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MÉRIEUX NUTRISCIENCES

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NITROSAMINES ANALYSES
on pharmaceutical products

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What are nitrosamines?

Nitrosamines, or more correctly *N*-nitrosoamines, refer to any molecule containing the nitroso functional group.

These molecules are of concern because **nitrosamine impurities are probable human carcinogens**, signifying that long-term exposure above certain levels may increase the risk of cancer development.

Although they are **also present in some foods and drinking water supplies**, their presence in medicines is nonetheless considered unacceptable.

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The facts

PRESENCE OF NITROSAMINE IMPURITIES IN HUMAN MEDICINAL PRODUCTS CONTAINING CHEMICALLY SYNTHETIZED API

June 2018

US manufacturer Prinston Pharmaceuticals Inc. had contacted FDA's Center for drug Evaluation and Research to inform the agency that it had stopped manufacturing valsartan products because it had detected trace amounts of a **nitrosamines impurity called NDMA (N-nitrosodimethylamine) in valsartan API supplied by Chinese manufacturer Zhejiang Huahai**.

July 2018

Authorities in Europe became aware of the presence of NDMA in valsartan manufactured at a facility based in China. Subsequently, another nitrosamine NDEA (N-nitrosodiethylamile) was detected and other sartans from more API manufacturers were implicated.

Late 2018 - early 2019

Companies that make sartan blood pressure medicines (also known as angiotensin II receptor blockers) are being required to review their manufacturing processes so that they do not produce nitrosamine impurities and many valsartan recalls continued both in EU and US.

September - November 2019

FDA has learned that some ranitinide medicines contain **a nitrosamines impurity called NDMA (N-nitrosodimethylamine)**. **FDA** has detected that also some nizatidine (which is chemically similar to ranitidine) medicines, contain **NDMA impurities**.

December 2019

EMA and **FDA** are aware concerning the **presence of low levels of NDMA in some metformin diabetes medicines outside the Europe and United States**. At this point, there are no data indicating that US and EU metformin medicines are affected, but authorities are in the process of working with companies to test medicines and provide more information.

Nitrosamines limits in API

LIMITS (PPM) IN API FOR SELECTION N-NITROSAMINE IMPURITIES

API	NDMA*	NDEA*	Limits applicable from 2022 for NDEA and NDMA	NDPIA	EIPNA or NIPEA
AI (ng/day)	**96**	**26,5**		**no tox data available, used the one for NDEA (26,5 ng/day)**	**no tox data available, used the one for NDEA (26,5 ng/day)**
Valsartan	0,300	0,082	0,03	0,082	0,082
Losartan	0,640	0,177	0,03	0,177	0,177
Olmesartan	2,400	0,663	0,03	0,663	0,663
Irbesartan	0,320	0,088	0,03	0,088	0,088
Candesartan	3,000	0,820	0,03	0,820	0,820

*Applicable from 1/1/2020 (2 years transitional period)

In the European Union (EU), following an **Article31 review of sartans (Directive 2001/83/EC) at risk of containing nitrosamine impurities** (those containing a tetrazole ring), manufacturers were asked to **review and make changes to their manufacturing processes to minimize nitrosamine impurities to the extent practically possible**. A **transition period of two years** has been allowed to make these changes.

During this transition period, interim limits as outlined in the table are being applied to products. Batches of product exceeding these limits for an individual impurity, or batches containing both NDMA and NDEA are not allowed in the EU.

Source: 20 August 2019 EMA/351053/2019 rev 1: Temporary interim limits for NMBA, DIPNA and EIPNA impurities in sartan blood pressure medicines.

Source of contamination

The review of some critical **APIs** (e.g. **Sartans**, **Ranitidine**) under **Article 31 of Directive 2001/83/EC** and information collected from MAHs identified several list of root causes of nitrosamine formation and contamination:

1. **during API synthesis** under certain processing conditions, and in presence of some types of raw materials, starting materials and intermediates
2. **use of sodium nitrite** ($NaNO_2$), or other nitrosating agents, in the presence of secondary or tertiary amines or in combination with reagents, solvents and catalysts, which are susceptible to degradation to secondary or tertiary amines, within the same or different process steps (if carry over can occur)
3. **use of contaminated raw materials** in the manufacturing process (e.g. DMP, DMF, DIPEA)
4. **use of recovered materials** (e.g. solvents, reagents and catalysts), including recovery outsourced to third parties
5. **use of contaminated starting materials and intermediates** supplied by vendors that use processing methods or raw materials which may allow nitrosamine formation
6. **cross-contaminations** due to different processes run on the same line and due to operator-related errors
7. **degradation processes** of starting materials, intermediates and drug substances, including those induced by inherent reactivity in combination with carry-over of sodium nitrite ($NaNO_2$), or other nitrosating agents (also during finished product formulation or storage)
8. **use of certain packaging materials** (case study: lidding foil containing nitrocellulose printing primer may react with amines in printing ink to generate nitrosamines, which would be transferred to the product under certain packaging process conditions, as during heat-sealing blistering processes via vaporization and condensation onto the drug product)

Source: https://www.ema.europa.eu/en/medicines/human/referrals/angiotensin-ii-receptor-antagonists-sartans-containing-tetrazole-group
https://www.ema.europa.eu/en/news/sartan-medicines-companies-review-manufacturing-processes-avoid-presence-nitrosamine-impurities

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The Risk Evaluation process

On September 26th, 2019 the CMDh (Heads of Medicines Agencies) published the notice "**Information on nitrosamines for marketing authorisation holders**" asking to all Marketing Authorization Holders (MAHs) of human medicinal products containing chemically synthesised active pharmaceutical ingredients to **evaluate the risk of the presence of nitrosamine impurities in their product**s. This includes generics and over-the counter (OTC) products.

The risk evaluation of all products should be concluded at the latest within 6 months of the publication of the CMDh notification (26th March 2020)

Taking into account their knowledge of the manufacturing processes and the potential sources of nitrosamine impurities, the manufacturers of API and finished products **should cooperate with MAHs to prevent nitrosamine formation and contamination of human medicinal products**.

The Risk Evaluation process

PRIORITIZING THE RISK EVALUATION
Knowledge of factors impacting the risk

QUALITY RISK MANAGEMENT
Failure Mode Effects Analyses (FMEA) tool

CONFIRMATORY TESTING
Determine which nitrosamines could potentially be present

REGULATORY SUPPORT
Change management Classification of changes Variation Application

CHEMICAL AND MANUFACTURING
Root causes of nitrosamine formation and contamination

ANALYTICAL REQUIREMENTS
Define the expected regulatory standard

ASSESSMENT & CONTROL OF MUTAGENIC IMPURITIES

(ICH M7 GUIDELINE)

Control limits and control strategy

Toxicology assessment

The Risk Evaluation process

THE RISK EVALUATION PROCESS STEPs

STEP 1 RISK EVALUATION

MAHs should perform risk evaluation of their medicinal products containing chemically synthesised API using quality risk management principles, as per ICH Q9 guideline and principles described in ICH M7 guideline related to toxicology assessment and control strategy. Prioritization of products is prescribed and risk evaluation of high risk / high priority products should be done immediately (e.g. higher daily exposure and chronic use). Risk evaluation documents should be made available upon request.

STEP 2 CONFIRMATORY TESTING

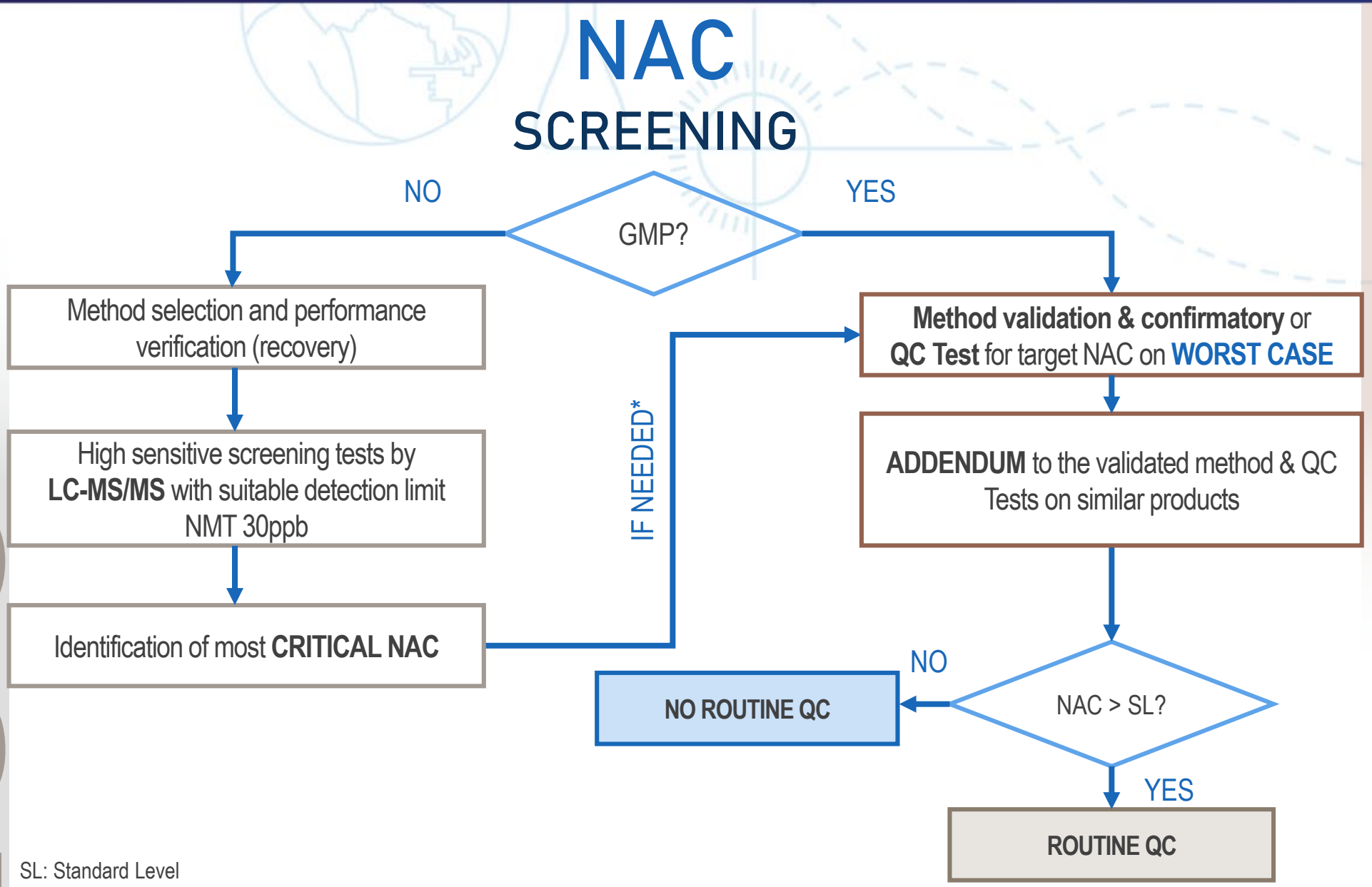
if a risk of presence of nitrosamines is identified, then confirmatory testing are required by the use validated and sensitive methods. MAHs should inform the competent authorities immediately if tests confirm the presence of an nitrosamine impurity irrespective of the amount detected.

STEP 3 CHANGES TO THE MARKETING AUTHORISATION

MAHs should apply for a variation in a timely manner to introduce any required changes, such as amendment of the manufacturing process or changes to product specifications.

Confirmatory testing and submission of Variations to the Marketing Authorization should be concluded at the latest within 3 years of the publication of the CMDh notification, by September 2022

Testing Strategy on Pharmaceutical Products



SL: Standard Level

Applicable testings for different phases of NAC evaluation

RISK EVALUATION

- GMP or NON-GMP screening limit tests on raw materials of drug products supporting the Risk Assessment process in case of missing information:
  - Multiresidual NACs analysis
  - Single NAC analysis (with or without reference standard)

CONFIRMATORY TESTING

- Method development & validation
- GMP quantitative tests with validated methods on high risk NAC(s)

NEW MARKETING AUTHORISATION AND BATCH RELEASE

- GMP screening quantitative tests to demonstrate absence of NACs before applying for new MA
- GMP QC tests for analytical batch release

ALERTS MANAGEMENT (e.g. Sartans, Rinatidine, Metformin)

- Target method development & validation (rush service)
- GMP quantitative tests on APIs and DPs on the market

Validation Strategies: Full Validation Protocols

PARAMETER	OPERATIVE CONDITIONS	ACCEPTANCE CRITERIA
SPECIFICITY	Injection of Reagent Blank, Reference Solution at LoQ, Reference Solution at SL, Test Solution, placebo (if applicable)	Response in reagent blank and control solution ≤ 30% of Reference Solution (LoQ)
MATRIX EFFECT	Comparison of response from solvent standards and matrix-matched standards (if necessary)	N/A
LINEARITY	Analysis of 5 Reference Solutions at concentration starting from LoQ (≤50% of the SL) to 150% of the SL	$R^2 \geq 0{,}990$ points randomly distributed around the calibration curve RSD of residuals ≤ 10%
REPEATABILITY	If the analyte is present in the sample as is at concentration higher than LoQ, 6 independent analyses of the sample, otherwise 3 independent analyses of 3 spiked samples at levels corresponding to LoQ, 100% and 150% of the SL	RSD (n=6) ≤ 20% RSD (n=9 Recoveries) ≤ 20%
ACCURACY/TRUENESS	3 replicate independent analyses of 3 spiked samples at levels corresponding to LoQ, 100% and 150% of the SL	Recovery between 70% and 130%
RANGE	Same procedure as for Accuracy evaluation	Precision, accuracy and linearity suitable across the verified range
QUANTITATION LIMIT (LoQ)	6 replicate injections of the Reference Solution corresponding to the LoQ specified	LOQ ≤ 50% of SL RSD (n=6) ≤ 20% S/N ≥ 10
INTERMEDIATE PRECISION	Same procedure reported for repeatability evaluation but performed by a different analyst and/or in a different day	Intermediate RSD (n=18) ≤ 20% or Intermediate RSD (n=12) ≤ 20%
ROBUSTNESS	Effect of a single variation in a specific operative condition (i.e. column temperature, mobile phase flow, mobile phase composition) evaluated on the Reference Solution at SL level	Variations of peak area ≤ 10%
STABILITY	Analysis of a reference solution and a test solution (or spiked test solution in case the sample doesn't contain the analyte) after 24 and 48 hours	Variations of peak area/concentration ≤ 10%

Validation Strategies: Limit Test Validation

PARAMETER	OPERATIVE CONDITIONS	ACCEPTANCE CRITERIA
SPECIFICITY	Injection of Blank Solution, Reference Solution at target concentration and Test Solution	No significant interference detected with the target analyte SST verified
REPEATABILITY	3 replicate independent analyses of 3 samples spiked at levels corresponding to the limit of specification	RSD (n=9) ≤ 15%
ACCURACY/TRUENESS	Same procedure as for Repeatability evaluation	Recovery between 70% and 130%
DETECTION LIMIT (LoD)	Analysis of the Reference Solution corresponding to the estimated limit of detection	S/N > 3 LoD ≤ SL

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Our capabilities

THE **GMP FACILITY**

OF MÉRIEUX NUTRISCIENCES

IS EQUIPPED WITH **ALL THE ANALYTICAL**

TECHNIQUES USED BY THE **OFFICIAL**

MEDICINES CONTROL LABORATORIES

Our capabilities

- **Dedicated Team & Lab** for analytical testing of NAC by:
  - LC-HRMS or GC-HRMS with Orbitrap and/or TOF Technology
  - LC-MS/MS or GC-MS/MS (Quadrupole Technology)
- Analysis of NDMA and NDEA in **Sartans** and related pharmaceutical products
- Analysis of NDMA in **Ranitidine** API and related pharmaceutical products (before and after accelerated degradation)
- Analysis of **NDMA in Metformin diabeted medicines**
- Method development and validation of target methods for the determination of specific nitrosamines in APIs and pharmaceutical products at low limits (NMT 30ppb)
- GMP analysis for analytical release of batches
- Risk Assessment with qualified partners

OUR CAPABILITIES

Our plus

Multiresidual analysis

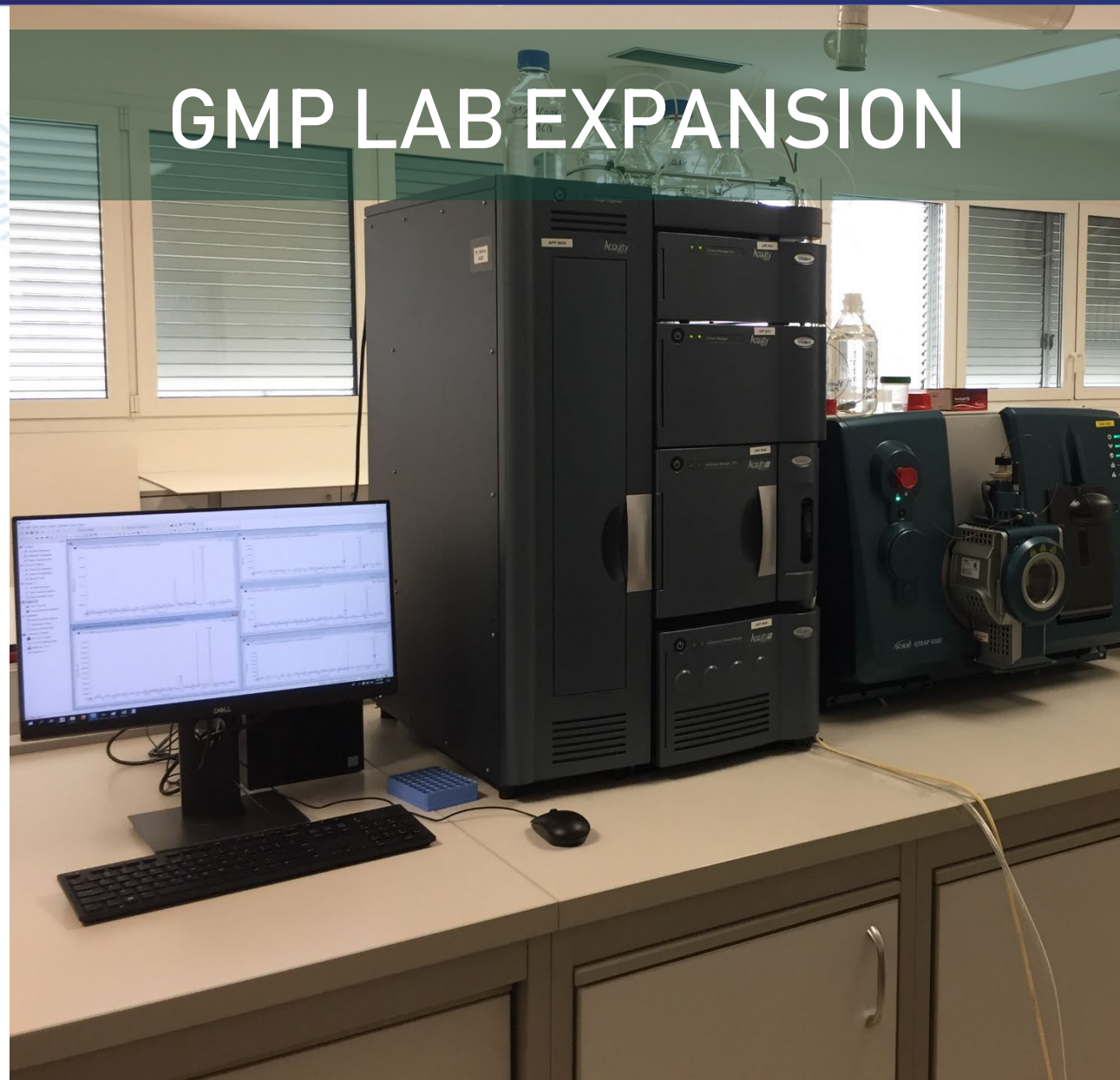
- N-Nitrosodimethylamine (NDMA)
- N-Nitrosodiethylamine (NDEA)
- N-nitrosoethylisopropylamine (EIPNA or NIPEA or NEIPA)
- N-methyl-4-aminobutyric acid (NMBA or BMSA)
- N-nitrosodiphenylamine (NDPhA)
- N-nitrosodi-n-propylamine (NDPA)
- N-nitroso-diisopropylamine (NDiPA or NDPIA)
- N-nitroso-di-n-butylamine (NDBA)
- N-nitrosomethylphenylamine (NMPhA)

Targeted analysis (e.g. NDELA)

Targeted screening by HRMS and/or MS/HRMS (for detection of NAC without available reference standards)

Our equipment

- LC-MS/HRMS and/or GC-MS/HRMS (Orbitrap and/or TOF Technology)
- LC-MS/MS and/or GC-MS/MS (Quadrupole Technology)
- GC-MS
- HPLC-UV



Our experties on NAC analyses

PRODUCT	SUBSTANCE	METHOD	LoQ (ppm)
APIs (e.g. Sartans, Ranitidine) & PHARMACEUTICAL PRODUCTS	N-Nitrosodimethylamine (NDMA) N-Nitrosodiethylamine (NDEA) N-nitrosoethylisopropylamine (EIPNA or NIPEA or NEIPA) N-methyl-4-aminobutyric acid (NMBA or BMSA) N-nitrosodiphenylamine (NDPhA) N-nitrosodi-n-propylamine (NDPA) N-nitroso-diisopropylamine (NDiPA or NDPIA) N-nitroso-di-n-butylamine (NDBA) N-nitrosomethylphenylamine (NMPhA)	LC-MS/MS HS-GC/MS LC-HRMS	0,030-0,200
FOOD (e.g. meet, fish, beer) & FEED	N-nitroso-di-methylamine (NDMA) N-nitroso-di-ethylamine (NDEA) N-nitroso-di-n-propylamine (NDPA) N-nitroso-diphenylamine (NDpheA)	LC-MS/MS	0,001-0,010
DRINKING WATER	N-nitroso-di-methylamine (NDMA)	LC-MS/MS	0,00002
TOBACCO	N'-nitroso-nornicotine (NNN) N'-nitroso-anabasine (NAB) N'-Nitrosoanabatine (NAT) 4-(N-methyl-N-nitrosoamino)-1-(3-pyridil)-1-butanone (NNK) 4-(N-methyl-N-nitrosoamino)-1-(3-pyridil)-1-butanol (NNO)	LC-MS/MS	0,15
FOOD CONTACT MATERIALS (rubber)	N-nitroso-di-methylamine (NDMA) N-nitroso-di-ethylamine (NDEA) N-nitroso-di-n-propylamine (NDPA) N-nitroso-piperidine (NPIP) N-nitroso-pyrrolidine (NPYR) N-nitroso-ethybutylamine N-nitroso-morpholine (NMOR) N-nitroso-N-methylaniline (NMA) N-nitroso-N-ethylaniline (NEA)	LC-MS/MS	0,001-0,020
COSMETICS	N-nitroso-di-ethanolamine (NDELA) N'-nitroso-nornicotine (NNN)	LC-MS/MS	0,02

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THANK YOU FOR YOUR ATTENTION

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