Nitrosamines: The race to regulation

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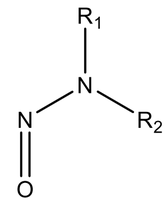
Following the discovery of N-nitrosodimethylamine (NDMA) in blood pressure medication, a “call for review” released by the European Medicines Agency (EMA) requested screening of all chemical and biological medicines for human use, for the presence of nitrosamines. Whilst nitrosamine impurities present a low hazard in the quantities found in medicinal products, the carcinogenic properties of nitrosamines has to be addressed.

This poster presents the techniques and expertise which have led nitrosamine impurity analysis to become an essential part of Pharmaceutical Chemistry Chromatography’s portfolio, ensuring RSSL are now an industry-trusted choice for nitrosamine screening.

What are nitrosamines?

Nitrosamines are organic compounds with a generic chemical structure of $R_2N–N=O$. They are generally produced by the reaction of nitrous acid and secondary amines. They are classified as 'probable human carcinogens' based on studies conducted on animals.



Nitrosamines are present in low levels in processed food, beer, tobacco, etc... The use of nitrites as preservative in food can cause the formation of nitrosamines under the right conditions. In pharmaceutical products, nitrosamines have been first identified in 2018, when EU regulators discovered the presence of NDMA in blood pressure medicines of the sartans family.

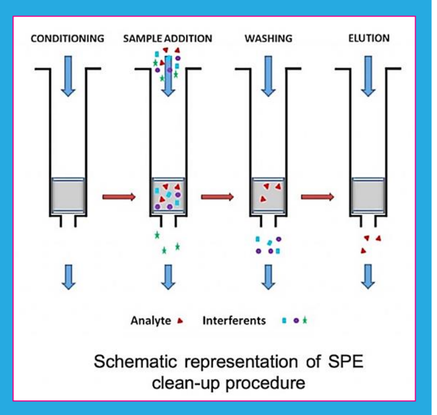
Following this discovery, the regulating authorities (EMA, MHRA, FDA, etc...) provided guidance to marketing authorisation holders. The guidance includes:

- risk evaluation, to identify any potential reaction or cross-contamination during manufacturing processes;
- confirmatory testing, to assess the presence or absence of nitrosamines in a product;
- changes in manufacturing process to reduce or remove the risk of nitrosamines formation.

Sample preparation

Solid Phase Extraction (SPE)

SPE is a common technique used to simplify sample matrix, reduce interference and improve analyte recovery and sensitivity. To extract nitrosamines, the team use strong cation exchange (SXC) cartridges with a low pKa sorbent, combined with aromatic groups and negatively-charged surface groups. By inducing acidic conditions, the resulting positively-charged nitrosamines are attracted to anionic surface groups and be retained on the sorbent material. A solvent with a higher pH is then used to draw out any nitrosamines into the clean solvent system, ready for analysis.



Schematic representation of SPE clean-up procedure

Liquid / liquid extraction

An alternative technique is liquid-liquid extraction. This method is used for samples that are not miscible in another solvent, e.g. dichloromethane (DCM), acetonitrile. On mixing, nitrosamines will transfer from the sample to the other solvent, minimising potential matrix effects from the sample. The solvent layer is then extracted, and the process is repeated twice more. The theory being, 90% of the analytes of interest will be removed in the first wash, 9% in the second and 0.9% in the third, resulting in a total theoretical recovery of 99.9% of the analyte of interest from the original sample.

Analysis

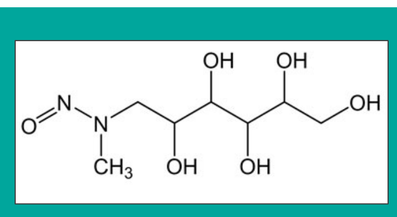
Liquid chromatography-mass spectrometry (LC-MS)

LC-MS is a powerful analytical technique that combines Liquid Chromatography for separation of mixtures containing multiple components, and Mass Spectrometry for identification and quantification of the components. Nitrosamines were traditionally analysed by GC-MS, however after the FDA mandated the investigation of nitrosamines, they released a LC-MS method, with this technique becoming the standard.

Using an LC-MS TQ (Triple Quadrupole) instrument allows the team to benefit from greater specificity in compound identification. The TQ identifies the ion of interest by its mass in the first quadrupole. In the second quadrupole, also known as the collision cell, the selected ion is fragmented in a predictable manner every time. Finally, in the third quadrupole the ion fragments are allowed to pass through whilst all other potential ions are filtered out. This final step enhances specificity allowing for the greater sensitivity of the instrument. The high sensitivity of the instrument is required as the team regularly analyses samples in the region of 3-4 ng/ml.

RSSL case study

RSSL were approached by a client with several contrast agent products in need of nitrosamine testing. These samples had previously been investigated unsuccessfully by another lab.



Development of a suitable sample preparation process to prepare LC-MS compatible samples was the first stage. The aim of the sample preparation was to provide sufficient clean up and removal of incompatible excipients and interferent species, as well as a suitable level of concentration to allow detect of nitrosamines at or below the threshold level

The final sample preparation method involved use of Carrez solution, a protein precipitation tool used to help precipitate out API and excipients that were not MS compatible. Following this, a liquid-liquid extraction was carried out using DCM. This liquid-liquid extraction enabled further clean-up of interferent species from samples as well concentration of nitrosamines within these samples to a detectable level. The concentration step is done by evaporating the DCM using nitrogen flow and then reconstituting the samples in water. This has the benefit of enabling testing at the regulatory threshold.

A specific challenge of the development process for sample preparation is the widely varying properties of the individual nitrosamines being tested for, while some nitrosamines readily go into DCM some nitrosamines do not and instead largely remain in the aqueous phase. N-nitroso-meglume is one such example.

Data interpretation

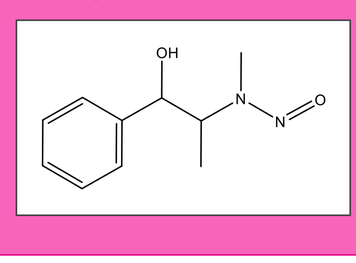
The analytical data produced requires interpretation to understand the results. First, a total ion chromatogram (TIC) is produced (Fig. 1), showing a summation of all the separate ion currents within the specified m/z range. A multiple reaction monitoring (MRM) chromatogram is then extracted from the TIC, showing data for multiple pre-set product ions from corresponding product ions (Fig. 2). Typically for nitrosamine analysis, clients only require data to be below an established threshold. By comparing sample chromatograms to a standard spiked at a known threshold, RSSL can provide clients with vital information to ensure products remain safe to sell.

Future of Nitrosamines

The assessment of nitrosamines in pharmaceuticals will be continue in the future. There is continuous testing for those nitrosamines that are present at low concentrations and for those products that have the propensity to form nitrosamines. There is a recent focus of API related nitrosamines with ever increasing toxicological data available that can alleviate the need to use the very low threshold of 18 ng/day. The long-term trend will focus on these substance related nitrosamines, where the understanding of chemical reactions generating nitrosamines has shown the possible formation of unique nitrosamines.

On example is N-Nitroso-ephedrine (structure seen),, which could form in the presence of nitrite under acidic conditions.

Nitrosamine analysis will continue to challenge in the impurity analysis of pharmaceuticals.



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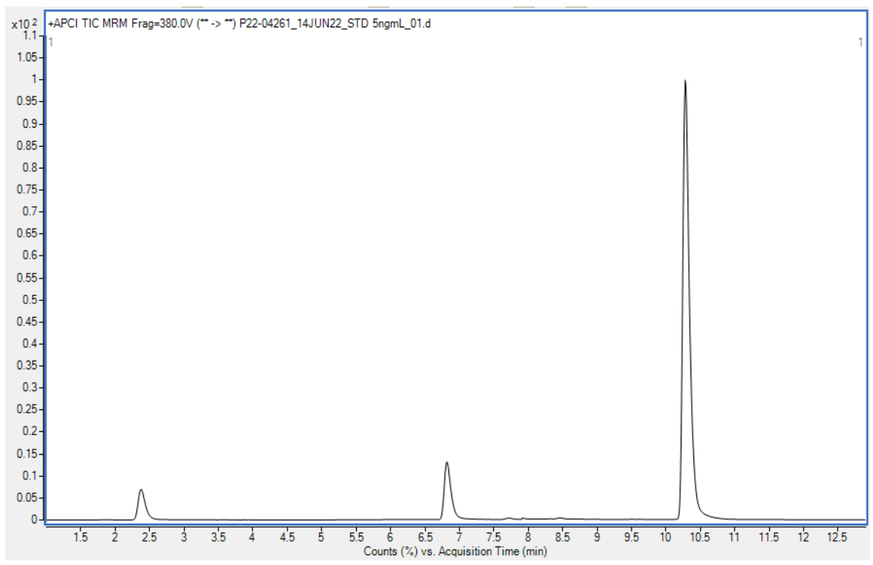

Figure 1 Total Ion Current (TIC) chromatogram.

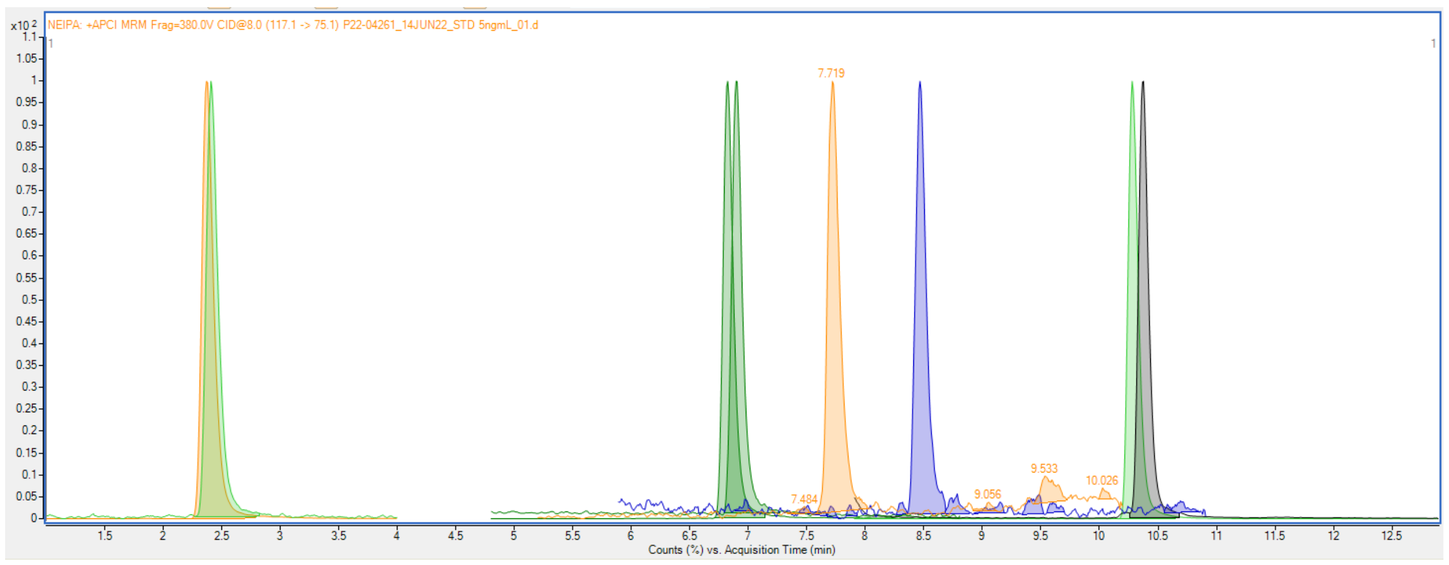

Figure 2 Multiple Reaction Monitoring (MRM) chromatogram extracted from the TIC, Fig 1.

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