LabAnalysis
LIFE SCIENCE

Delivering scientific excellence

NITROSAMINES

Unacceptable levels of nitrosamine impurities were first reported in June 2018. Further nitrosamine impurities were subsequently detected in other medications.

The current regulatory approach (e.g. EMA/FDA) for Carcinogenic Potency Categorization (CPCA) and the corresponding Acceptable Intake (AI, ng/day) is based on:

- **Structure–Activity Relationship (SAR) principles.**
- **Animal carcinogenicity data (e.g. Carcinogenic Potency Database - CPDB).**

This framework assumes that the α-hydroxylation step in the metabolic activation pathway is primarily responsible for the high mutagenic and carcinogenic potency observed in many N-nitrosamines.

Structural features that increase or decrease the likelihood of α-hydroxylation are therefore considered to have a corresponding impact on carcinogenic potency.

Accordingly, the mutagenic and carcinogenic potential of a nitrosamine can be predicted from its structural characteristics.

Based on these principles, five Predicted Potency Categories and their associated AI limits have been established for nitrosamines.

LabAnalysis Life Science has at disposal internal methods already validated (for standard solution part) to be used based on sample type (e.g. API, tablets, injectable solutions, creams etc.) for determination of the small nitrosamines listed in the guidelines (e.g. EMA/FDA).

From revision **16 of document EMA/409815/2020** new changes were implemented to Chapter 10, "What Limits Apply to Nitrosamines on Medicinal Products?"

Taking advantage from the multiannual experience, LabAnalysis supports Customers to evaluate the genotoxic potential of API-specific and generic nitrosamines using the **Enhanced Ames Test** to evaluate **nitrosamines safety** as impurities in medicinal products.

Equipment for nitrosamines determination:

- **8 LC-MS/MS (Triple Quadrupole)**
- **3 LC-HRMS (High Resolution Mass Spectrometry)**
- **2 GC-MS (Single Quadrupole)**
- **2 HS-GC-MS (Head Space) (Single Quadrupole)**
- **3 GC-MS/MS (Triple Quadrupole)**
- **1 GC-NCD (Nitrogen Chemiluminescence Detector)**

In addition, LabAnalysis Life Science has a dedicated team of experts fully involved in the development and validation of ad hoc methods for the determination of NDSRIs (Nitrosamine Drug Substance-Related Impurities). More than 60 methods for NDSRIs determination have been already developed and validated.

WHY LABANALYSIS?

- **+60 NDSRIs experience**
- **Screening and confirmation of small nitrosammine and NDSRIs in API, intermediate and final products**
- **High sensitivity quantification with LC-MS/MS, GC-MS/MS methods etc.**
- **Development and validation of analytical methods accordingly to ICH Q2(R2)**
- **Formation studies of NDSRIs (e.g. NAP test)**

LEACHABLE & EXTRACTABLE

During drug production phase or during drug storage phase, by direct or indirect contact between the packaging material and the active ingredient or the formulated product, numerous contaminants can be released from the materials.

These molecules are **Extractables** or **Leachables** and their determination is one of the regulatory requirements for patient safety.

Extractables are all compounds that can be extracted from materials if placed in contact with solvents or simulants under controlled time and temperature conditions, but worse than the conditions of normal use. Extractables can potentially migrate into the drug.

Contrarily, **Leachables** are all compounds that migrate into the pharmaceutical product from any material because of direct or indirect contact between the material and the product itself during the production phase or during the storage of the pharmaceutical product.

LabAnalysis, with its consolidated experience, acquired from the delivery of hundreds of Extractables and Leachables studies, and with a state-of-the-art analytical instrumentation, will perform **any type of analysis on a very wide type of materials** (primary and secondary packaging, dispensing and dosing systems, industrial components, filtering systems, etc.) and **pharmaceutical products** (active pharmaceutical ingredients and formulated products), according to USP <1663>, <1664> and <1665>.

LabAnalysis will share with the Client the technical approach of the **Extractable** studies defined on a **Study Protocol** containing information regarding the purpose of the activity, the stress (number and chemical properties of the extracting solvents) and the analytical techniques.

- **HPLC-MS-MS in positive for non- volatile compounds**
- **HPLC-MS-MS in negavtive form non-volatile compounds**
- **CS-MS for semi-volatile compounds**
- **ICP-MS for elemental impurities**
- **GC-MS or HPLC-MS for nitrosamines**
- **HS-GC / MS for volatile compounds (directly on the packaging)**
- **IC for ionic compounds**

At the end of the experimental activity, LabAnalysis will draft a related Study Report containing all the experimental information, the numerical data obtained, the mass balances, the chromatographic traces and the considerations on the result.

Based on the chemical compounds obtained from the Extractable study, LabAnalysis can perform the **Leachables** studies to conducted to monitor any substances that leach out of a material into the final drug product during a long-term contact.

In this way it will be possible to evaluate the presence in the finished drug products of substances coming from packaging material.

METHODS DEVELOPMENT AND VALIDATION

Thanks to the expertise of the laboratory, acquired and significantly increased over the years, as well as the preparation of committed teams, we are able to offer methods development and validation services.

Aiming at better understanding the problems and needs, once established the Customer's requests, through direct dialogue and technical support, and based on the current regulations / guidelines, LabAnalysis will develop specific methods, with the use of classical techniques (HPLC-UV, GC-FID, ICP-AES, AAS, Etc...) or more innovative techniques (ICP-MS, LC-MS, LC-MS / MS, LC-HRMS, GC-MS / MS).

The developed methods are thus validated.

The validation activities of analytical methods are carried out in accordance with the requirements of the most common ICH guidelines (the parameters frequently evaluated are: specificity, linearity, accuracy, instrumental precision, repeatability, intermediate precision, limit of quantification, limit of detection, robustness, stability and system suitability) or specific requests by the Customer or specific requests from regulatory entities. The approach proposed by LabAnalysis consists in drafting the **validation protocol** that is shared with the Customer for approval, followed by the validation activity. After the statistical evaluation of the data obtained, LabAnalysis always takes care of drafting the final **validation report**, which will also be shared with the customer.

LabAnalysis can support the Customers with the analytical method transfers, with activities that are similar to what is reported for validations.

STRESS TEST

Forced Degradation (or **Stress Test**) is a process that involves the degradation of active pharmaceutical ingredients or products formulated in a worse environmental condition compared to the condition of normal use of the products. Stress condition mostly involve the use of physical agents (heat, light) and chemical agents (moisture, acids, bases, oxidants). The stress test generates degradation products, known or unknown, which can be investigated to determine the pathways of drug degradation or to prevent it. The stress test also finds applications to support the validation of analytical methods to demonstrate that the method is "stability indicating", which means that is able to quantitatively detect the loss of content of the pharmaceutical active ingredient and the consequent increase in degradation products.

LabAnalysis, with its consolidated experience, acquired from the delivery of hundreds of forced degradation studies, and with a state-of-the-art analytical instrumentation, will perform any type of stress test on active pharmaceutical ingredients or products formulated to **support the submission of registration files all over the world** (our studies are regularly viewed by the main entities such as FDA, ANVISA, AIFA, etc).

For the realization of a stress study, LabAnalysis guarantees the:

- **Use of the best state-of-art technologies for the qualitative and quantitative evaluation of active pharmaceutical ingredients and their products of degradation.**
- **Numerical evaluation and rational discussion of mass balances.**
- **Transfer analytical methods adapting it to mass spectrometers to obtain useful structural information of the main degradation products obtained.**
- **Isolation and characterization of the higher impurities via NMR.**

LabAnalysis will share with the Client the technical approach of the forced degradation studies by drafting a **Study Protocol** containing information regarding the activity purpose, the analytical methods, the stress and processing conditions of the samples, the processing and presentation results, regulatory references. At the end of the experimental activity, LabAnalysis will draft a related **Study Report** containing all the experimental information, the numerical data obtained, the mass balances, the chromatographic traces and the considerations on the results.