

Application Note

NDMA separation from DMF

Introduction

Nitrosamines have come under enormous scrutiny in the last few years since they were found in API's and drug products. Widely suspected of being a human carcinogen, Nitrosamines must now be monitored for their presence and potential introduction in manufacture since being found in Ranitidine and Metaformin, amongst other drugs products, by the US FDA¹.

Nitrosodimethylamine (NDMA) is one of the more common nitrosamines and has a potential problem with co-elution with N,N-dimethylformamide (DMF) which is a

“Nitrosamine separation from DMF is challenging due to the high concentration of DMF often present”

common solvent and a precursor to NDMA. Resolution between the pair needs to be high to ensure no coelution when high concentrations of DMF are present. In this study we show how high resolution was achieved on a Evosphere AQUA column sufficient to not cause issues, Fig 2.

Experimental Analysis

In this application note we show the ability of the new Evosphere[®] AQUA column in conjunction with a simple mobile phase to produce full resolution and offer good sensitivity. Evosphere is built around a new Monodisperse Fully Porous Particle (MFPP) which is designed to provide more efficiency than traditional polydisperse particles. So in this application note a 3µm Evosphere particle is providing the efficiency and sensitivity of what would be expected if using a UHPLC sub 2µm particle, but with less backpressure. If you run with a UHPLC particle then you can get a much elevated backpressure and the potential for blockage and robustness issues,

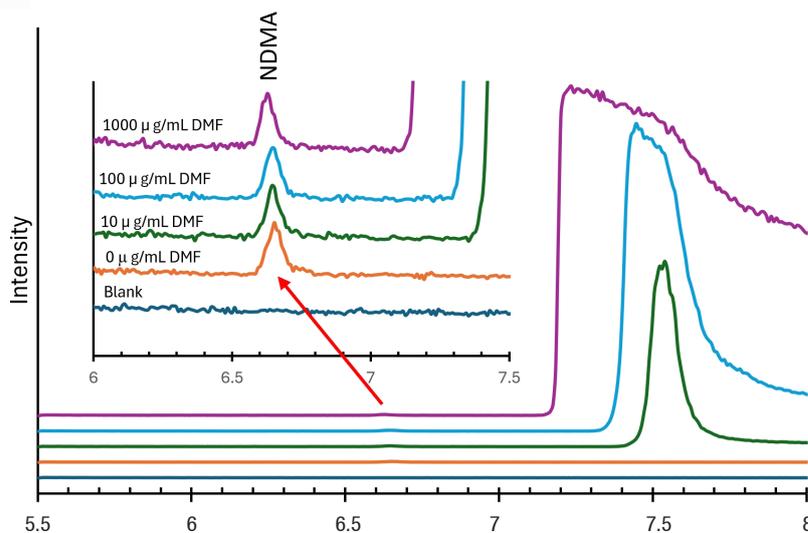
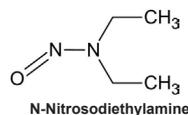


Figure 1. Separation of NDMA and DMF

leading to a lack of confidence in the method.

The MFPP will provide better packed columns, less band broadening and 40-50% greater efficiency than other equivalent silica particles in HPLC, therefore giving higher resolution and sensitivity. Bonded to this MFPP is a polar endcapped alkyl chain, providing the ability to enhance resolution for critical pairs



of closely related polar compounds, ideal for the Nitrosamines which can have very similar structures.

False positives in NDMA analysis caused by the interference of DMF have been discussed in detail in a publication by the FDA². To avoid interference and any potential ion suppression better separation of NDMA and DMF is desired. Fig 2, shows how several columns registered as compatible with 100% aqueous mobile phases were screened taking into

account for the negative LogP of -0.57 for NDMA.

The best retention and resolution were obtained with Evosphere AQUA. Since the retention time of DMF decreases when its concentration increases due to mass overload and may cause co-elution, high resolution between NDMA and DMF was essential.

Experimental Conditions

Column: 3µm Evosphere[®] AQUA 150x4.6mm
 p/n EVOAQUA-050703
 Mobile phase
 A: 0.05% TFA in water
 B: MeCN
 Flow Rate: 1.0ml/min
 Gradient:
 100% A for 1minute
 100% - 90% A in 9minutes
 Temp: 25°C
 Injection : 50ul
 Detection: MS +ESI m/z=75 for NDMA & m/z=81 for d6 NDMA

Conclusion

In this application note we have shown a robust LC-MS method for separation of Nitrosodimethylamine. The analysis is completed quickly and with excellent resolution between analytes. Interference of DMF at concentrations upto 1,000,000 times higher than NDMA can be achieved. The limit of quantitation (LOQ) achieved is 3ng/g and the limit of detection (LOD) is 1mg/g for metaformin products utilising a single quadrupole mass spectrometer.

The use of a monodisperse HPLC particle has provided a significant gain in performance in terms of resolution and sensitivity between these compounds. With the simple mobile phase used it should promise a straightforward, reproducible and robust method.

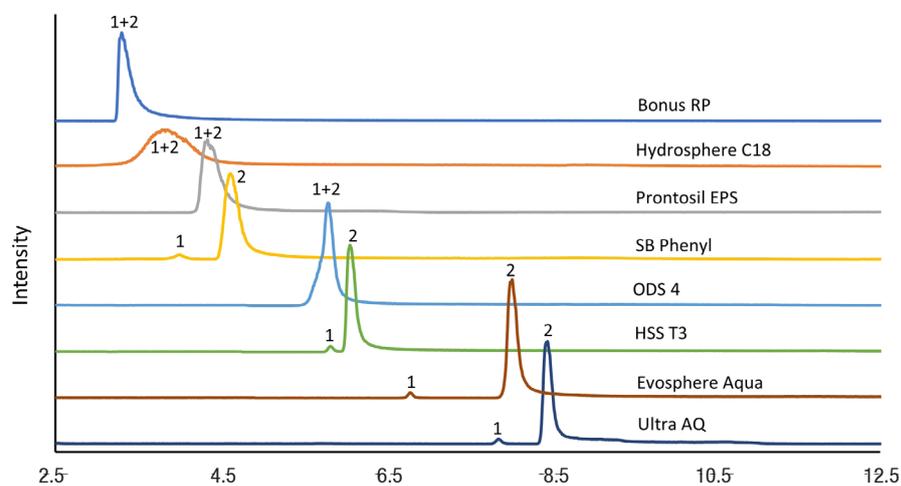


Figure 2. Initial choice of HPLC Column

1. Control of Nitrosamine Impurities in Human Drugs, Guidance for Industry. February 2021 Pharmaceutical CGMP, Rev1.

2. Yang, J; Marzan T.A; Ye,W; Sommers, C; Rodriguez, J; A Cautionary Tale; Quantitative LC-MHRS Analytical procedure for the Analysis of N-Nitrosodimethylamine in Metaformin. *Aaps. J* 2020, 22(4)89

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