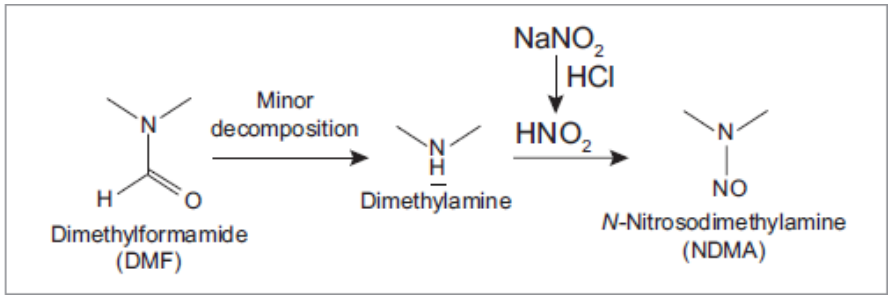
- Process impurities can develop during the synthesis
  - Intermediates
  - Reactants
  - By-products
- Degradation products may form during storage

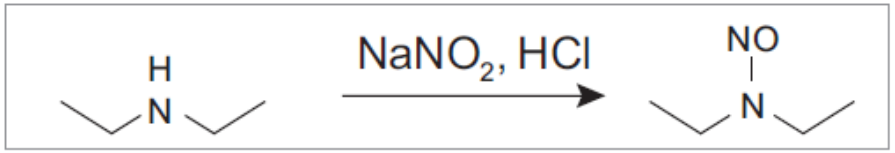
Impurities in Drug product

- May form during the formulation of the dosage forms
  - Degradation products of a drug substance
  - Reaction products of a drug substance with excipients or container closure
- Degradation products can develop during storage & aging

Nitrosamines Regulatory Updates

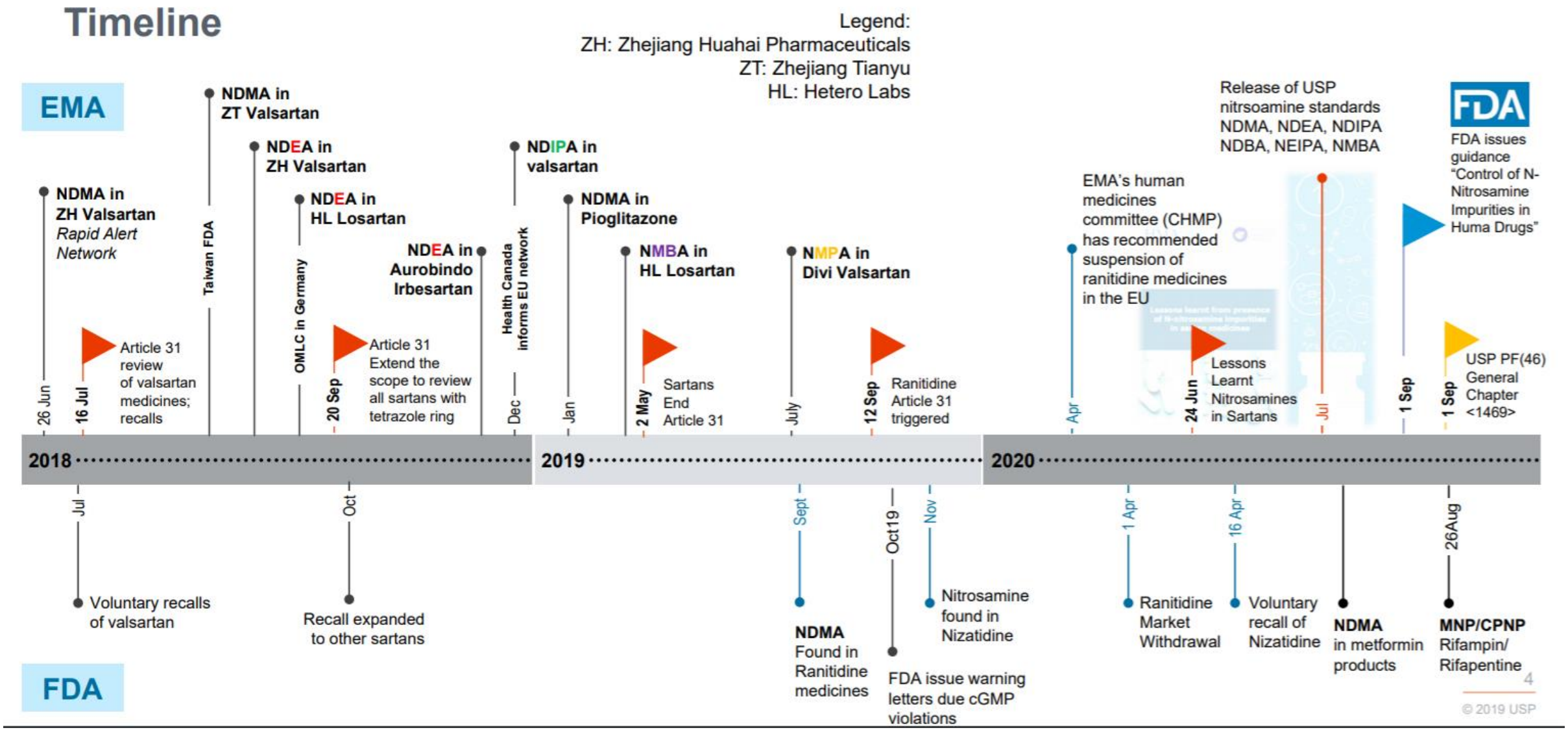
- World Health Organization (WHO) classifies as “probable human carcinogens”
- Reports of nitrosamine impurities (NDMA, NDEA) in commercialized medicines began in **mid-2018**
- Dimethylformamide (DMF) was introduced **to decrease manufacturing costs** of the API, Valsartan.
- **Nitrosamine carcinogens** (known genotoxic impurities - GTIs)*, **were then detected** by companies who purchased the API and **traced to the new manufacturing process**.



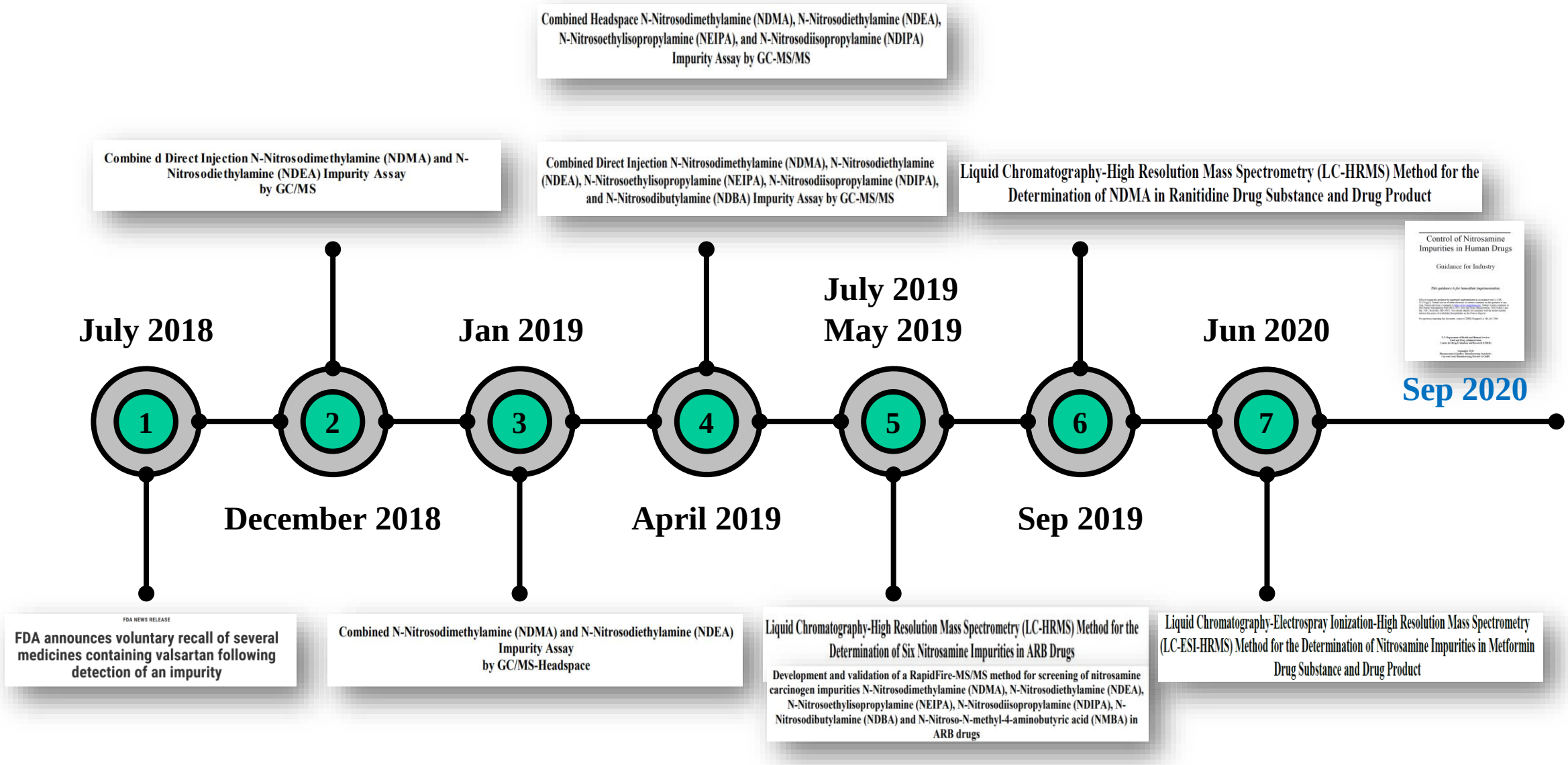


- **Process related impurities** of DMA or DEA (secondary amines) react with $NaNO_2$ (nitrosating agent) to potentially form N-nitrosamines impurities in drug substance
- NDMA later found in **recycled solvents** for sartans synthesis, **unrelated API** such as Raniditine (could be product degradation) and Metformin suggest wider spread of NDMA contamination beyond just process impurities related to sartans product family

❑ General Chapter <1469> “Nitrosamines Impurities” by USP became official on Dec. 2, 2021



❑Timeline


Combined Headspace N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), and N-Nitrosodiisopropylamine (NDIPA) Impurity Assay by GC-MS/MS
Combine d Direct Injection N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS
Combined Direct Injection N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA) Impurity Assay by GC-MS/MS
Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of NDMA in Ranitidine Drug Substance and Drug Product
Control of Nitrosamine Impurities in Human Drugs
Guidance for Industry
Sep 2020
July 2018
Jan 2019
July 2019
May 2019
Jun 2020
1
2
3
4
5
6
7
December 2018
April 2019
Sep 2019
FDA NEWS RELEASE
FDA announces voluntary recall of several medicines containing valsartan following detection of an impurity
Combined N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS-Headspace
Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs
Development and validation of a RapidFire-MS/MS method for screening of nitrosamine carcinogen impurities N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosodibutylamine (NDBA) and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA) in ARB drugs
Liquid Chromatography-Electrospray Ionization-High Resolution Mass Spectrometry (LC-ESI-HRMS) Method for the Determination of Nitrosamine Impurities in Metformin Drug Substance and Drug Product

FDA Method	Method	Technique	Drug product	Compounds
117843	Combined headspace method	GC-MS	Valsartan	NDMA, NDEA
117807	Combined direct injection method	GC-MS/MS	Valsartan	NDMA, NDEA
123409			Valsartan	NDMA, NDEA, NDIPA, NEIPA, NDBA
124025	Headspace GC-MS/MS method	GC-MS/MS	Valsartan	NDMA, NDEA, NDIPA, NEIPA
125477	Rapid Fire-MS/MS method	LC-MS/MS	Losartan	NDMA, NDEA, NEIPA, NDIPA, NDBA, NMBA
125478	LC-HRMS method	LC-HRMS	Losartan	NDMA, NDEA, NEIPA, NDIPA, NDBA, NMBA
130801			Ranitidine	NDMA
131868	LC-MS/MS method	LC-MS/MS	Ranitidine	NDMA
138617	LC-HRMS method	LC-HRMS	Metformin	NDMA, NDEA, NEIPA, NDIPA, NDPA, NDBA, NMBA, NMPA,

Control of Nitrosamine Impurities in Human Drugs

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

February 2021
Pharmaceutical Quality/ Manufacturing Standards/
Current Good Manufacturing Practice (CGMP)

Revision 1

AI limits of common N-Nitrosamines align with limit given by EMA

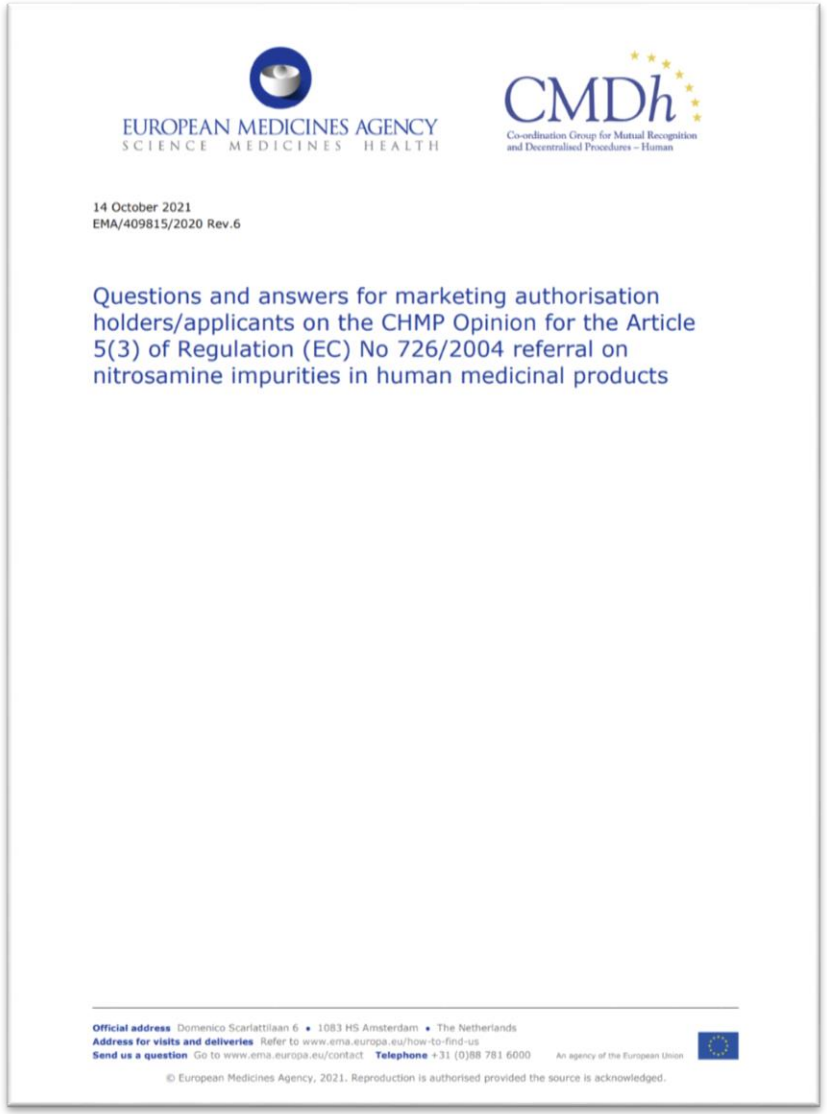
Table 1. AI Limits for NDMA, NDEA, NMBA, NMPA, NIPEA, and NDIPA in Drug Products

Nitrosamine	AI Limit (ng/day)[1,2]
NDMA	96
NDEA	26.5
NMBA	96
NMPA	26.5
NIPEA	26.5
NDIPA	26.5

[1] The AI limit is a daily exposure to a compound such as NDMA, NDEA, NMBA,NMPA, NIPEA, or NDIPA that approximates a 1:100,000 cancer risk after 70 years of exposure. Appendix B includes a description of the AI derivation for NDMA, which is an example of how FDA applied ICH M7(R1) to set a limit.
[2] The conversion of AI limit into ppm varies by product and is calculated based on a drug's maximum daily dose (MDD) as reflected in the drug label (ppm = AI (ng)/MDD (mg)).

FDA U.S. FOOD & DRUG ADMINISTRATION

❑ Questions and Answers for Marketing Authorization Holders



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

CMDh
Co-ordination Group for Mutual Recognition and Decentralised Procedures – Human

14 October 2021
EMA/409815/2020 Rev.6

Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products

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Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

These limits are applicable only if a FP contains a single N-nitrosamine.

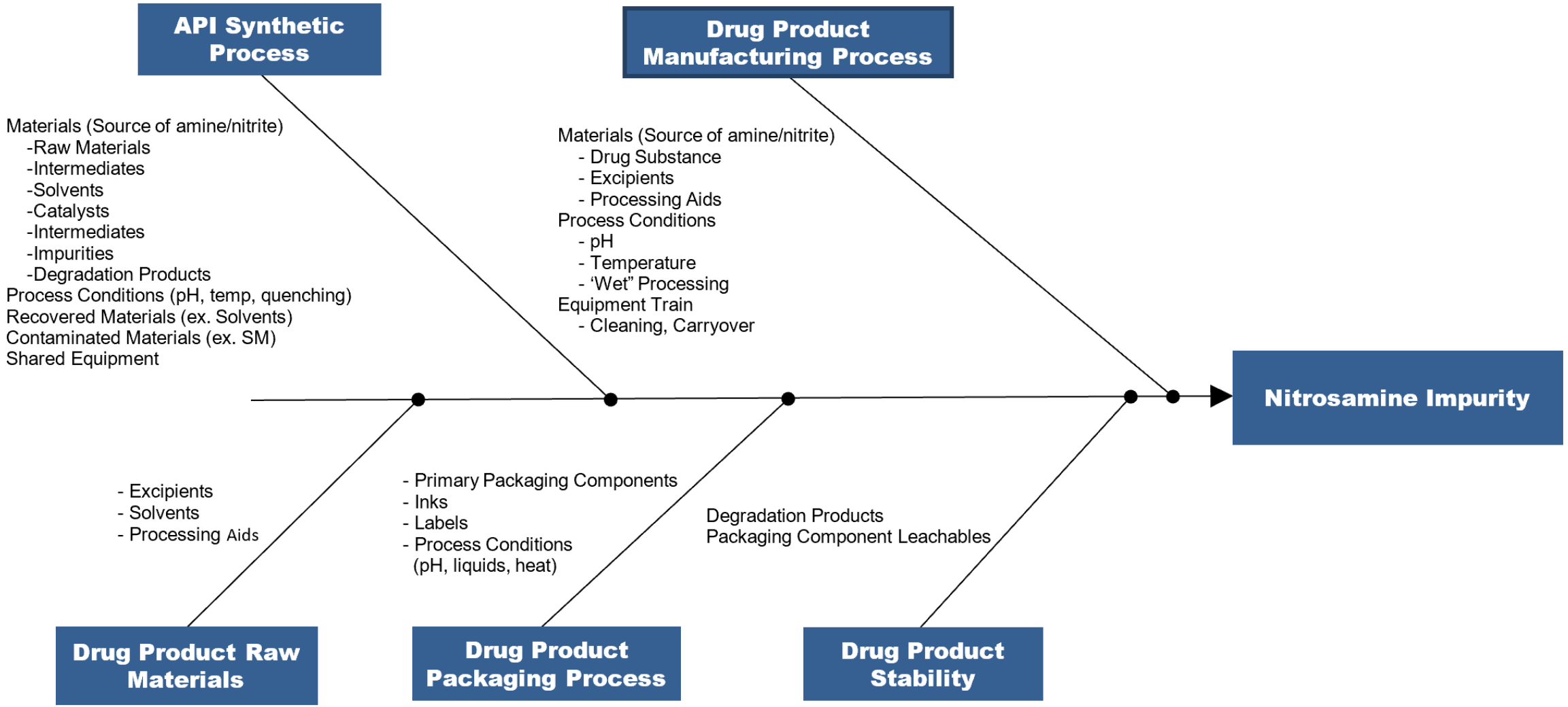
***N*-Nitrosamine (CAS number)**	**ng/day***
N-Nitrosodimethylamine, NDMA[1] (62-75-9)	96.0
N-Nitrosodiethylamine, NDEA[1] (55-18-5)	26.5
N-Nitrosoethylisopropylamine, EIPNA[2] (16339-04-1)	26.5
N-Nitrosodiisopropylamine, DIPNA[2] (601-77-4)	26.5
N-Nitroso-N-methyl-4-aminobutyric acid, NMBA[3] (61445-55-4)	96.0
1-Methyl-4-nitrosopiperazine, MeNP[2] (16339-07-4)	26.5
N-Nitroso-di-n-butylamine, NDBA[2] (924-16-3)	26.5
N-Nitroso-N-methylaniline, NMPA[1] (614-00-6)	34.3
N-nitrosomorpholine, NMOR[4] (59-89-2)	127
N-nitroso-varenicline, NN [5]	37.0

❑PPM Example Calculations

$$\textbf{ppm} = \frac{\text{Acceptable Intake or AI Limit (ng/day)}}{\text{Maximum Daily Dose or MDD (mg/day)}}$$

Medication	MDD (mg)	AI (ng) NDMA	ppm	AI (ng) NDEA	ppm
Valsartan	320	96.0	**0.300**	26.5	**0.083**
Losartan	150	96.0	**0.640**	26.5	**0.177**
Metformin	3000	96.0	**0.032**	26.5	**0.009**
Ranitidine	300	96.0	**0.320**	26.5	**0.088**

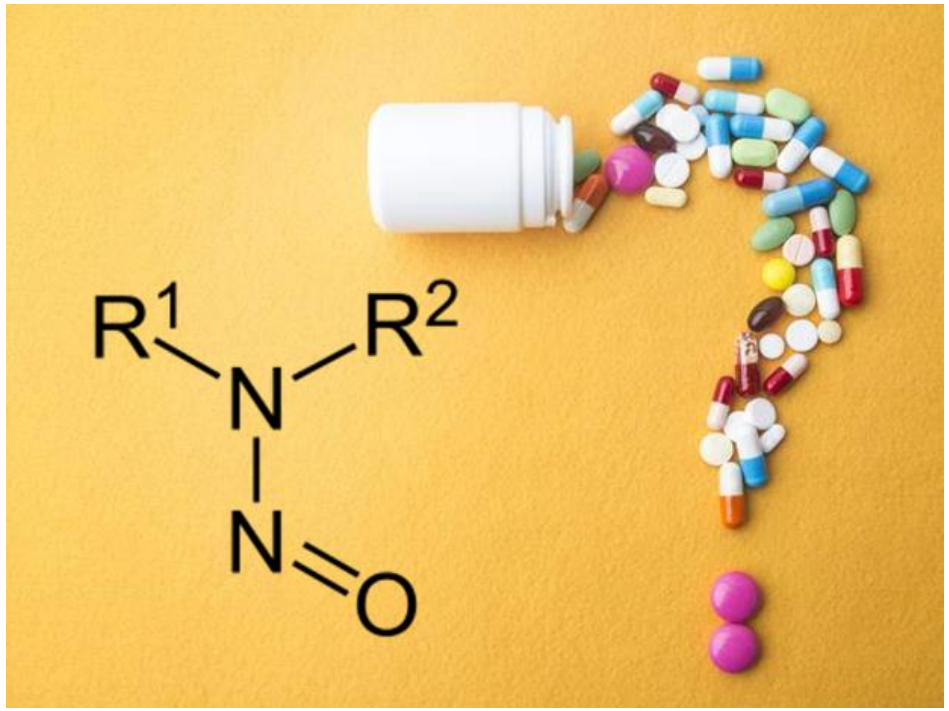
Step 1 : Root Cause Analysis - *Risk Assessment Process*



Step 2 : Confirmatory Testing - *How should confirmatory tests be conducted*

- Testing on Finished product (Testing of the API or its intermediates is also recommended if the risk evaluation indicates that the API or its intermediates are a potential source of nitrosamine impurities in the FP)
- 10% of annual batches or 3 batches (should includes retained sample)
- Methods for determination of various nitrosamines/Appropriately sensitive analytical methods
- LoQ should be at or below the acceptable limit for the respective nitrosamine impurity.
- Interference caused by presence of trace amounts of nitrosamines in testing materials utilised (e.g., water, airborne sources, plastics and rubber/elastomeric products)
- Contamination during sample preparation (avoiding cross contaminations from gloves, membranes, solvents etc.) which could lead to false positive results
- In situ formation of nitrosamines during analysis
- Use of accurate mass techniques are required (MS/MS or high-resolution accurate mass systems) in order to overcome interference in the identification of the specific peak of a certain nitrosamine (e.g., false positives have been observed from DMF co-eluting with NDMA).

- Small MW, from 74 to 158
- Low fragmentation pattern
- High solubility in water and organic solvents
- Highly volatiles (low boiling points)
- Low LOQ to be achieved (10 ppb)
- DS/DP properties (solubility, high concentration…)
- High concentration of DS/excipient in sample to be analyzed
- Non exhaustive list of nitrosamines



❑ Multiple lots of sartan drugs recalled in UK, US, Canada, Korea, Taiwan

UK Regulators Recall Batches of "Sartan" Drug for Azido Impurities

The UK Medicines and Healthcare products Agency (MHRA) is recalling 25

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Multiple lots of irbesartan, losartan and valsartan drugs recalled

Starting date:	May 30, 2021
Posting date:	October 26, 2021
Type of communication:	Advisory
Subcategory:	Drugs
Source of recall:	Health Canada
Issue:	Product Safety
Audience:	General Public
Identification number:	RA-75715

Report a Concern

Last updated: 2021-10-27

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Risk of the presence of mutagenic

Back

nd Drug Safety

home > administration > Ministry of Food and Drug Safety

rtan, valsartan, etc. Sartan

e drugs... Detection of genetic

ng impurities

ed drug products containing 'vareniclin tartrate', a smoking cessation treatment

ty begin safety investigation of both products

om | Registration 2021.06.22 09:28:07 Copy URL

iphenyl]-2yl)-1H-tetrazole) , a property that causes genetic mutations in

sartans such as irbesartan, losartan, and valsartan) and other impurities are

out the wavelength.

한국판뉴딜

식품의약품안전처

보 도 자 료

식약처, 고혈압치료제(사르탄류)와 금연치료보조제(바레니클린) 안전성 조사 실시

관련 성분 의약품 대상 불순물 시험 조속히 진행

□ 식품의약품안전처(처장 김강립)는 고혈압치료제(사르탄류)와 금연치료보조제(바레니클린)에 대한 **안전성 조사**를 **진행** 중이라고 밝혔습니다.

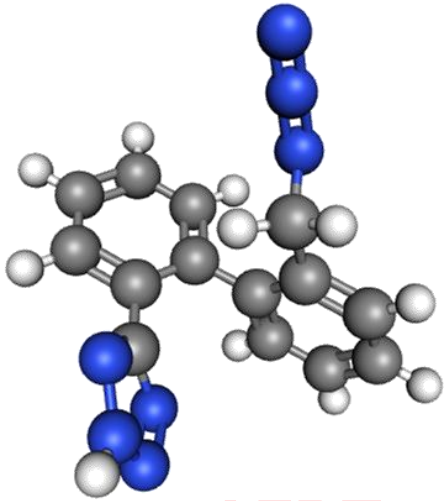
- 이르베사르탄, 로사르탄, 발사르탄

○ 이번 조사는 해외의 관련 성분 의약품에서 잠정관리기준을 초과한 불순물(AZBT, N-nitroso-varenicline, 불임)이 검출됨에 따라 국내 제품의 안전성을 확인하기 위해 실시하게 되었습니다.

□ 식약처는 관련 업체에 신속한 시험검사와 불순물 안전관리에 대한 사전예방조치를 지시했으며, 관련 업체와 긴밀히 협력하여 시험결과를 조속히 얻을 수 있도록 하고 결과에 따라 안전을 최우선으로 하여 필요 조치를 신속히 실시하도록 하겠습니다.

- 1 -

- About AZBT
  - Azidomethyl biphenyl tetrazole (AZBT) occur as a
    - ✓ by-product in the synthesis steps during the production of sartan active substances
  - AZBT is considered mutagenic and should be controlled
    - ✓ at or below the Threshold of Toxicological Concern (TTC)
    - ✓ as outlined in ICH M7 guidelines



AZBT

  - Manufacturer of …sartan drugs need to ensure that impurity is below the TTC. Laboratories need to adopt high sensitive detection methods for identification and quantification of AZBT.
- Europe and North America adopting tougher impurity limits in an attempt to identify and remove possible contaminants. In the UK, the current limits for AZBT (33 ppm for losartan and 66 ppm for irbesartan) are only interim standards and are under discussion by the MHRA's Commission on Human Medicines.
- Korea MFDS Set the allowable daily Intake 1.5 µg/Day

Analytes

- Reactivity/instability
- Volatility
- Polarity
- Detectability

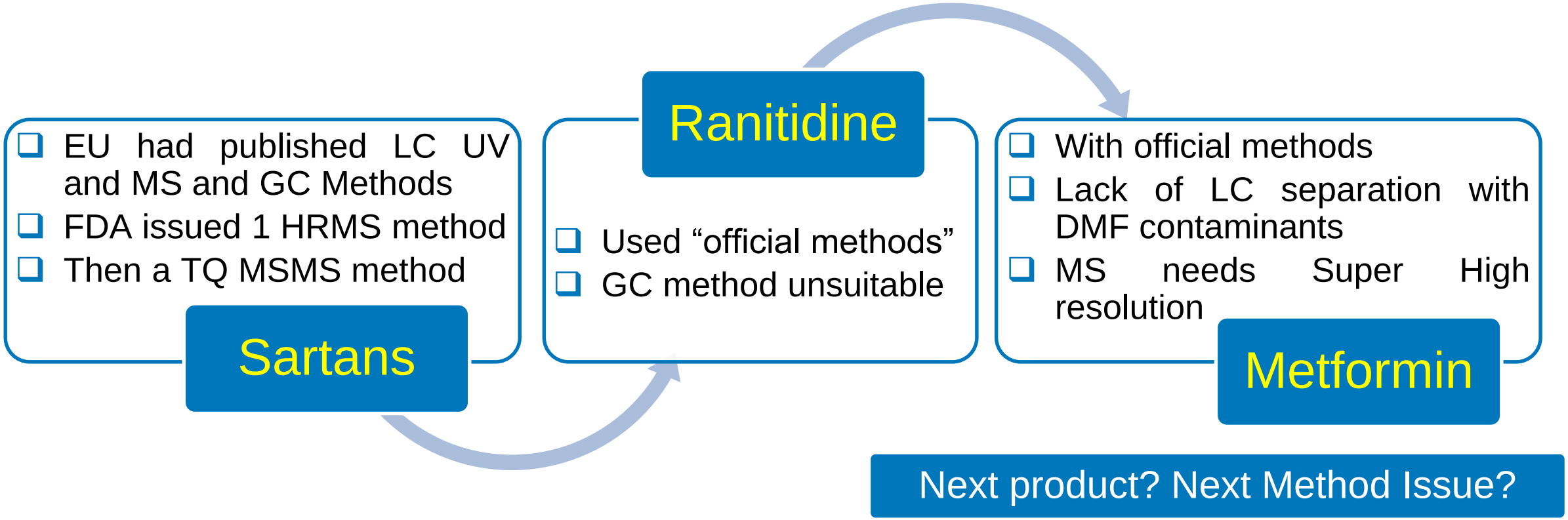
Matrix Effects

- Suppression & enhancement
- High amount of API in sample
- Degradation of API
- Poor recovery

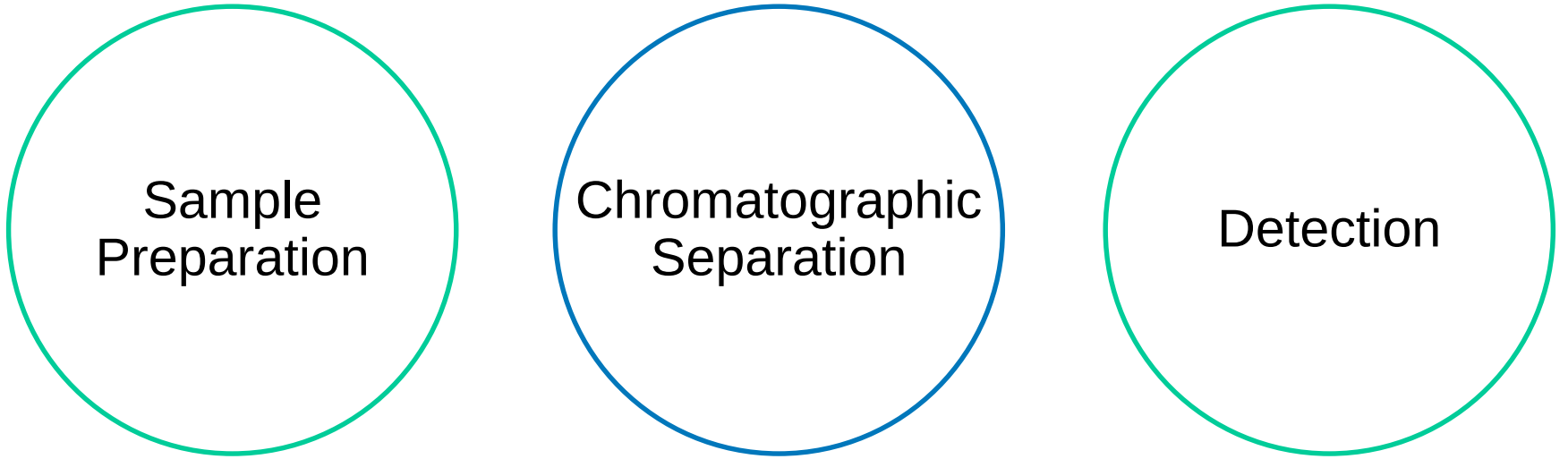
Analytical Methods

- Trace level ppm
- Sophisticated technology
- Limit test/Quantification
- Method validation
- Technology transfer

- With the different Excipients, Solvents, APIs and Drug Products, and the number of Nitrosamines to analyze:
  - there is not one analytical method suitable of doing all the work!
  - is there one analytical system capable?



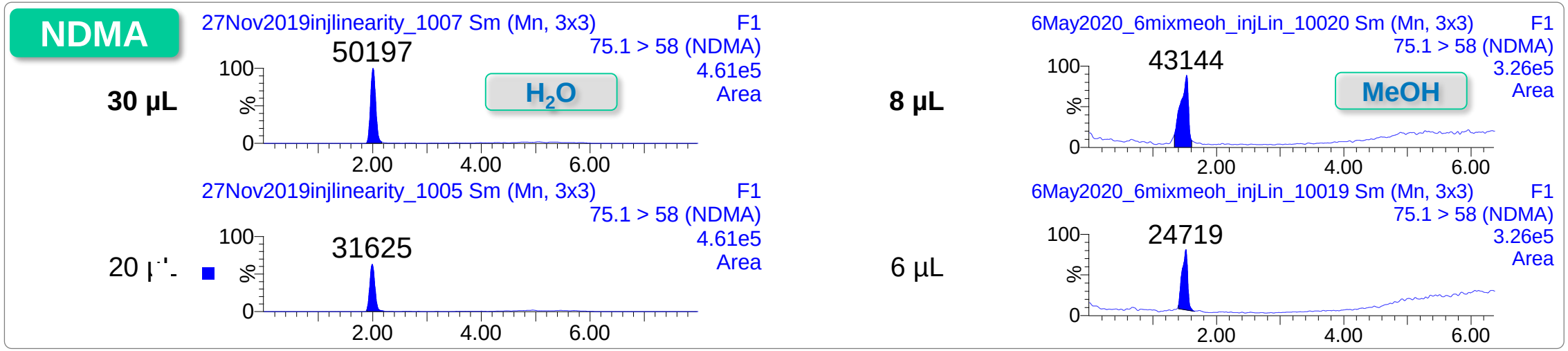
❑ Steps Developed and Optimized for Methods



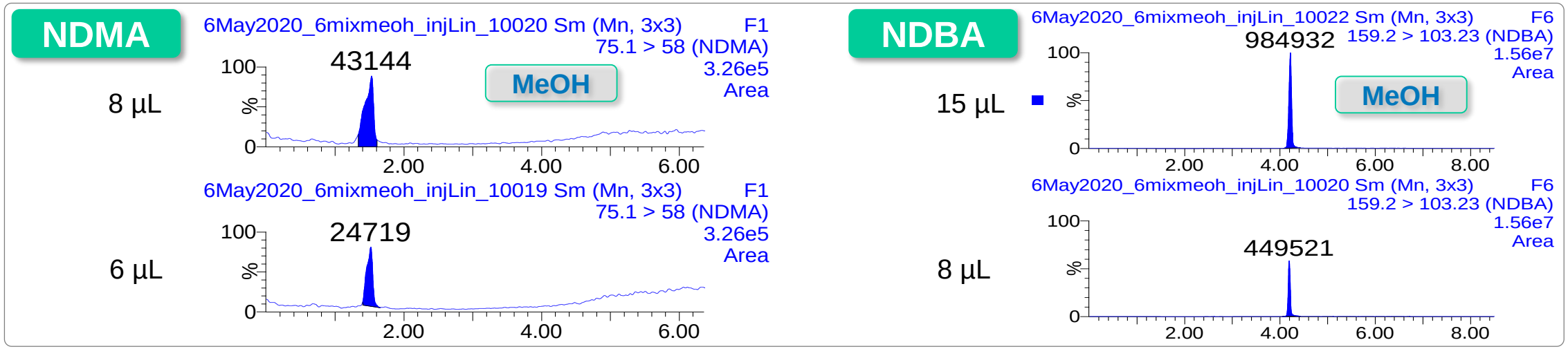
- ❑ Solubility
- ❑ API concentration
- ❑ Sample Handling

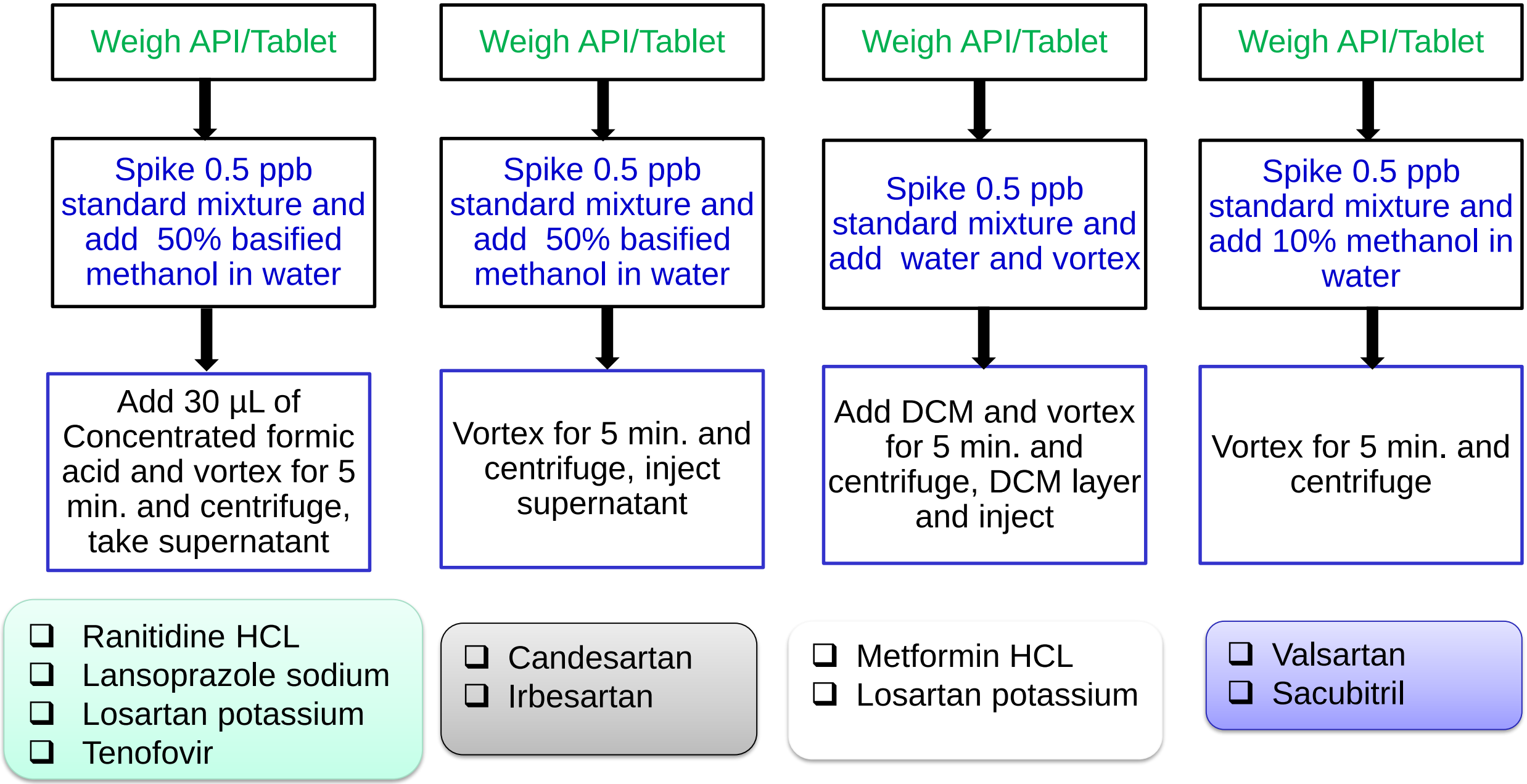
- ❑ Column chemistry
- ❑ Mobile phase

- ❑ Analytical technique
- ❑ Detector settings



Injection volume limited with organic diluent, particularly for polar NDMA (peak fronting)




Weigh API/Tablet
Spike 0.5 ppb standard mixture and add 50% basified methanol in water
Add 30 µL of Concentrated formic acid and vortex for 5 min. and centrifuge, take supernatant
Ranitidine HCL
Lansoprazole sodium
Losartan potassium
Tenofovir
Weigh API/Tablet
Spike 0.5 ppb standard mixture and add 50% basified methanol in water
Vortex for 5 min. and centrifuge, inject supernatant
Candesartan
Irbesartan
Weigh API/Tablet
Spike 0.5 ppb standard mixture and add water and vortex
Add DCM and vortex for 5 min. and centrifuge, DCM layer and inject
Metformin HCL
Losartan potassium
Weigh API/Tablet
Spike 0.5 ppb standard mixture and add 10% methanol in water
Vortex for 5 min. and centrifuge
Valsartan
Sacubitril

❑ Acceptable Intake over Maximum Daily Dose gives ppm level

N-Nitrosamine (CAS number)	AI (ng/day)
NDMA (62-75-9)	96.0
NDEA (55-18-5)	26.5
EIPNA (16339-04-1)	26.5
DIPNA (601-77-4)	26.5
NMBA (61445-55-4)	96.0
MeNP (16339-07-4)	26.5
NDBA (924-16-3)	26.5

Medication	MDD (mg)
Valsartan	320
Losartan	150
Metformin	3000
Ranitidine	300

ppm Valsartan	ppm Losartan	ppm Metformin	ppm Ranitidine
0.300	0.640	0.032	0.320
0.083	0.177	0.009	0.088
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0.083	0.177	0.009	0.088

$$\textbf{ppm} = \frac{\text{Acceptable Intake or AI Limit (ng/day)}}{\text{Maximum Daily Dose or MDD (mg/day)}}$$

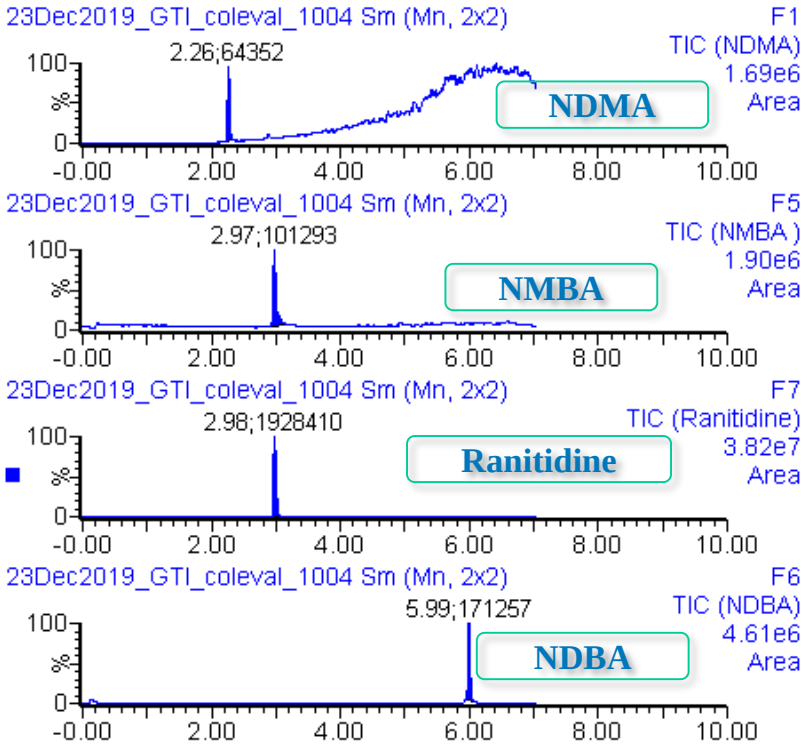
Solubility of API is critical for Limit of Impurity

Impurity Limit in ppm	API in sample (mg/mL)	Impurity Limit (ng/mL)
10 ppm	1	10 ng/mL
1 ppm	10	
0.5 ppm	20	
0.1 ppm	1	0.1 ng/mL
0.01 ppm	10	
0.005 ppm	20	

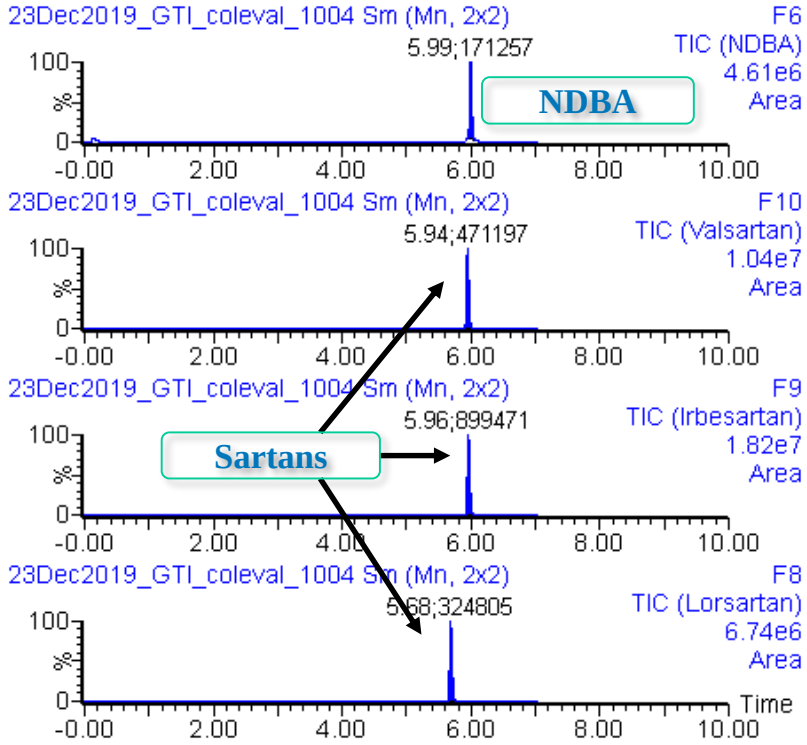
Limit of Impurity (ng/ml) = ppm (ng/mg) x solubility (mg/mL)

- It is important to select the correct column for the targeted impurity and API

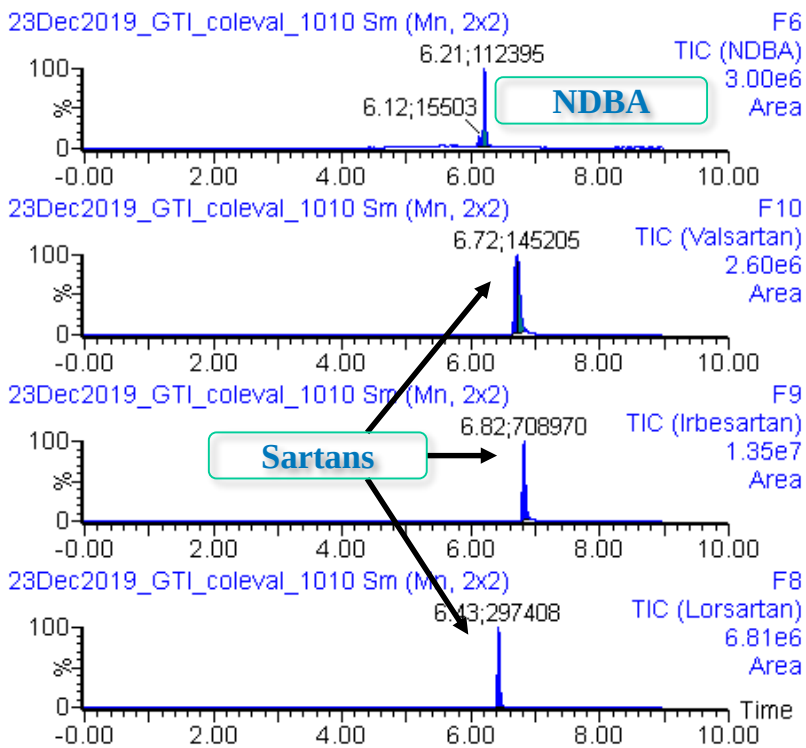
HSS T3 (C18)
NDMA resolved from Ranitidine



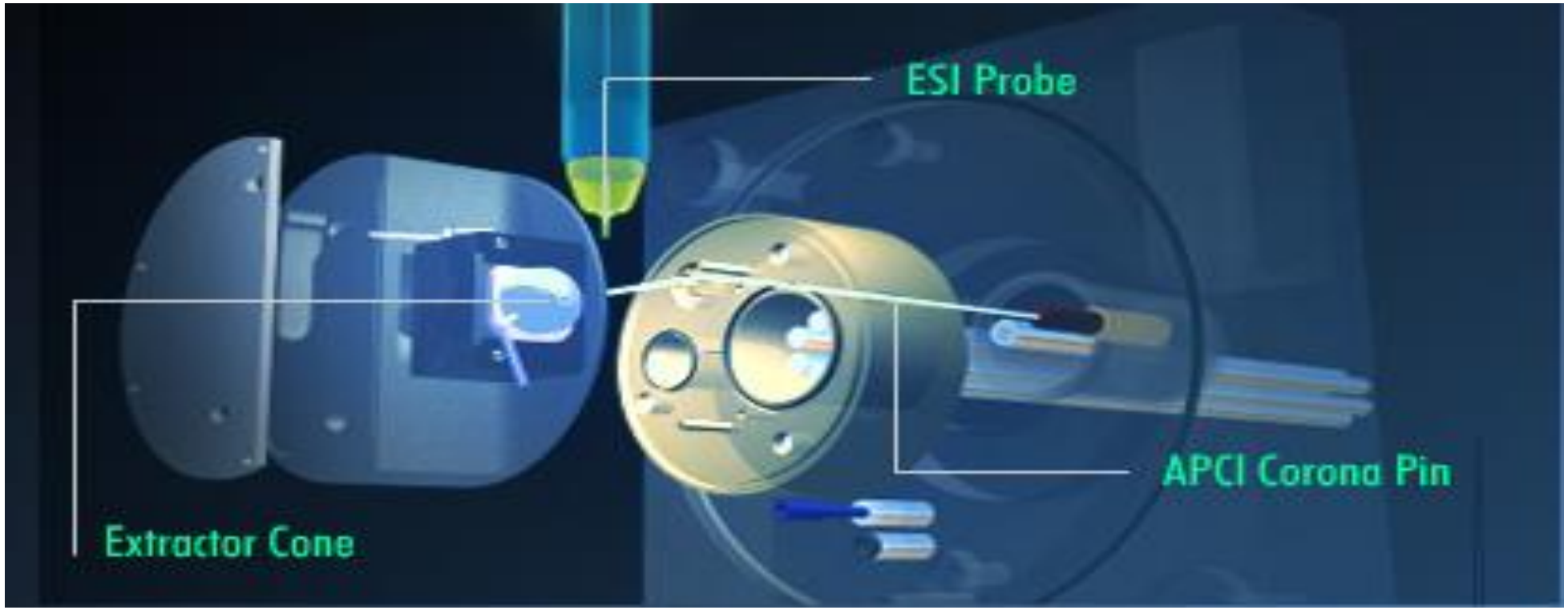
HSS T3 (C18)
NDBA not resolved from Sartans



Atlantis PREMIER BEH C18 AX
NDBA resolved from Sartans



- ESI and APCI scans in the same acquisition
- Allows wider range of compounds to be ionised

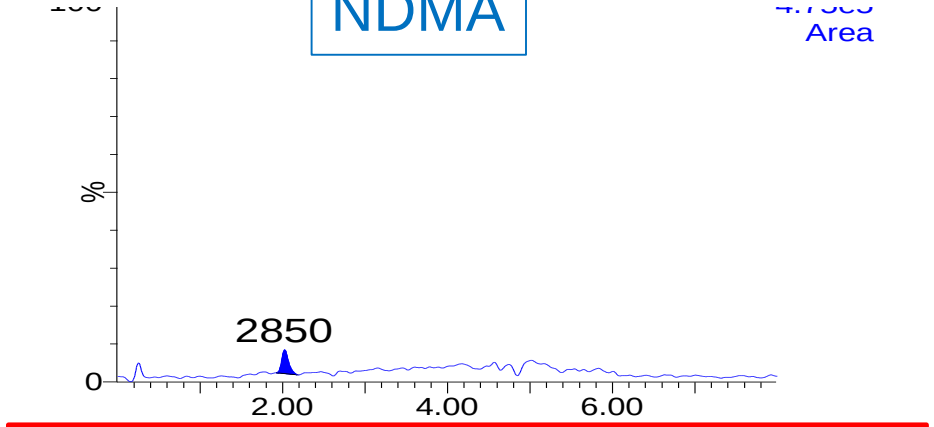


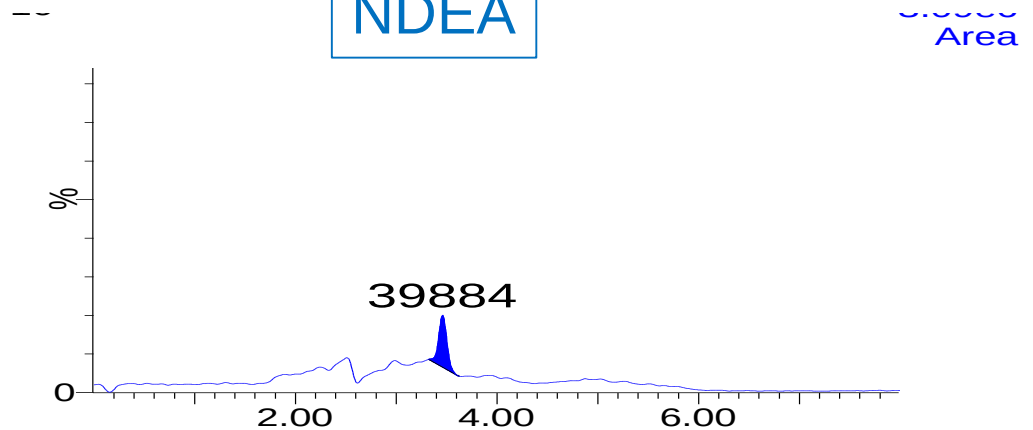
APCI 10X > sensitivity vs. ESI Probe

NDMA

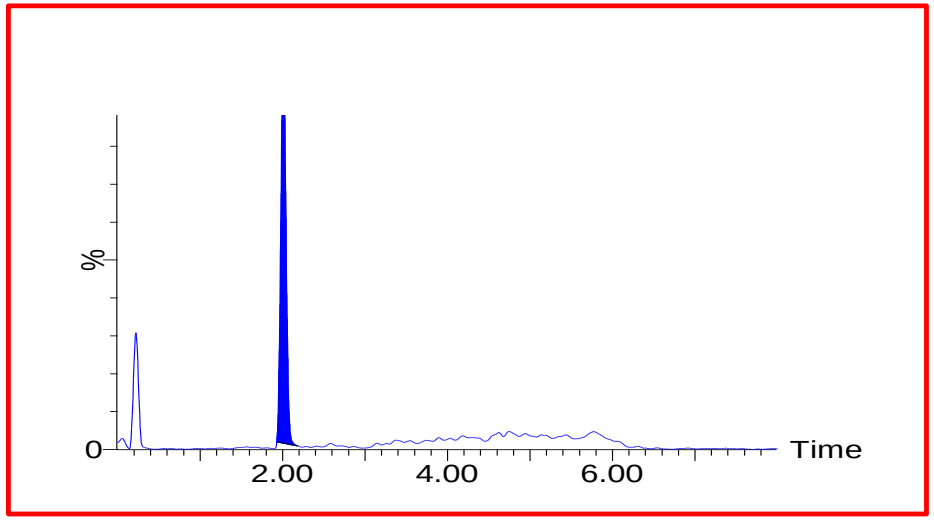
NDEA

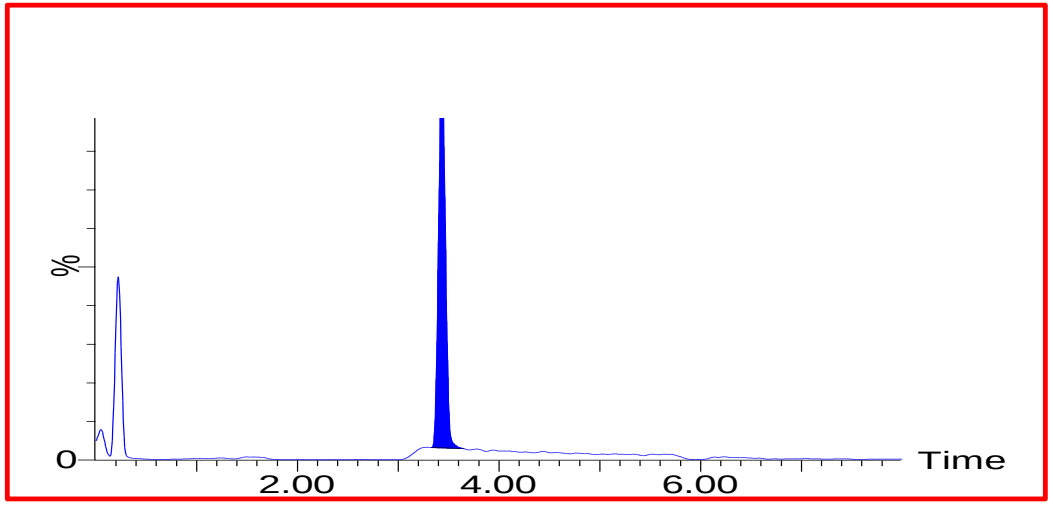
ESI





APCI

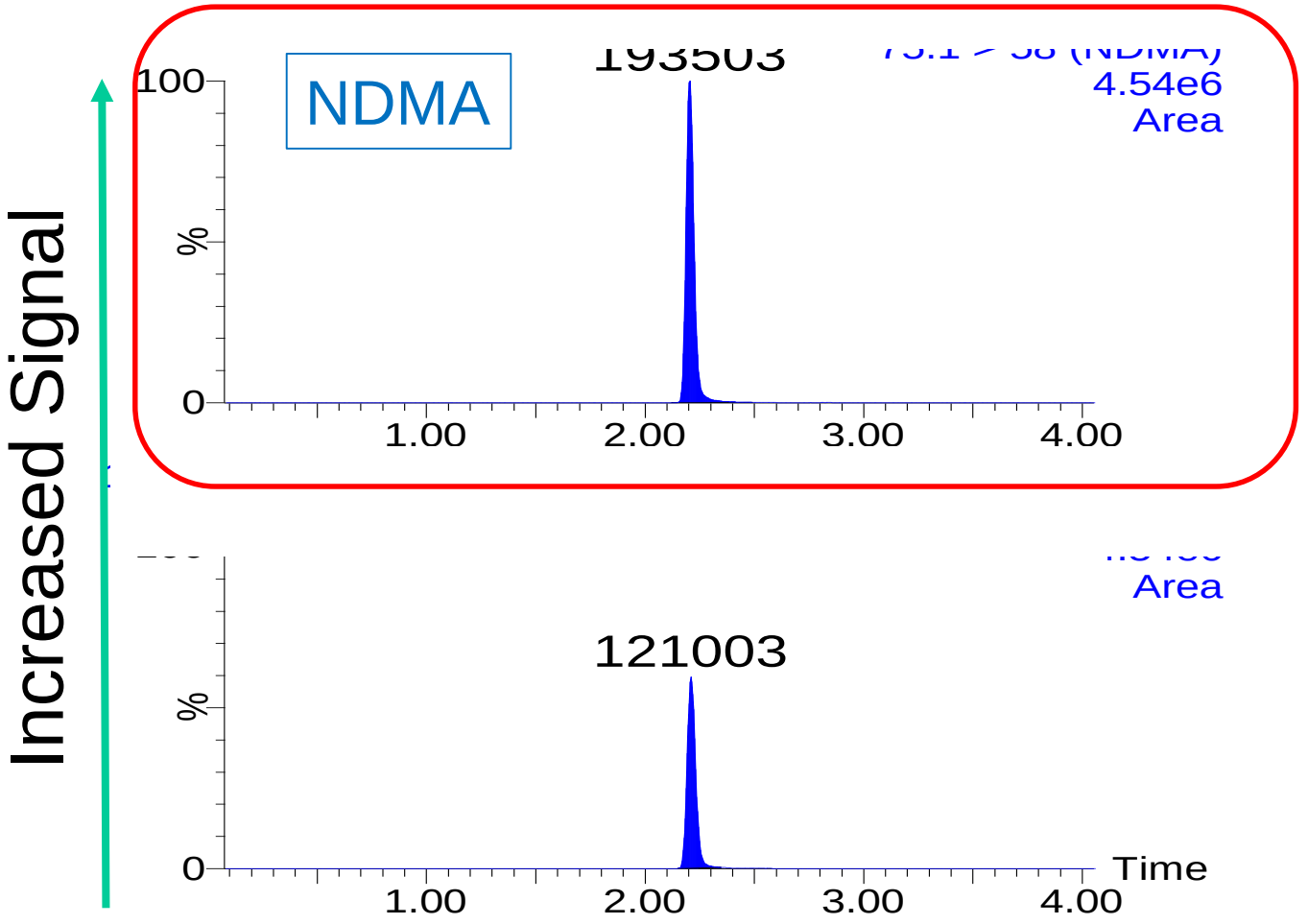




❑ Probe and Source Temperature

Probe 250°C
Source 130°C

Probe 400°C
Source 150°C



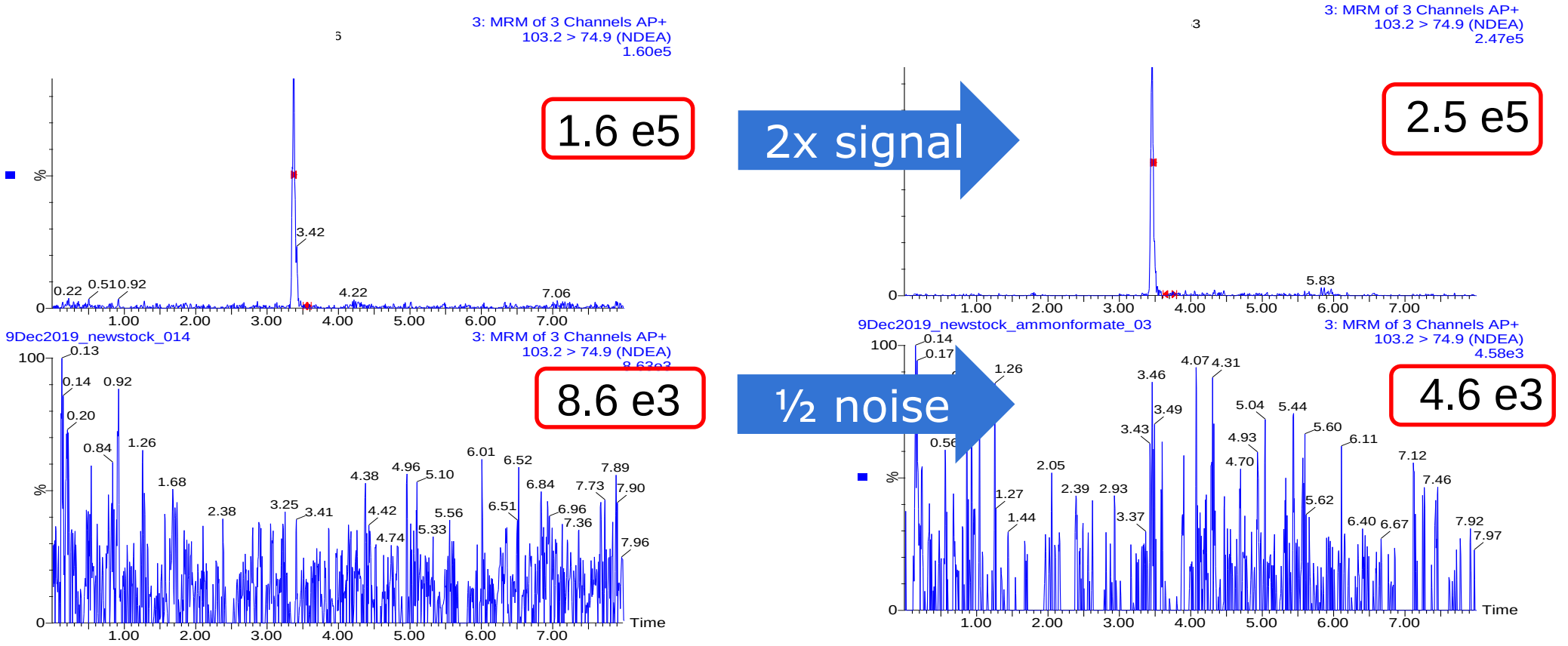
Nitrosamine MS response increase with lower probe and source temps

❑ Improved Peak Response/ Reduced Noise using Ammonium Formate (better S/N)

0.1% Formic Acid

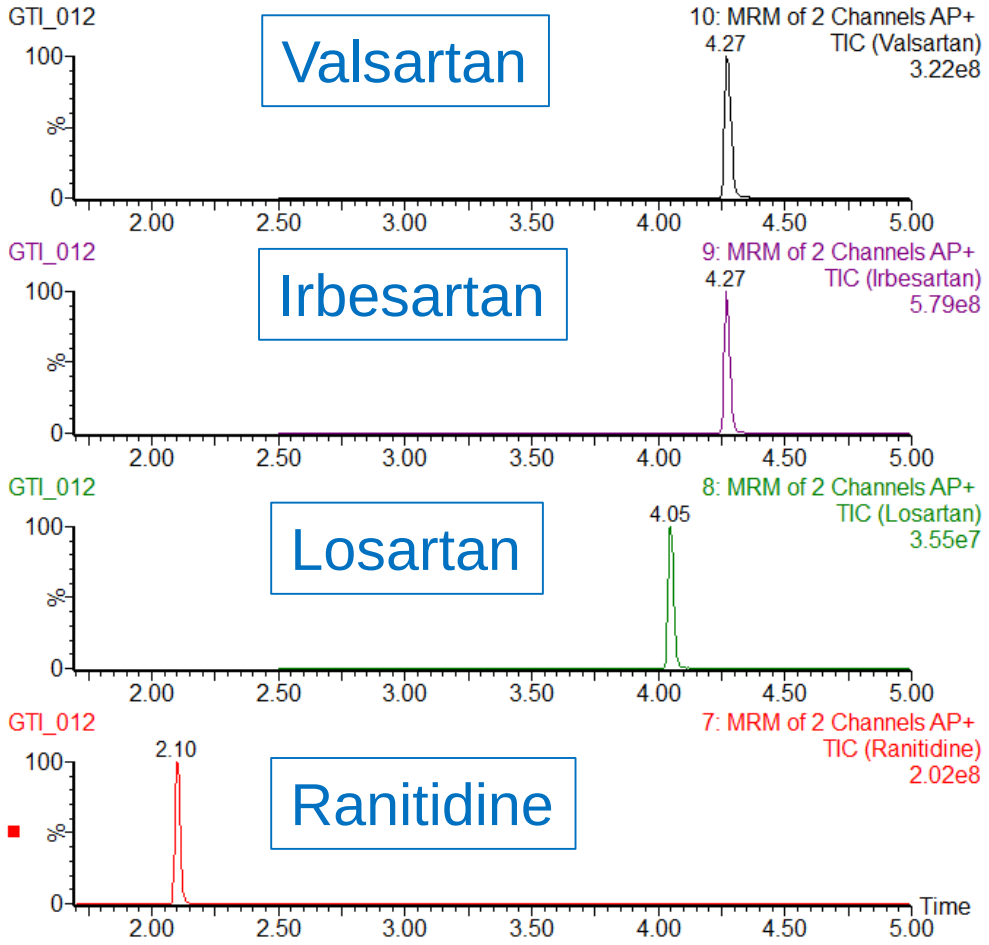
NDEA

0.1% Formic Acid with Ammonium Formate

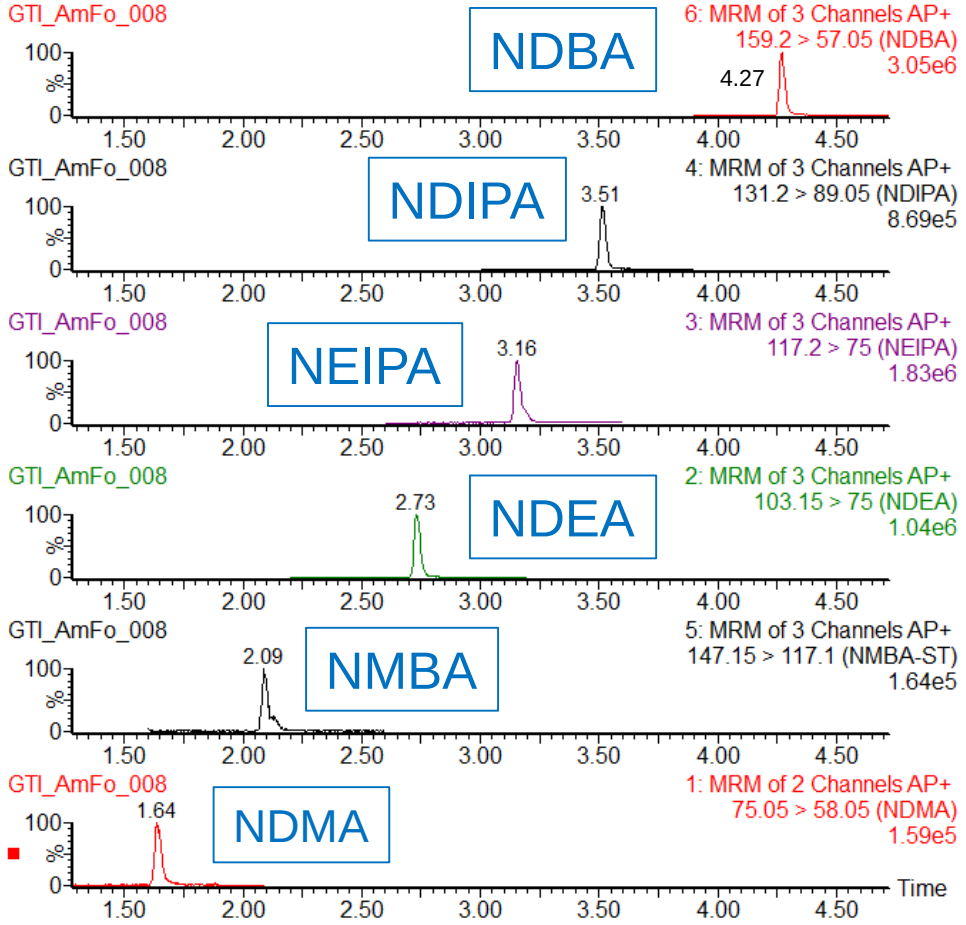


❑ UPLC-MS/MS

Drug Substance



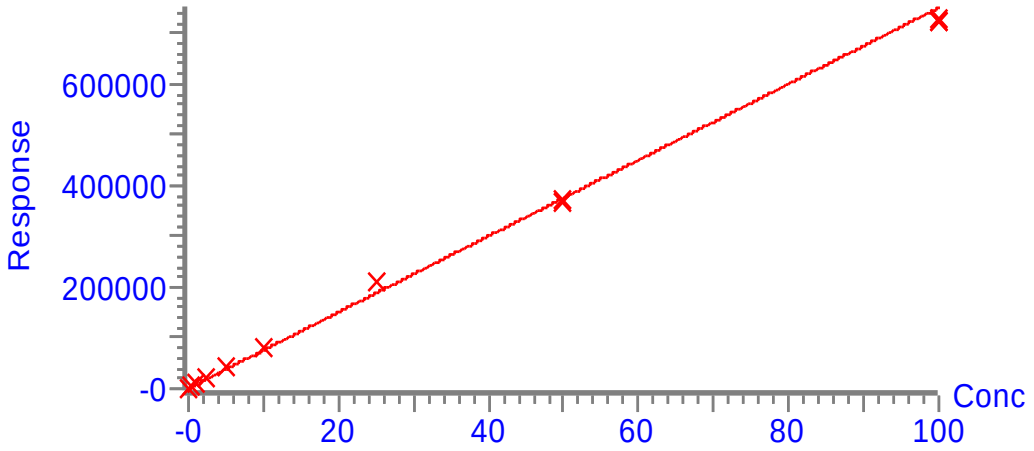
Nitrosamine Impurities



❑ UPLC-MS/MS

Standard Curve Performance: Neat Solutions

GTI Quantification Performance				
GTI	**Std Curve Range (ng/mL)**	**Weighting**	**Linear Fit (R^2)**	**MRM Transition**
NDMA	<0.025-100	1/x	≥ 0.99	75.1>58.0 75.1>43.1
NDEA				103.2>74.9 103.2>46.9
NDBA				159.2>103.2 159.2>57.1
NMBA				147.1>117.1 147.1>44
NEIPA				117.2>74.9 117.2>43.1
NDIPA				131.2>89.1 131.2>47.1

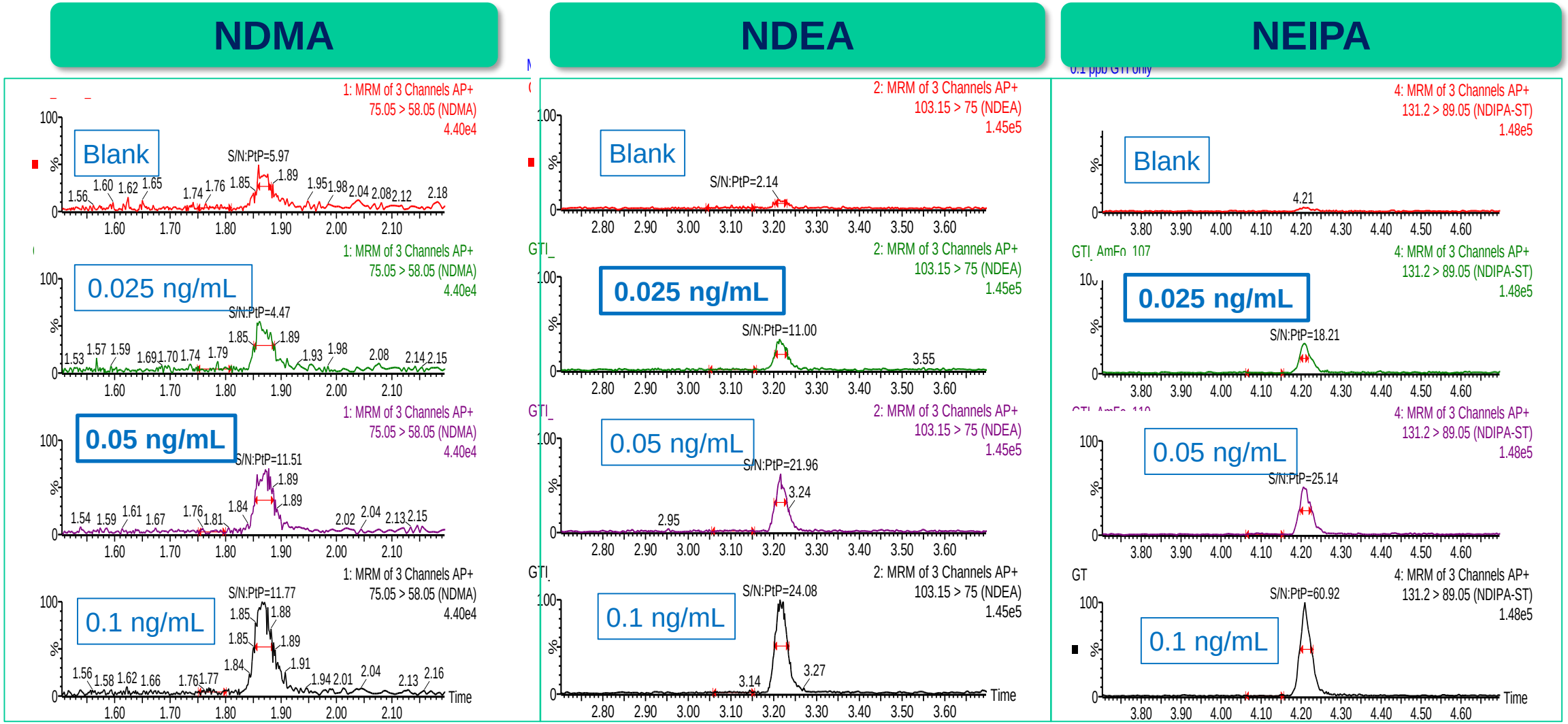


Impurity limit for <u>0.1 ng/mL</u> LLOQ (based on 40 mg/mL dose) would be = <u>0.0025 ppm</u>

❑ UPLC-MS/MS

LODs/LLOQs 0.025-.05 ng/mL (< 1 pg on column)

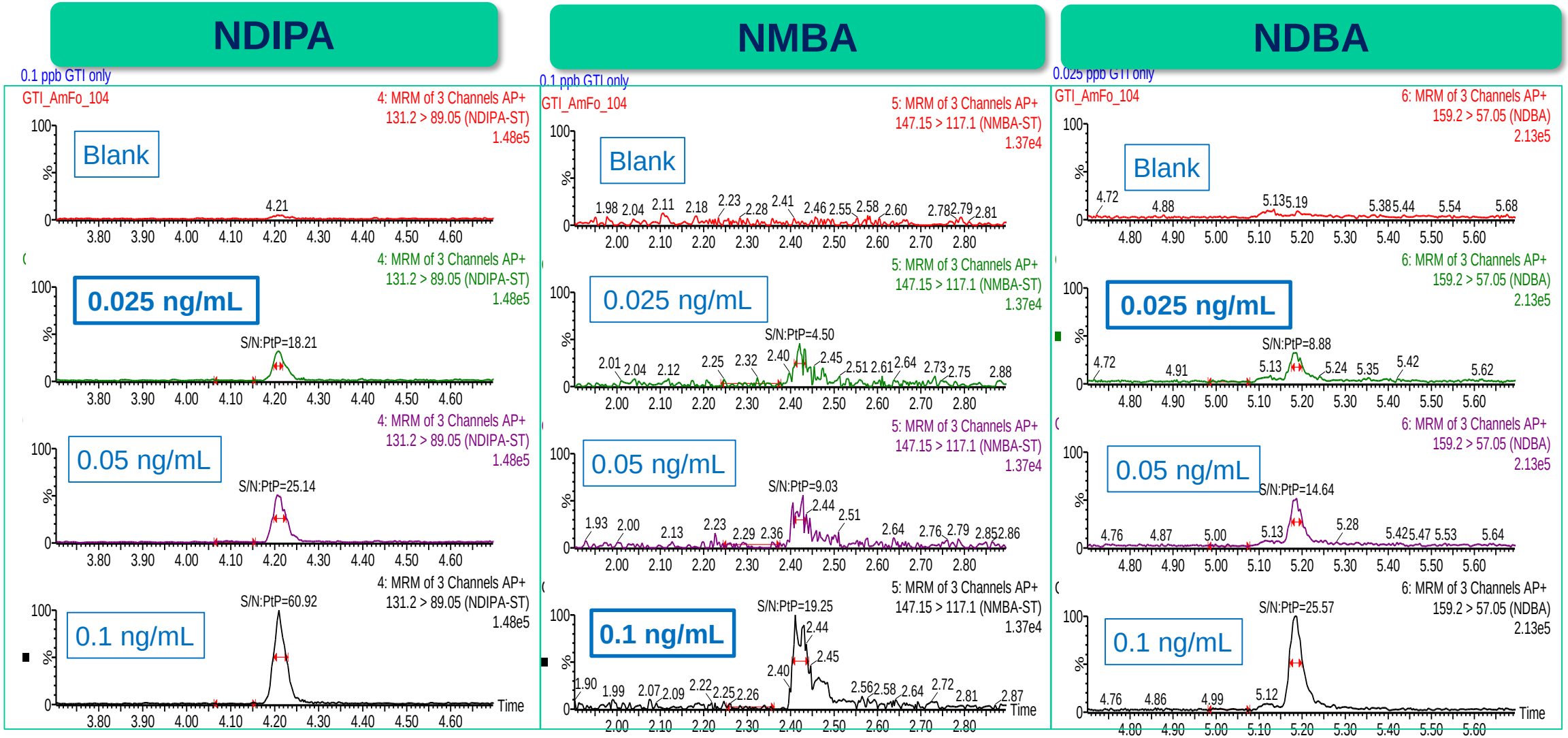
Absolute not relative to API



❑ UPLC-MS/MS

LODs/LLOQs 0.025-0.1 ng/mL (< 1 pg on column)

Absolute not relative to API



Method Review

- **Test Sample Preparation**
  - 30 mg/mL of API ranitidine
    - ✓ Drug Substance
    - ✓ Drug Product
  - Prepared in 100% water
  - Injection volume: 10.0 µL
- **Dynamic Range**
  - 1.0 - 100 ng/mL (0.033-3.33 ppm)
- **Limits of detection/quantification**
  - LOD 0.3 ng/mL (0.01 ppm)
  - LOQ 1.0 ng/mL (0.033 ppm)

FDA U.S. FOOD & DRUG ADMINISTRATION

10/17/2019

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method for the Determination of NDMA in Ranitidine Drug Substance and Solid Dosage Drug Product

	NDMA
LOD (ng/mL)	0.3
(ppm)	0.01
LOQ (ng/mL)	1.0
(ppm)	0.033
Range (ng/mL)	1.0 - 100
(ppm)	0.033 – 3.33

Drug substance sample preparation
Accurately weigh 120 mg of drug substance into a 15 mL glass centrifuge tube. Add 4.0 mL of water and mix the solution using a vortex mixer until dissolved.

Drug product sample preparation
Crush the appropriate number of tablet(s) to obtain a target concentration of 30 mg/mL of API in water, and transfer into a 15 mL glass centrifuge tube. Add the appropriate volume of water and mix for about a minute using a vortex mixer. Shake the sample for 40 minutes using a mechanical wrist action shaker.

After extraction, centrifuge the sample for 15 minutes at 4500 rpm. Filter the supernate using a 0.22 µm PVDF syringe filter, discard the first 1 mL and transfer the filtered sample into an hplc vial for analysis.

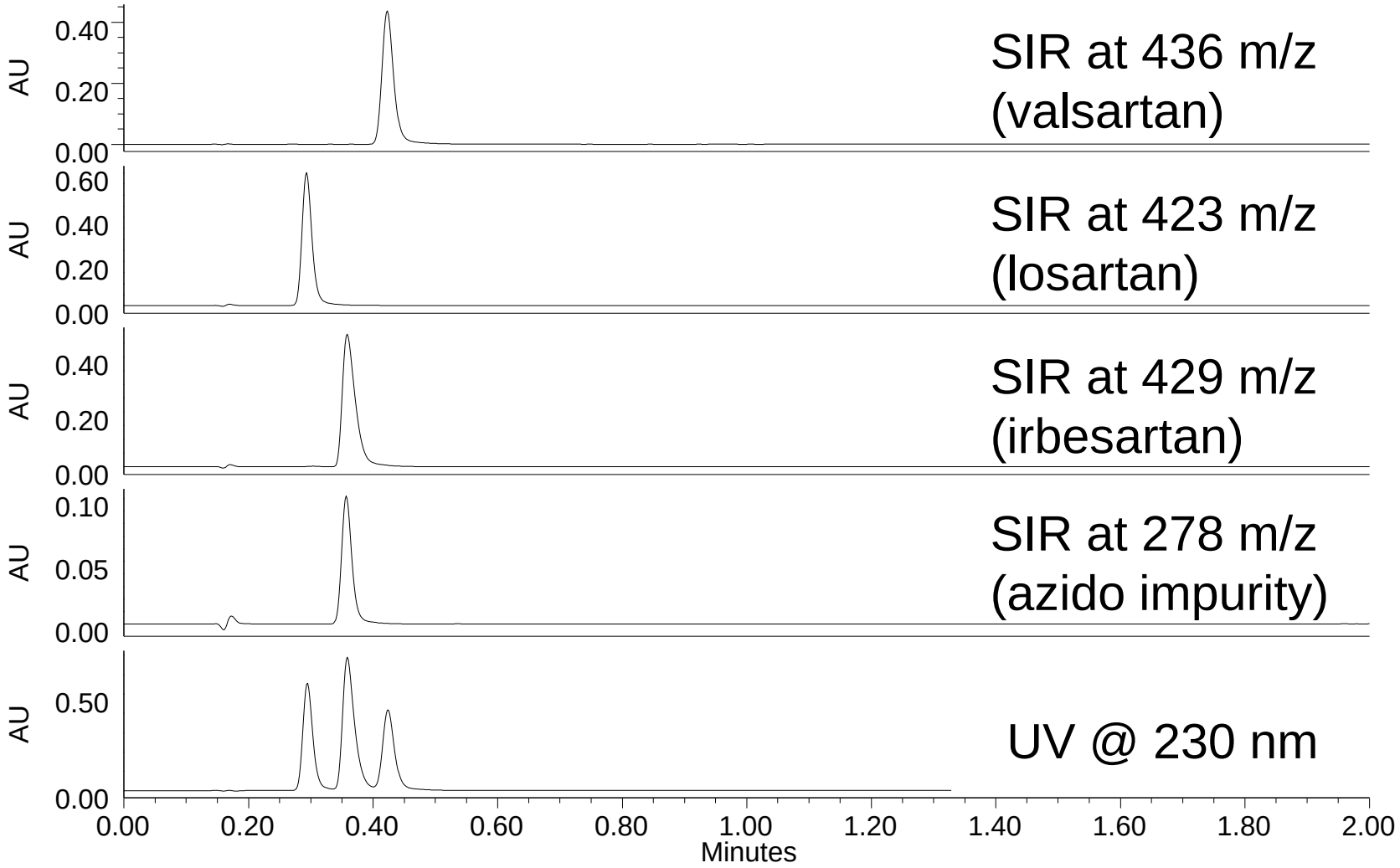
Impurity	Valsartan /Sacubitril		Losartan potassium		Irbesartan		Candesartan		Pirfenidone	
	Regression (R2)	Linearity Range (ng/mL)	Regression (R2)	Linearity Range (ng/mL)	Regression (R2)	Linearity Range (ng/mL)	Regression (R2)	Linearity Range (ng/mL)	Regression (R2)	Linearity Range (ng/mL)
NDMA	0.9946	0.05-1.0	0.9982	0.05-10	0.9977	0.02-5.0	0.9977	0.02-5.0	0.9932	0.05-5.0
NDEA	0.994	0.05-1.0	0.9985	0.05-10	0.9965	0.02-5.0	0.9965	0.02-5.0	0.9904	0.02-5.0
NIEA	0.9994	0.05-1.0	0.999	0.05-10	0.9944	0.02-5.0	0.9944	0.02-5.0	0.9951	0.02-5.0
NDPA	0.9993	0.05-1.0	0.9942	0.05-10	0.9936	0.02-5.0	0.9936	0.02-5.0	0.9992	0.02-5.0
NDBA	0.9964	0.05-1.0	0.992	0.05-10	0.9919	0.02-5.0	0.9919	0.02-5.0	0.9931	0.02-5.0
NMBA	0.9936	0.05-1.0	0.9982	0.05-10	0.9908	0.02-5.0	0.9908	0.02-5.0	0.9996	0.05-5.0

Impurity	Metformin HCL		Ranitidine		Lansoprazole		Tenofovir	
	Regression (R2)	Linearity Range (ng/mL)	Regression (R2)	Linearity Range (ng/mL)	Regression (R2)	Linearity Range (ng/mL)	Regression (R2)	Linearity Range (ng/mL)
NDMA	0.9902	0.05-10	0.9918	0.05-10	0.9986	0.05-10	0.992	0.05-5.0
NDEA	0.9929	0.05-10	0.9932	0.05-10	0.9936	0.05-10	0.9911	0.02-5.0
NIEA	0.9944	0.05-10	0.991	0.05-10	0.9945	0.05-10	0.9903	0.02-5.0
NDPA	0.9936	0.05-10	0.9902	0.05-10	0.9902	0.05-10	0.993	0.02-5.0
NDBA	0.9919	0.05-10	0.9991	0.05-10	0.995	0.05-10	0.9941	0.02-5.0
NMBA	0.9952	0.05-10	0.9919	0.05-10	0.9921	0.05-10	0.9996	0.05-5.0

6 Nitrosamines with a LOQ of 0.05 ppm relative to valsartan

- The Current Regulatory requirements is Manufacturers of APIs and Drug Products should use methods with LOQs at or below 0.03 ppm. In other words, the analytical procedures should ideally allow LOQs in the range of 10 to 30 ppb.
- However these instruments (Tandem Quad or HRMS) may not be readily available or affordable to many Pharmaceuticals; in addition, the need for highly skilled operators for method optimization and subsequent data interpretation have further limited their use in routine pharmaceutical analysis of large sample set.
- USP has recently published UPLC-MS (adopted the chromatographic conditions as per FDA LC-HRMS method) for 6 Nitrosamines with a LOQ of 0.05 ppm relative to valsartan.
- The method was validated within a wide dynamic range (0.05 – 3.6 ppm) and can be applied to commercial valsartan samples in GMP QC Environment.

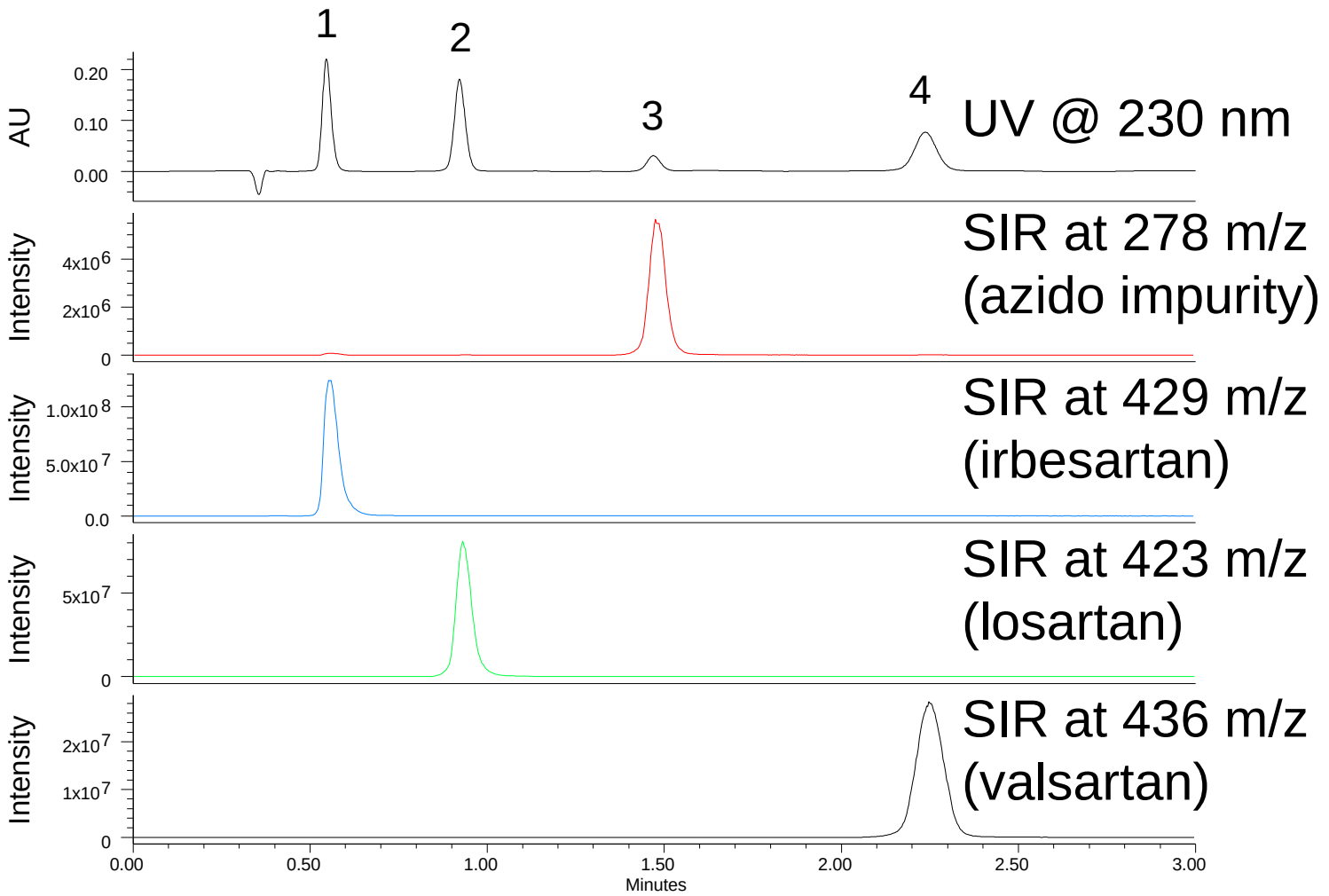
❑ HPLC-PDA-MS: Separation using Valsartan USP Monograph Conditions



- ❑ Irbesartan and azido impurity co-elute.
- ❑ APIs at 0.2 mg/mL (monograph standard concentration), Impurity at 0.02 mg/mL

Assay conditions: XBridge BEH C18 (3.0 x 50 mm; 2.5 µm), Acetonitrile – Water - Acetic Acid (50:50:0.1), 1.7 mL/min

❑ HPLC-PDA-MS: Developing Isocratic Separation Based on Initial Screening with Alternate Column Chemistry



1. Irbesartan (0.10 mg/mL)
2. Losartan (0.10 mg/mL)
3. Azido Impurity (0.016 mg/mL)
4. Valsartan (0.10 mg/mL)

40% acetonitrile with 0.1% formic acid, 0.85 mL/min, 30°C,
XSelect CSH Phenyl-Hexyl (3.0 x 50 mm; 2.5 µm)

❑ Separation and Identification of Degradation Products, Known and Genotoxic Impurities

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Development and validation of a stability indicating HPLC method for organic impurities of erythromycin stearate tablets

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Stability indicating
Erythromycin stearate tablets

ABSTRACT

A rapid, sensitive, and accurate high-performance liquid chromatography (HPLC) method was developed and validated for the separation and analysis of organic impurities in erythromycin stearate tablets. The method separates Erythromycin, Erythromycin B, Erythromycin C and nine impurities (EP Impurity A, B, C, D, E, F, H, I and M). The chromatographic separation was achieved on a Waters XBridge C18 (100 mm × 4.6 mm, 3.5 µm) column. The mobile phase comprised of 0.4 % ammonium hydroxide in water and methanol delivered in a gradient mode. The compounds of interest were monitored at 215 nm. The stability-indicating capability of this method was evaluated by performing stress studies. Erythromycin was found to degrade significantly under acid, base, and oxidative stress conditions and it was only stable under thermal and photolytic degradation conditions. The degradation products were well resolved from the erythromycin peaks. In addition, the major degradants formed under stress conditions were characterized by ultra-high-performance liquid chromatography coupled with Single-Quadrupole Mass Spectrometer (UHPLC-QDa). The method was validated to fulfill International Conference on Harmonization (ICH) requirements and this validation included specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and robustness. The developed method could be incorporated into the USP monograph and applied for routine quality control analysis of erythromycin stearate tablets.

Introduction

Erythromycin Stearate ((3S,4R,5R,6S,7S,9S,12R,14S)-6-[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione;octadecanoic acid) is the stearate salt form of erythromycin, macrolide antibiotic with broad spectrum antibacterial activity. Erythromycin is produced by a strain of *Streptomyces erythraeus* and is used for the treatment of pneumonias, streptococcal pharyngitis, skin infections and erythrasma. Erythromycin is a basic compound which is degraded to inactive anhydrous erythromycin in acidic fluids. Hence, ester type prodrugs are preferred and used for oral administration [1]. Erythromycin stearate, a prodrug of erythromycin, diffuses through the bacterial cell membrane and reversibly binds to the 50S subunit of the bacterial ribosome, preventing bacterial protein synthesis. Erythromycin stearate may be bacteriostatic or bactericidal in action, depending on the concentration of the drug at the site of infection and the susceptibility of the organism involved [2].

Currently, development of efficient and economical analytical methods to cope with a large number of samples has become an important part in the pharmaceutical industry [3]. Although erythromycin stearate is a well-known drug in the world, very few analytical methods appeared in literature regarding separation and determination of its organic impurities (EP Impurity A, B, C, D, E, F, H, I and M). A literature survey reveals several analytical methods for the determination of erythromycin stearate in pharmaceutical preparation and biological fluids. These methods are time-consuming (a run time of 75 min) and not all the impurity peaks are baseline resolved form each other and the erythromycin peak [4–6]. In addition, no official methods are included in major pharmacopeias for the determination of organic impurities in Erythromycin Stearate tablets [7,8]. Hence, in this work a novel stability-indicating reverse-phase HPLC method was developed for the determination of degradation products of erythromycin stearate. Altogether, this new method would be of great value to ensure the safety and quality of erythromycin stearate in raw material and dosage forms.

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0731-7085/

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Development and Validation of a Stability-indicating Method for Ibrutinib: Identification and Separation of Degradation Products, Known and Genotoxic Impurities Using RP-HPLC/PDA and QDa Mass Detectors

Naresh Konduru, Rambabu Gundla, Naresh Kumar Katari, Kalyani Paidikondala, Annem Siva Reddy & Varaprasad Jagadabi

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A NOVEL STABILITY-INDICATING RP-UPLC METHOD FOR THE QUANTIFICATION OF IMPURITIES AND A NEW QDa MASS DETECTOR COUPLED WITH LC-PDA FOR IDENTIFICATION OF MASS OF DEGRADATION PRODUCTS IN A FIXED DOSE COMBINATION OF EMPAGLIFLOZIN AND LANIGLIPTIN TABLETS USED AS SECOND-LINE THERAPY FOR TYPE-2 DIABETES

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ABSTRACT

- A single UPLC-MS/MS method can successfully be developed for the accurate, robust, and highly sensitive quantification of six nitrosamine impurities, achieving LLOQs of 0.1 ng/mL
- This method offers a practical starting point for high sensitivity quantification of nitrosamines or similar compounds.
- The HSS C18, BEH C18 and CSH Phenyl Hexyl column provided excellent retentivity and selectivity for six nitrosamine impurities.
- USP has published UPLC-MS for analysis of 6 Nitrosamines with a LOQ of 0.05 ppm relative to valsartan.
- A single HPLC-PDA-MS method can be applied for analysis of Azido Impurity in sartan Drugs
- A Mass Detector for Forced Degradation Studies is useful for early-stage identification of new impurities in drug substance and drug product


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