# Robust HPLC–MS Method for the Determination of Trace NDMA in the Presence of High Levels of DMF Using Monodisperse Particles.

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## Introduction

Nitrosamine impurities, particularly N-nitrosodimethylamine (NDMA), have become a critical concern in pharmaceutical analysis due to their potential carcinogenicity and stringent regulatory limits. A major analytical challenge is the accurate quantification of NDMA in the presence of N,N-dimethylformamide (DMF), a commonly used solvent and known precursor, which is often present at significantly higher concentrations and can cause co-elution, ion suppression, and false positive or negative results.

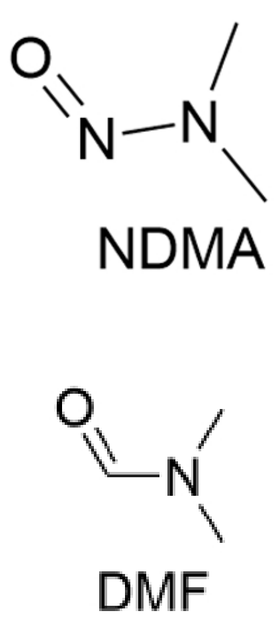
In this study, a robust and sensitive HPLC–MS method was developed using a monodisperse fully porous particle (MFPP) The column chemistry and particle morphology were optimized to enhance retention and resolution of polar analytes, enabling effective separation of NDMA from DMF, even under extreme concentration disparities. Chromatographic conditions employed a simple aqueous/organic mobile phase with MS detection in positive electrospray ionisation mode.

## Method Development

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 a new Monodisperse Fully Porous Particle (MFPP) designed to provide increased efficiency over traditional polydisperse particles.

These monodisperse particles generate better packed column beds, leading to less band broadening and greater efficiency than equivalent silica particles in HPLC. Bonded to this MFPP is a novel polar endcapped C18 alkyl chain, providing the ability to enhance resolution for critical pairs. Fig 2.

**FIGURE 1. Structure of NDMA + DMF**




## Experimental Conditions

**Column**: 3µm Evosphere® AQUA 150x4.6mm p/n EVOAQUA-050703

**Mobile phase A**: 0.05% TFA in water

**Mobile phase B**: MeCN

**Flow Rate**: 1.0ml/min

**Gradient**: 100% A for 1minute
100% - 90% A in 9minutes

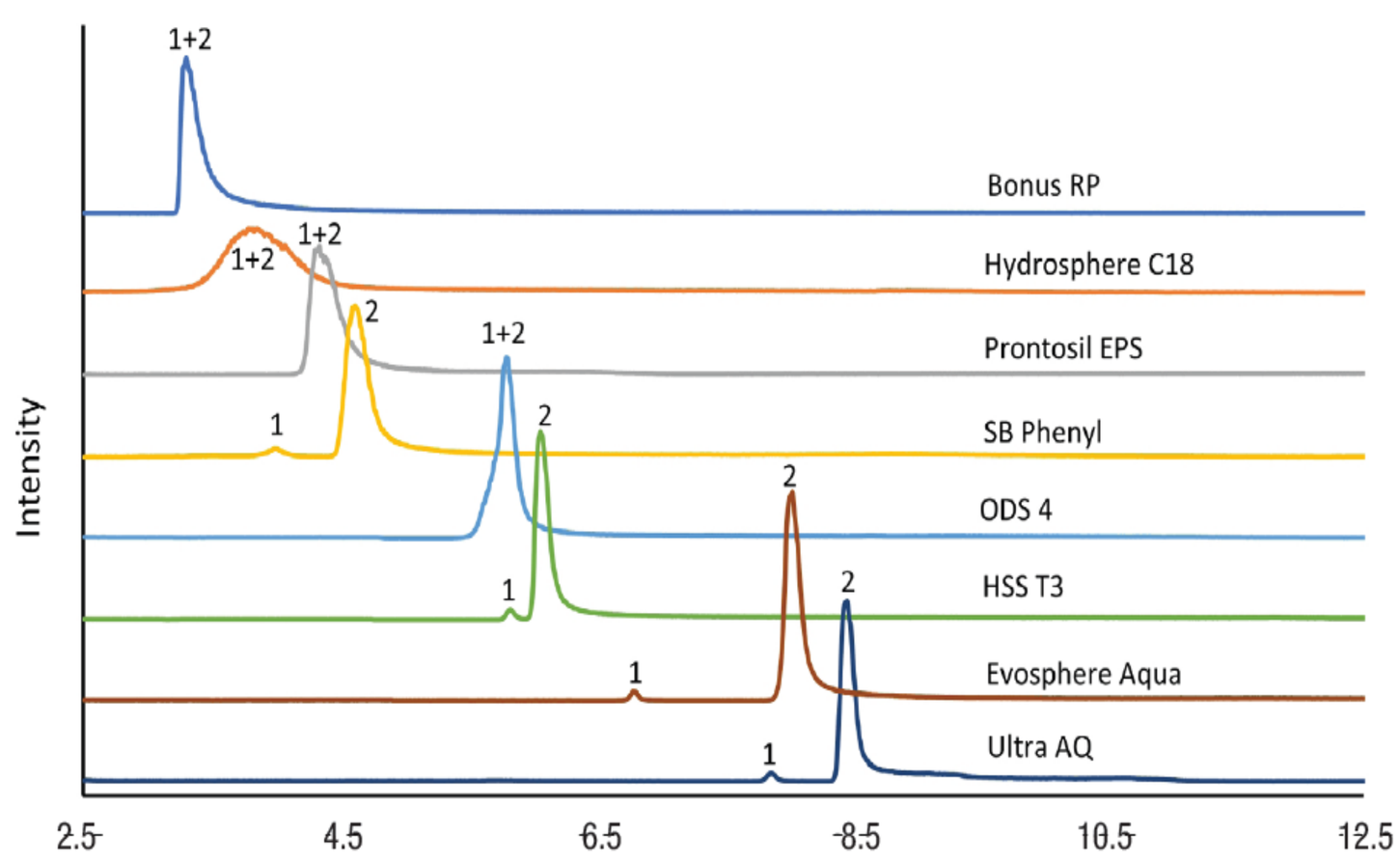
**Temp**: 25oC

**Injection** : 50ul

**Detection**: MS +ESI m/z=75 for NDMA
m/z=81 for d6 NDMA

The particle size for all columns evaluated was either 3um or 3.5um depending on manufacturers specifications. UHPLC columns were avoided to prevent issues with backpressure.

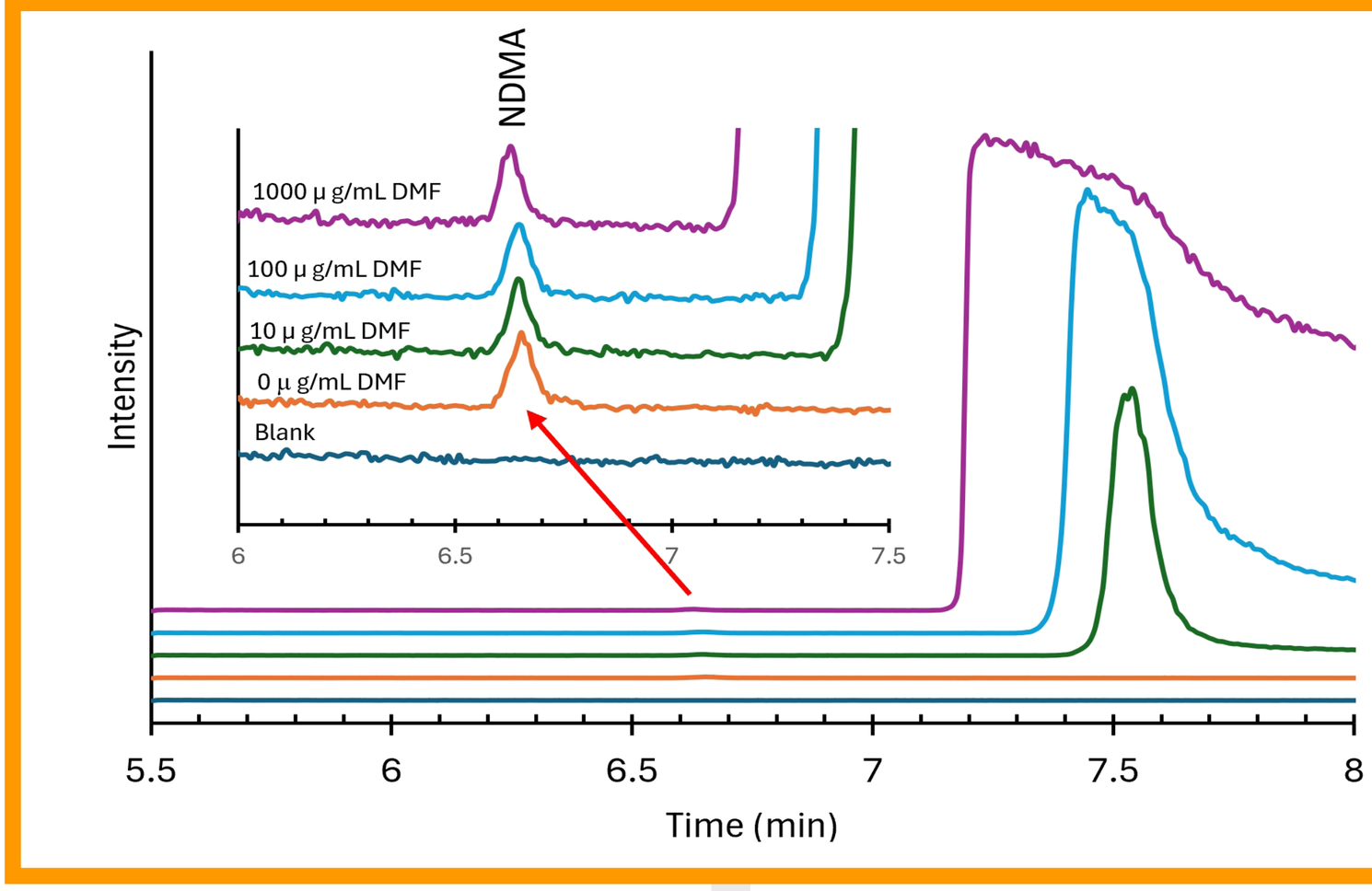
**FIGURE 2. Initial Column study to gain maximum resolution**




Initial evaluations of eight stationary phases led to only four phases being able to resolve NDMA and DMF from each other. The Evosphere AQUA gave the most resolution of the four phases which increases confidence that the separation will be maintained when in overload conditions.

Figure 3. highlights this resolution at several concentration levels of DMF in the presence of 0.01ng of NDMA. If sufficient selectivity was not achieved before overload, then the ability to quantitate would quickly be compromised by co-elution.

**FIGURE 3. Separation of DMF + NDMA, under DMF overload conditions**




## Conclusion

The method demonstrated excellent chromatographic performance, achieving baseline separation of NDMA and DMF and maintaining resolution even when DMF was present at up to $10^6$-fold excess relative to NDMA. Limits of quantitation and detection were determined at 3 ng/g and 1 ng/g, respectively, for metformin drug products using a single quadrupole mass spectrometer. The use of MFPP technology provided improved efficiency, reduced band broadening, and enhanced sensitivity compared to conventional particle technologies, while maintaining manageable backpressure and method robustness.

This approach offers a practical, high-performance solution for routine quality control laboratories, enabling reliable monitoring of NDMA in complex pharmaceutical matrices without the need for high-resolution MS instrumentation. The method is well-suited for regulatory compliance and supports ongoing efforts to ensure drug safety.

All work carried out by JinJian Zheng and colleagues, Merck Analytical Rahway, NJ.

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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