Ellutia
Chromatography Solutions

Whitepaper

Testing for nitrosamines in pharmaceuticals

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Nitrosamines, or more correctly N-nitrosoamines, are compounds that feature a nitroso group bonded to a deprotonated amine. Certain nitrosamines belong to the so-called "cohort of concern," which is a group of potent mutagenic carcinogens that have been classified by the WHO's International Agency for Research on Cancer as probable human carcinogens. [i]

The WHO Medicine Regulatory Authorities first became aware of the presence of the nitrosamine impurity N-nitrosodimethylamine (NDMA) in products containing valsartan in July 2018. Valsartan is an angiotensin II receptor blocker (ARB) and belongs to a family of compounds commonly referred to as sartans. Further nitrosamine impurities have been detected in other medicines belonging to the sartan family, as shown in Table 1 below along with the allowable daily intake values set by the WHO to form the basis of the risk assessment.

In September 2019 the European Medicines Agency (EMA) issued instruction EMA/189634/2019[ii] to marketing authorisation holders of all medicines containing chemically synthesised active substances to conduct a risk assessment to evaluate the possibility of nitrosamines being present in every concerned medicine. The deadline for compliance was 26 March 2020, six months after the risk assessment instruction was issued, and companies will then have another two years to take mitigation measures where risks are identified,[iii] starting with the highest risk products. In the US, the Food and Drug Administration (FDA) has acted to call in ARB drugs for testing, enforcing product recalls for popular brands.

Mandatory risk assessment for potential nitrosamines

There is potential for nitrosamines to be present in active pharmaceutical ingredients (APIs) for medicines, depending on the API and the product manufacturing processes. If the product contains a small molecule API made by chemical synthesis, it is at risk. Because of this risk, all relevant pharmaceutical marketing authorisation holders (MAHs) are responsible for conducting a risk assessment.

How do nitrosamines enter the pharmaceutical supply chain?

Nitrosamines are formed by the interaction of secondary or tertiary amines with nitrite ions.

A source of contamination in pharmaceutical manufacturing includes nitrosamine impurities occurring during API processing. However, the possibility of nitrosamines being created goes broader than the presence of both nitrites and amines during synthesis of the API. Nitrites or amines may also be present in the raw materials themselves, or in the reagents or solvents.

For example, using sodium nitrite ($NaNO_2$) in the presence of secondary or tertiary amines creates a potential source of nitrosamines. Secondary amines can either be present as contaminants in reagents and solvents or may actually be part of them. Amine solvents can degrade to secondary amines which are known sources of nitrosamines. Dimethylamine in the common solvent dimethyl formamide (DMF) can cause nitrosamines to form. Tertiary amines include common bases which allow nitrosamine formation.

Nitrosamines may also be present in APIs following the use of contaminated raw materials in manufacturing. Recovered solvents, reagents and catalysts may pose a risk largely due to their quenching with nitrous acid on completion of the manufacturing process, and if the recycling is outsourced to a shared facility, the risk of cross-contamination increases.

The raw materials themselves may be a source. There are no generally recognised standards relating to nitrosamine levels in the potable water used in pharmaceutical product manufacture. WHO

Impurity (abbreviation)	Chemical name	Allowable daily intake
NDMA[6]	N-nitrosodimethylamine	96.0 ng/day
NDEA[6]	N-nitrosodiethylamine	26.5 ng/day
NMBA[7]	N-Nitroso-N-methyl-4-aminobutyric acid	96.0 ng/day
DIPNA[7]	N-nitrosodiisopropylamine	26.5 ng/day
EIPNA[7]	N-nitrosoethylisopropylamine	26.5 ng/day

Table 1: Interim allowable daily intake limits for selection N-Nitrosamine impurities

guidelines suggest a limit of 100 parts per billion, but different countries may set their own interpretation. A key issue here is that the API manufacturer may not be aware of the risk of nitrosamines in the raw materials, particularly if nitrosamines cannot otherwise form during the manufacturing process, so will not realise the need for risk assessment.

Where does the risk responsibility lie?

The risk assessment introduced for nitrosamines in pharmaceuticals by the EMA places responsibility firmly in the hands of the MAH. This confirmatory testing should be carried out as soon as possible, starting with priority risks.

Current recognised methods for testing small molecule pharmaceutical products include gas chromatography-mass spectrometry (GC-MS), liquid chromatography (LC)-MS/MS or high resolution (HR) LC-HRMS. Introducing a new step, however, or having to outsource testing at different stages of production, can be expensive and can introduce production delays.

Pre-screening with thermal energy analysis

The Ellutia 800 Series thermal energy analyser (TEA) detector can be used in-house to perform both routine pre-screening and more detailed speciated analysis of nitrosamines in pharmaceutical samples. Its selectivity and sensitivity for nitroso-containing compounds has led it to become the standard for nitrosamine analysis in many industries, used extensively in GMP environments including food safety and materials manufacturing.

Rather than start with a full speciated analysis, the apparent total nitrosamine content (ATNC) method using TEA detection is a screening test that determines the total nitrosamine content in a sample.

ATNC gives a rapid and accurate result for the total nitrosamine content of a sample showing both volatile and non-volatile components in a single test. Any sample showing an ATNC below the specified level of concern cannot possibly contain any one compound above the total level, and so it can be deemed safe. Any sample testing positive for the ATNC value can be further analysed using the TEA interfaced to a GC where volatile nitrosamines such as NDMA (N-Nitrosodimethylamine) can be separated and quantified.

To perform the ATNC analysis the 800 Series TEA is interfaced to a proprietary chemical stripping system. There is no need for complex and lengthy sample preparation. A chemical reaction is used to reduce the nitrosamine by reflux reaction with hydrobromic acid in ethyl acetate. The nitrosamine sample is injected into the reaction vessel to produce NO, a secondary amine and bromine. The NO is carried in a gas stream to the TEA, passing through a cold trap to remove vapour. In the reaction chamber the NO is oxidised by ozone to produce NO_2, releasing a photon of light. This light energy is detected by the TEA as shown in Figure 1.

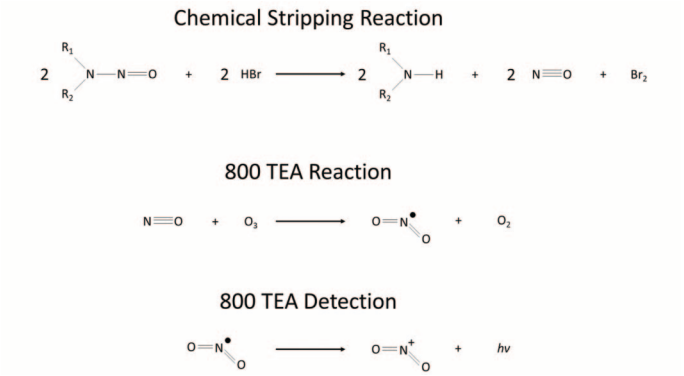

Figure 1: Chemical reaction employed to strip down nitrosamine source

Optimising the extraction procedure for the specific pharmaceutical product is important to ensure the nitrosamine has dissolved into the extraction solvent, which is then typically filtered to facilitate the injection. Because the liberated NO is a molar function, semi-quantitation of the resultant peaks can be performed as shown in Figure 2.

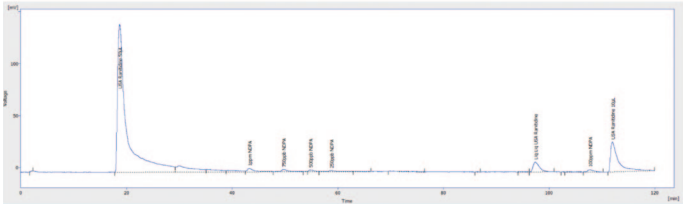

Figure 2: Example chromatogram obtained from a TEA Chemical Stripping Analysis

If further speciated analysis is required, the TEA detector can be interfaced to a GC or HPLC system. In these configurations after the compounds are separated a pyrolyser is used rather than a chemical reaction to break the bonds to liberate the NO. The reaction of the NO with Ozone within the TEA Remains the same. The selectivity and sensitivity for nitroso compounds makes the TEA an ideal choice for this application.

The future of nitrosamine testing

The recommendations of the WHO are clear regarding nitrosamine impurities in medicines. If the level of any nitrosamine impurity is below the interim acceptable limits, products are generally considered safe and may remain on the market. In cases where the levels of nitrosamines exceed acceptable limits, these products should in general not be permitted on the market.

This new risk assessment for the nitrosamines within the WHO 'cohort of concern' is just the starting point. As well as the EMA, authorities in Canada, South Korea and Switzerland have also issued similar instructions. In the USA, the Food and Drug Administration (FDA) has implemented a recall of medicines with levels above accepted interim limits. It is clear that MAHs must implement a risk assessment programme and be able to demonstrate the results of their assessment. The 800 Series TEA is uniquely capable of a pre-screen assessment in accordance with the EMA mandate.

The Ellutia 800 Series TEA detector interfaced with the unique chemical stripping system offers a pass/fail prescreen of pharmaceutical compounds for nitrosamines. Further speciated nitrosamine analysis is also possible for full identification and quantification.

References

[i] WHO Information Note: Update on Nitrosamine Impurities, 2020, https://www.who.int/medicines/publications/drugalerts/InformationNote_Nitrosamine-impurities/en/

[ii] Information on nitrosamines for marketing authorisation holders, EMA/189634/2019

[iii] WHO Information Note: Update on Nitrosamine Impurities, 2020, https://www.who.int/medicines/publications/drugalerts/InformationNote_Nitrosamine-impurities/en/

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