Nitrosamines
Analysis for Extractables and Leachables

White Paper

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Abstract

Nitrosamines are potentially carcinogenic and mutagenic compounds [1] that have been shown to occur in many products, including the rubber seals and stoppers used on process lines, and in some medical devices. As such, there is a risk of nitrosamines appearing in pharmaceutical (and other) products through leaching, and this possibility must be properly evaluated and mitigated by manufacturers prior to marketisation of any product.

The methods traditionally used for the analysis of nitrosamines have been characterised by high cost and complexity. However, the Chemiluminescence detector makes it much easier to develop methods for routine analysis and, also, improves the selectivity and sensitivity of the test.

This selective detection method greatly improves the prospect of detecting nitrosamines in pharmaceutical products and medical devices that incorporate rubber or latex.

Nitrosamines Background

An association between NDMA (N-Nitrosodimethylamine) exposure and liver damage in rats was first discovered in the late 1950s. Then, during the 1970s, following outbreaks of liver disorders in farm animals in Norway, researchers discovered a link between their diet of fishmeal and formation of cancer. The fishmeal had been preserved with sodium nitrite, which it was later revealed, had led to the formation of NDMA. Subsequently, many other compounds having the same functional group, (see Figure 1) have been identified as potential carcinogens. These nitrosamines have also been shown to occur in a number of product types (see Table 1).

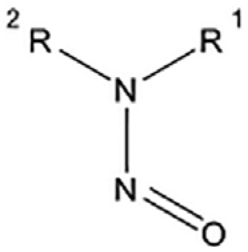


Figure 1

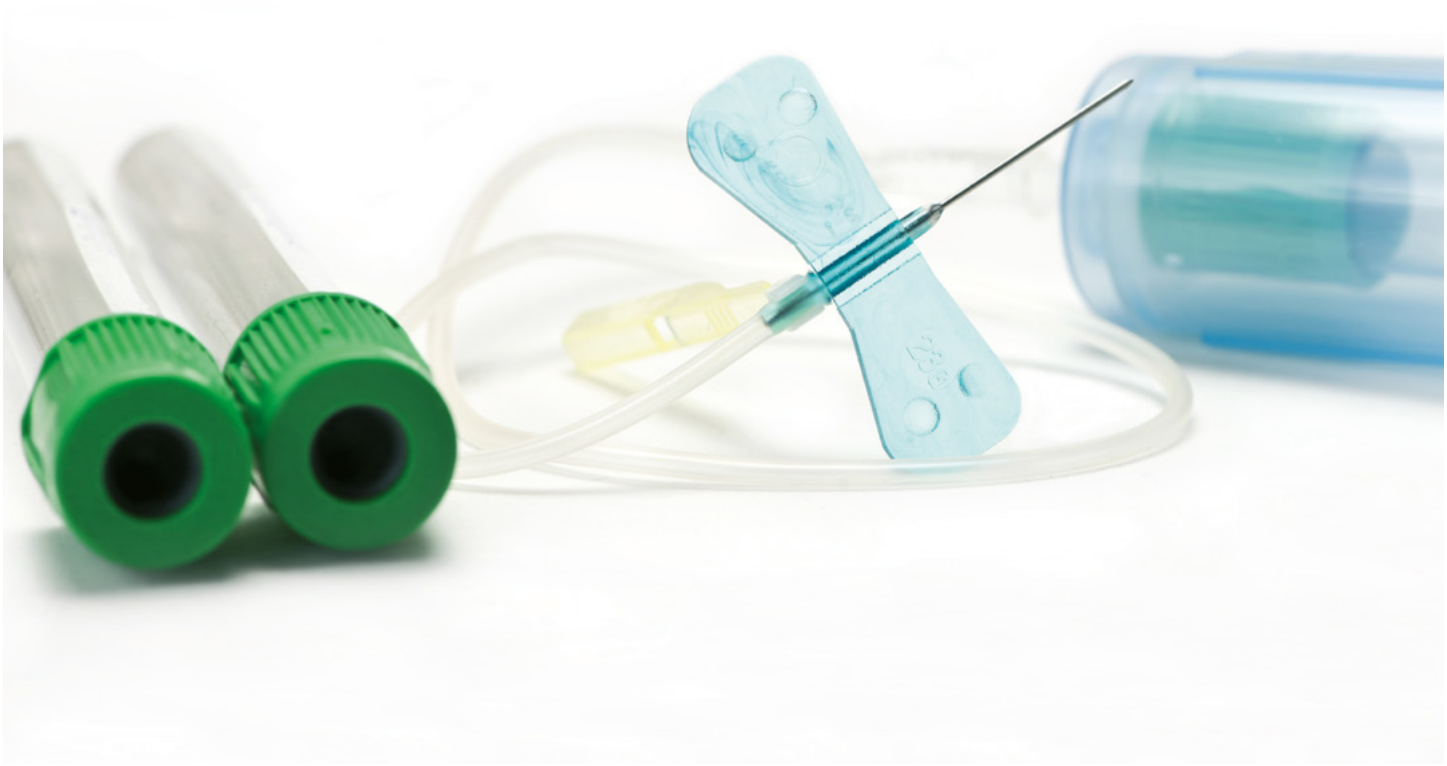
Matrix
Foodstuffs e.g. meat, grain, oils
Pharmaceuticals e.g. antihistamines, tetracycline, piperazine
Biological specimens e.g. animal tissue
Environmental samples e.g. wastewater
Industrial chemicals e.g. pesticides, coolants
Other matrices e.g. pacifiers, cosmetics

Table 1

Nitrosamines are not added to products, but are formed in situ. For example, they are formed in food products from nitrites which are typically applied as preservatives. They are formed in cosmetics and in beer through reaction of nitric oxides with alkaloids. In the pharmaceutical and healthcare sectors, one source of nitrosamines is rubber which can be found in a variety of medicinal devices such as stoppers and in parts of inhalation devices. Studies of carbon black which was used as a filler in valve elastomers showed that they could contain some or all of the following:[2]

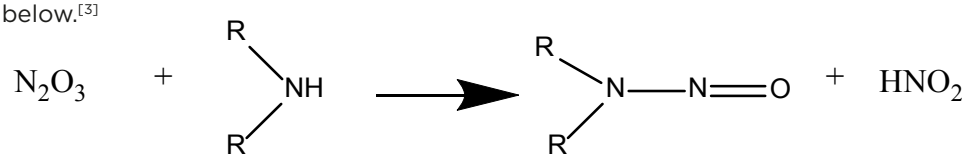
N-nitrosodimethylamine	N-nitrosomorpholine
N-nitrosodiethylamine	N-nitrosomorpholine
N-nitrosodi-n-butylamine	N-nitrosopyrrolidine

The risks associated with rubber products was highlighted most famously, by a German study on the occurrence of volatile N-nitrosamines (nitrosamines) in rubber baby bottle teats back in 1981. A follow-up investigation by the Food and Drug Administration (FDA) revealed the presence of nitrosamines in similar products marketed in the United States and showed that the nitrosamines could migrate into foods, such as milk and infant formula, from contact with the rubber teat during the conventional sterilisation process. This ultimately led to manufacturers altering product formulas and manufacturing processes to reduce nitrosamine formation, and to adopt a limit of 10 ppb or less.



Nitrosamine Formation

Nitrosamines are formed when secondary amines for example dimethylamines react with nitrosating agents such as nitrogen oxides. Secondary amines can be found in accelerators which are used to increase the speed of vulcanisation (cross linking to create a three dimensional network of polymers) so this is why one of the main materials in which nitrosamines are found is rubber products. They are also formed in foods due to the presence of nitrite which is added as a preservative, this reacts with amines and amides forming N-nitrosamines. The formation of nitrosamines is shown below.[3]



Detection of Nitrosamines

The analysis of nitrosamines has typically involved the use of a Gas Chromatography-Thermal Energy Analyser (GC-TEA) or Gas Chromatography Mass Spectrometer (GCMS). However, older TEA instruments were often complex to repair, and required manual adjustment of method settings e.g. gas flows, thereby making method development slow.

Methods have also been documented for the use of Liquid Chromatography Mass Spectrometry (LCMS) and GCMSMS but both require a large initial outlay of capital.

Nitrochemiluminescence Detectors

A better solution to nitrosamine detection and quantitation is offered by modern Nitrochemiluminescence Detectors, which can be attached to existing chromatography instrumentation and combine electronic pressure control with simplified detector design. This means they provide a robust and cost effective route to developing methods for detection of nitrogen containing compounds, and running routine samples.

Unlike a conventional Flame Ionisation Detector (FID) type detector the Nitrochemiluminescence Detector (NCD) is highly selective, only responding to nitrogen containing compounds. NCD detectors work by converting nitrogen atoms within the analyte in an oxygen and hydrogen plasma to NO. The NO is then reacted with ozone which produces NO_2 in an excited state. This then emits light at a specific frequency (600-3200nm). The reactions are displayed below.

$$R\text{-}N + O_2 \longrightarrow NO + CO_2 + H_2O$$

$$NO + O_3 \longrightarrow NO_2 + O_2 + light$$

Not all nitrogen containing analytes will react with ozone in the gas phase hence a conversion step (pyrolysis) is needed, this 'oxidative combustion' converts all nitrogen containing analytes to NO which can then be converted to NO2 in its excited state. A catalyst is used to prevent secondary species from being formed. A typical burner can be seen opposite (Figure 2).

The NCD detector is valuable, not only for detecting nitrosamines in the context of extractables and leachables, but any nitrogen containing compound of relevance to pharmaceutical products, medical devices and raw materials testing e.g. Trolamine.

So, this might mean other extractable/leachable molecules that contain nitrogen, as well as any other nitrogen containing active or its analytes. Clearly, with the abundance of nitrogen containing molecules in nature, there is huge scope for analysis using this highly specialised and selective detector.

Returning to the specific case of nitrosamine analysis, this selectivity reduces matrix interferences and enables low detection levels to be reached.

It is possible to improve the selectivity for nitrosamine compounds further by breaking only the N-NO bond which is typically weak. Lowering the pyrolysis temperature allows the following conversion to occur. [4]

$$R\text{-}N\text{-}NO \longrightarrow NO + R\text{-}N$$

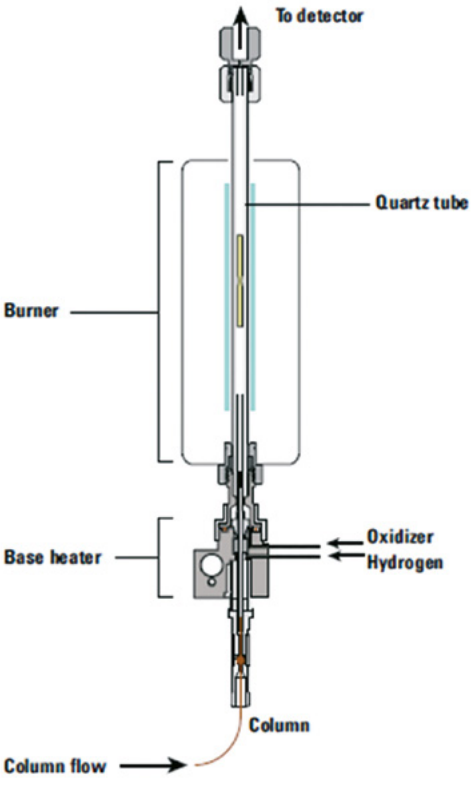


Figure 2

This produces only one NO molecule for each Nitroso group present, reducing the overall detector response but improving the selectivity. Below we can see the increase in sensitivity as we move from 700°C to 900°C burner temp (Figure 3).

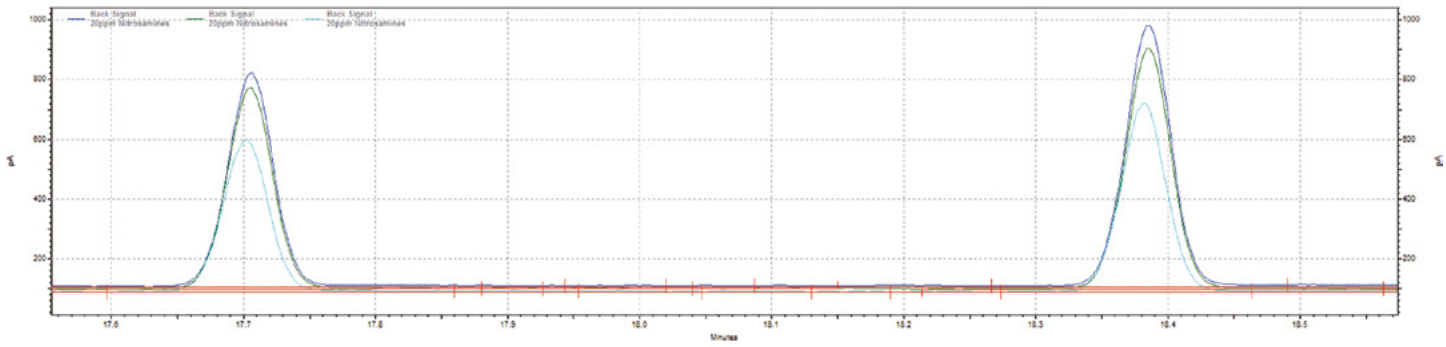


Figure 3: Increase in peak response with increasing detector temperature for a 1ppm standard.

The example below compares a sample run with a burner temperature of 900°C (green trace) to the sample with a burner temperature of 400°C (light blue trace). This sample was chosen to provide a complex matrix to challenge the selectivity of the instrument. Figure 4 shows a marked reduction in the presence of nitrogen containing peaks whereas the nitrosamines are still visible. The third chromatogram (dark blue) is a nitrosamine standard run to provide retention time information.

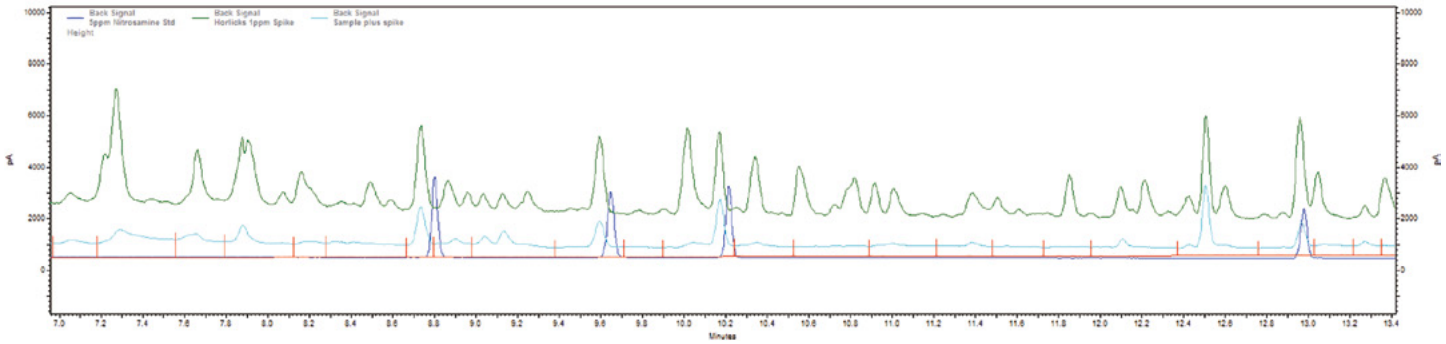


Figure 4: Complex matrix run at 2 different detector temperatures.

The selectivity of the detector can be further demonstrated below, a sample spiked at 1ppm with a nitrosamine mix was analysed by GCMS, then GC-NCD. The chromatogram in figure 5 shows the GCMS trace of the peak for N-Nitrosodimethylamine at 11.11 minutes. This can only be detected when the molecular ion at 74m/z is extracted from the trace. Without knowledge of its presence in the sample it could go undetected.

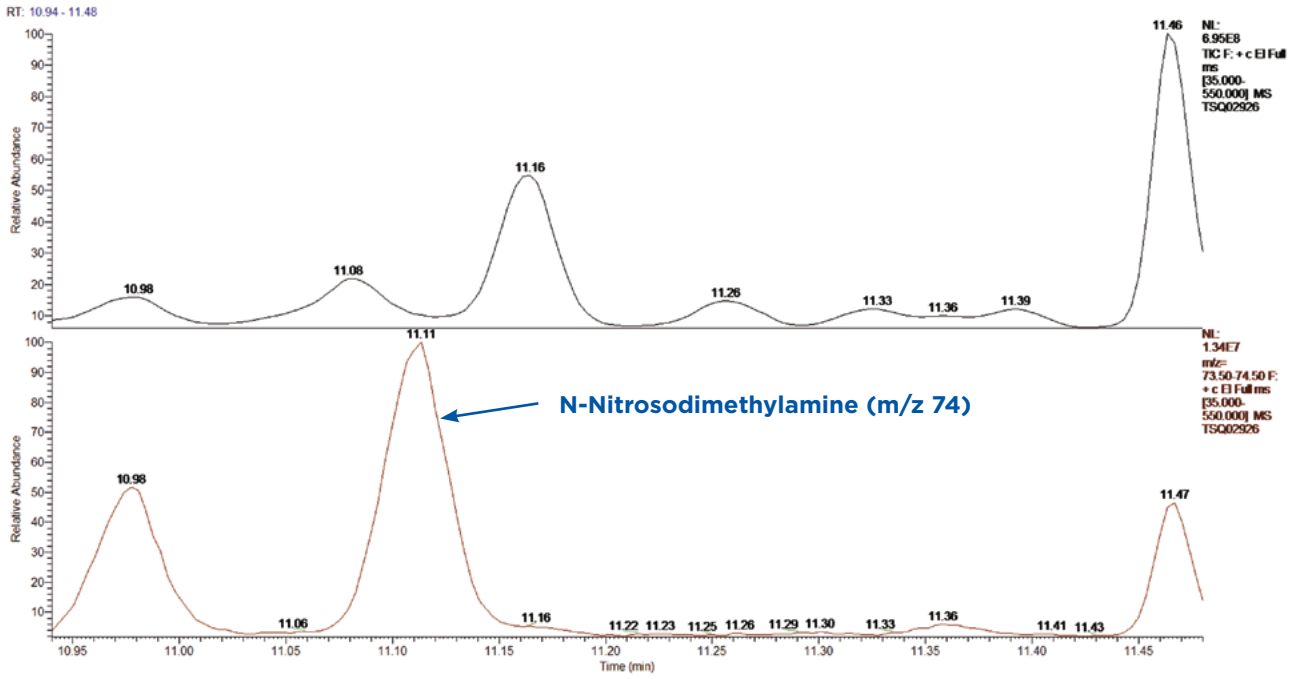


Figure 5: GCMS trace with extracted ion to show nitrosamine peak.

Conversely the sample run on the NCD shows a clear peak in figure 6 for N-Nitrosodimethylamine which is not seen in the unspiked sample (blue trace), demonstrating the superior selectivity of the detector.

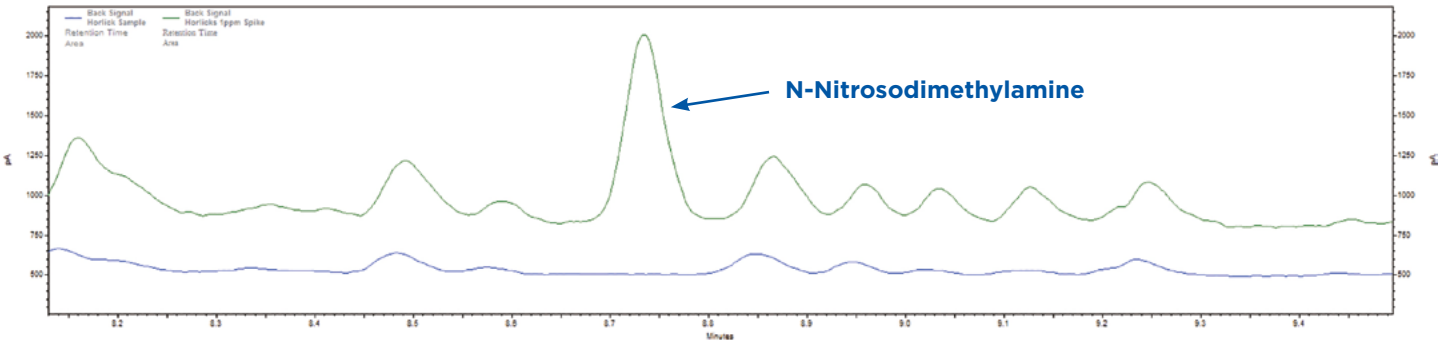


Figure 6: NCD trace of unspiked (blue) and spiked (green) sample.

Conclusion

To reach the low detection levels required for the analysis of nitrosamines a detector that is both sensitive and selective is required. The Nitrochemiluminescence detector demonstrates sub ppm levels of detection whilst selectively responding to nitrogen containing compounds even in a complex matrix.

RSSL is an established expert in this field, we have performed controlled extractables and leachables studies on a wide variety of containers, closures and medical combination drug delivery devices using a range of analytical technologies (LC-MS, GC-MS, ICP-MS), including nitrosamine analysis using GC-NCD.

To find out more about our extractables and leachables services, please contact us on: +44 (0)118 918 4076, email enquiries@rssl.com, or visit www.rssl.com

References

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About the author

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Paul has over 20 years' experience as an analytical scientist, having graduated in 1994 from the University of Birmingham with a Bachelor's Degree in Chemistry. He has worked in a number of sectors including defense, pharmaceutical, environmental and agricultural to date. Paul specialises in chromatographic analysis and is a qualified NVQ assessor working with the RSC on accreditation of training courses.

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