TECHNOLOGY BRIEF

TRACE LEVEL ANALYSIS OF NDMA USING GAS CHROMATOGRAPHY AND THE LUMA™ MULTI-CHANNEL VACUUM ULTRAVIOLET (VUV) DETECTOR

GOAL

Demonstrate measurement of nitrosamine impurities, specifically NDMA, using gas chromatography (GC) and vacuum ultraviolet (VUV) detection.

BACKGROUND

Mutagenic impurities, such as nitrosamines are thought to cause DNA mutations, potentially leading to cancer. Since 2018 there has been a concerted effort by various regulatory bodies including the ICH and FDA to regulate the average daily intake of nitrosamines in a wide range of drug products. This added scrutiny has resulted in a need for sensitive and reliable instrumentation capable of the detection and quantification of nitrosamine impurities along with other potential genotoxic impurities (PGIs) at trace levels.

Measuring nitrosamines in drug products presents several challenges due to the complex nature of pharmaceutical formulations and the low levels of nitrosamines typically present in these products. Some of the major challenges include:

1. **Sensitivity:** Nitrosamines are typically present in drug products at very low levels, often in the parts per billion (ppb) or parts per trillion (ppt) range, which makes their detection and quantification challenging. Sensitive analytical methods and instrumentation are required to accurately measure such low levels of nitrosamines.

2. **Matrix interference:** Pharmaceutical products contain a complex matrix of excipients and other ingredients, which can interfere with the measurement of nitrosamines. These interferences can lead to false positives or negatives and can reduce the accuracy of the measurement.

3. **Stability:** Nitrosamines are not stable and can degrade over time, making it difficult to accurately measure their levels in drug products. This degradation can occur during sample preparation, storage, and analysis.

Figure 1 — LUMA Multi-Channel Vacuum Ultraviolet Absorbance Detector

4. **Analytical Methods:** There are several analytical methods available for measuring nitrosamines, including gas chromatography (GC), liquid chromatography (LC), and mass spectrometry (MS). Each method has its advantages and disadvantages, and selecting the appropriate method depends on the specific drug product and the type of nitrosamine being measured.

5. **Regulatory Compliance:** There are strict regulations on the allowable levels of nitrosamines in drug products, and meeting these regulatory requirements can be challenging. Manufacturers must ensure that their products are consistently below the regulatory limits and that they have reliable methods for measuring nitrosamine levels.

In this work, we present a preliminary GC method using the LUMA™ Multi-channel Vacuum Ultraviolet detector for the trace level detection of N-nitrosodimethylamine (NDMA).

SOLUTION

The GC-VUV separation was performed using an Agilent 8860 Gas Chromatograph equipped with a LUMA Multi-Channel Vacuum Ultraviolet Absorbance detector, a Supelco SPB-5 (30m x 0.25mm, 0.25µm) column and the OpenLab Chromatography Data System software. Method conditions for this experiment are described in Table 1.

LUMA is a powerful multi-channel VUV detector that is both sensitive and universal. LUMA acquires data using twelve independent wavelength bands. Each band or channel represents a different wavelength range. While Nitrosamines show absorbance in several wavelength bands on LUMA, absorbance is particularly strong in Band 10 which includes wavelengths from 232nm to 261nm. Using band 10 therefore provides for the best sensitivity for this analysis.

GC Conditions	
Injection Volume	4µL
Inlet Temperature	200°C
Splitless Injection	0.75 min
Column	Supelco SPB-5 (30m x 0.25mm, 0.25µm)
Carrier Gas	Hydrogen @ 1.2mL/min Constant Flow
Oven Program	40°C, hold 0.1 min; 35°C/min to 250°C (8 min)
Run Time	14 minutes
LUMA Conditions	
Makeup Gas / Pressure	N_2 / 17 PSI
System Gas / Pressure	N_2 / 58 PSI
Flow Cell Temperature	275°C
Transfer Line Temperature	275°C
Acquisition Frequency	10 Hz
Acquisition Band	10 (232-261nm)

Table 1 — Instrument conditions for analysis of NDMA.

LINEARITY, SENSITIVITY, AND REPEATABILITY

To determine to lower limits of detection a standard test mixture was prepared at a concentration of 100 ppm. Serial dilutions were then made to determine both the lower limit of detection (LOD) and linearity. The concentrations used for the linearity test were 15, 25, 45, and 65 ng/mL (ppb) respectively.

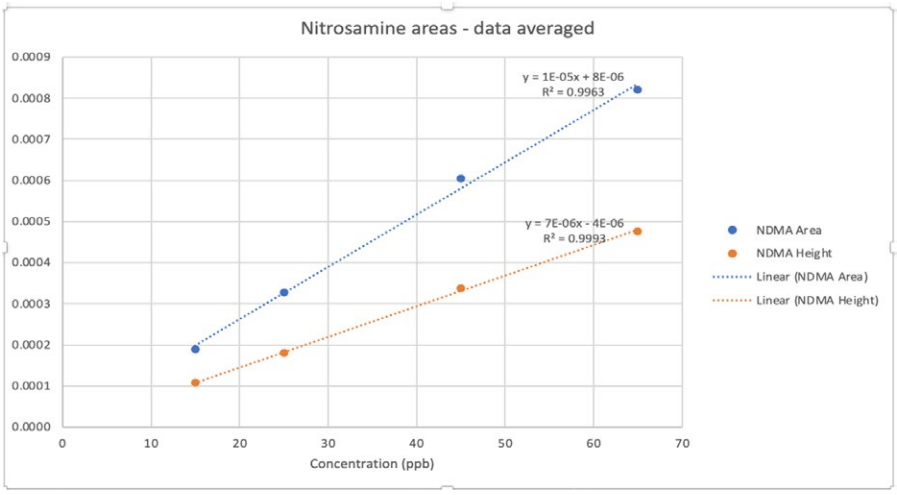

Figure 2 — NDMA Linearity for Area and Height.

Very good linearity was obtained using external calibration for the following concentrations of NDMA: 15, 25, 45, and 65 ng/mL (ppb), as shown in Figure 1. The correlation coefficient R2 of NDMA was 0.9963 (area) and 0.9993 (height) respectively.

Sensitivity of the method was calculated by assessing the S/N of the compounds of interest in the sensitivity test solution. The limit of detection (defined as LOD, S/N ≥ 3) of NDMA was 15 ppb shown in Figure 2.

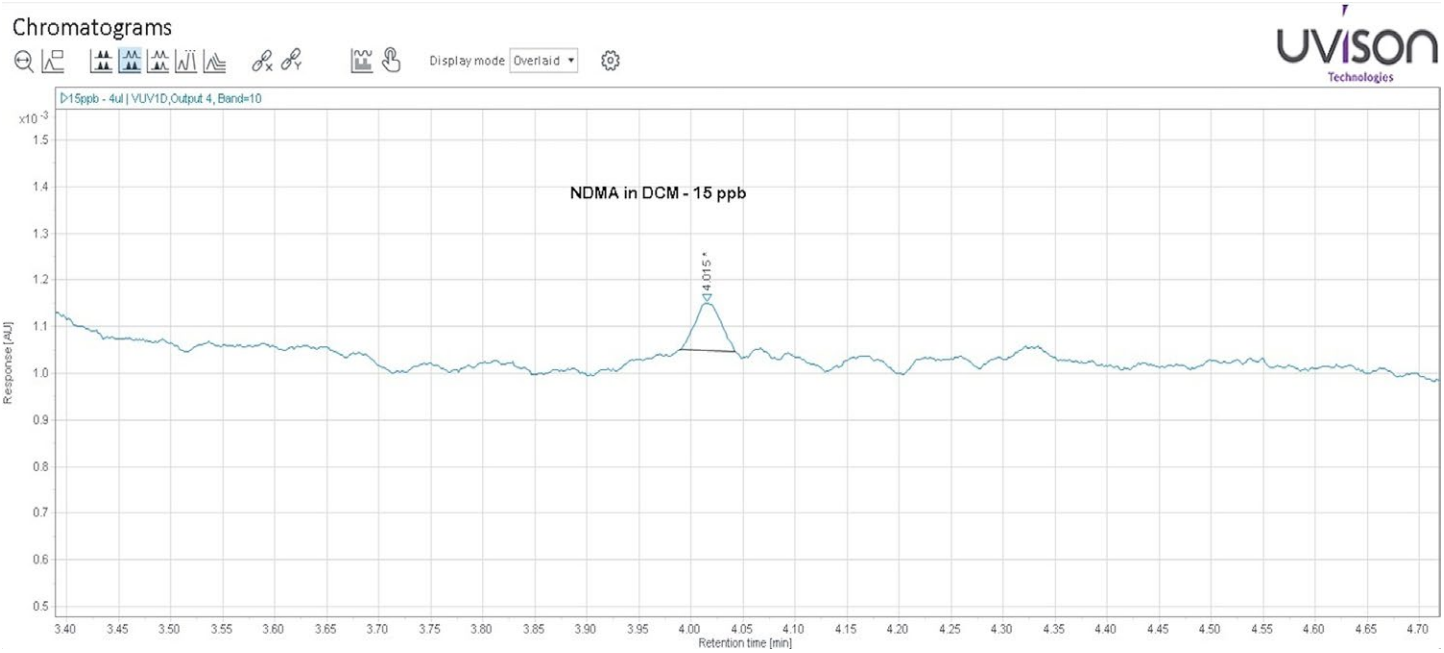

Figure 3 — NDMA @15ppb, no concentration step.

The repeatability of the method was also investigated. The %RSD of the absolute peak area for consecutive n=6 injections of reference solution at 45 ppb on column was 9.28% for NDMA (Table 2). Repeatability can be improved with further method optimization.

Peak Areas		Repeatability of NDMA	
Run	RT (Min)	Area	Height
1	4.007	5.855E-04	3.105E-04
2	4.011	5.065E-04	2.925E-04
3	4.000	5.966E-04	3.416E-04
4	4.003	4.727E-04	2.681E-04
5	4.004	5.418E-04	3.054E-04
6	3.999	5.003E-04	3.058E-04
Average	4.004	5.339E-04	3.040E-04
Std dev	0.00447	0.00005	0.00002
%RSD	0.11%	9.28%	7.90%

Table 2 — N=6 replicate injections @45ppb.

CONCLUSIONS

The results presented in this work clearly demonstrate that the LUMA Multi-Channel Vacuum Ultraviolet Absorbance detector can be used to detect nitrosamines like NDMA at trace levels without the need for a concentration step. Additional development is needed to optimize this method, perhaps driving lower levels of detection and quantification for NDMA and other nitrosamines. While 15ppb is above the lower limit as required by regulatory bodies, the desired LOD for NDMA in formulated products can be achieved with a simple concentration step.

While the analysis of nitrosamines requires a high degree of detector sensitivity to meet stringent regulatory requirements, most pharmaceutical impurities do not need to be measured to this level. As such, LUMA's excellent sensitivity combined with the fact that it is a universal detector, where nearly all compounds absorb make it an excellent detector for the analysis of a wide range of mutagenic and potential genotoxic impurities without adding any additional operational complexity to your analytical workflow.

Future work will continue to highlight the sensitivity advantages of LUMA but will also focus on the ability to analyze multiple types of impurities, traditionally performed by multiple analytical techniques in a single analysis.

ACKNOWLEDGEMENTS

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