Nitrosamines on Evosphere AQUA Monodisperse HPLC Column

Nitrosamines

Introduction

Nitrosamines have come under enormous scrutiny in the last few years since they were first 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 FDA[1].

Although hundreds of nitrosamines exist they can vary widely in their chemical nature being hydrophilic or hydrophobic in nature. Therefore presenting a challenge to developing a method that can function for many of these groups. Another key criteria was to develop a simple screening HPLC method that could be used quickly and efficiently even at low concentration levels without the requirement for LC-MS detection.

> "Nitrosamines have become a hot topic due to risk of carcinogenic properties, and potentially being so prevalent"

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 that would be expected if using a sub 2µm UHPLC particle. If you run with a UHPLC particle then you get a much elevated backpressure and the potential for blockage and robustness issues, wghich can lead to a lack of confidence in the method.

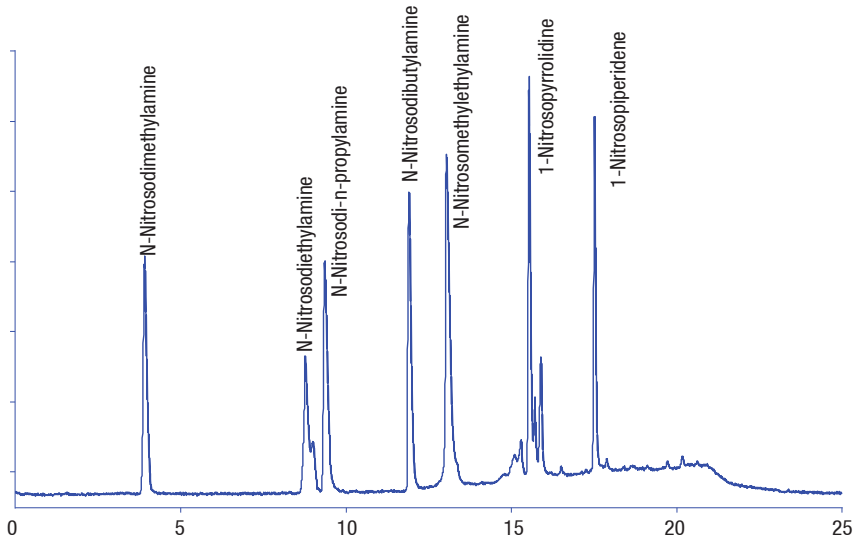

Figure 1. Separation of Nitrosamines

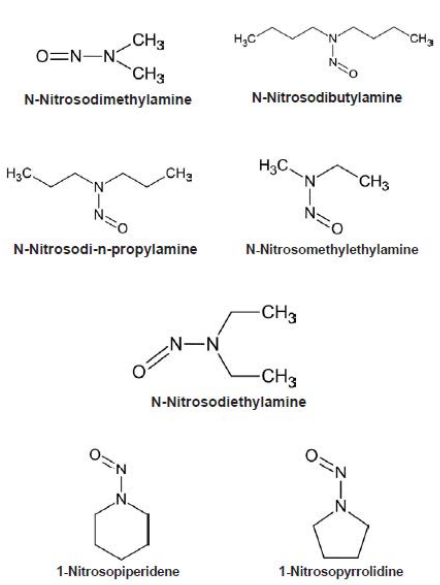

Figure 2. Nitrosamines structures

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 selection of new stationary phase choices, providing the ability to enhance resolution for critical pairs of closely related compounds, ideal for the Nitrosamines which can have very similar structures.

Experimental Conditions

Column: 3µm Evosphere® AQUA 100x2.1mm
p/n EVOAQ020503
Mobile phase
A: 0.1% formic acid in water
B: 0.1% formic acid in MeOH

Time	%B
0.5	5
14	95
15	95

Flow Rate: 0.4ml/min
Temp: 40ºC
Detection: 254nm

The 2nd part of this method development work was to run the Nitrosamines with a sample of Ranitidine to see if detection of both species could be achieved. For this the gradient was changed to:

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

EVOSPHERE

Technical Note

Nitrosamines on Evosphere AQUA Monodisperse HPLC Column

Column: 3µm Evosphere® AQUA 100x2.1mm
p/n EVOAQ020503
Mobile phase
A: 0.1% formic acid in water
B: 0.1% formic acid in MeOH

Time	%B
0	5
5	5
15	45
30	95

Flow Rate: 0.4ml/min
Temp: 40°C
Detection: 254nm

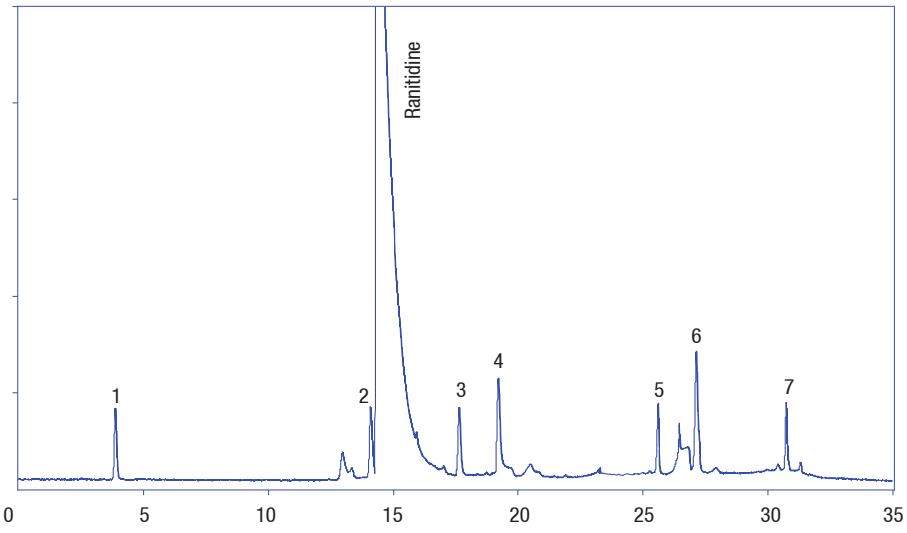

Figure 3. Separation of Nitrosamines and Ranitidine

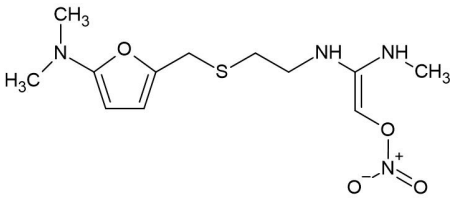

Figure 4. Ranitidine

Conclusion

In this application note we have shown a robust LC-UV method for separation of some of the Nitrosamines. The analysis is completed quickly and with good overall resolution between analytes, however if further gains in speed were required there is scope to decrease the size of the column, or increase the organic proportion of the mobile phase or increase the temperature to obtain a faster run time. If other metabolites were present then there is also plenty of scope to shallow the gradient to gain increased resolution.
When looking for the Nitrosamines in a drug substance sample, in this case Ranatidine, good loading of the parent drug and sharp efficient peak shapes for the nitrosamines allow a high sensitivity, high resolution method to be developed.

The use of a monodisperse particle has provided a significant gain in performance in terms of resolution and sensitivity for these compounds. With the simple mobile phase utilised it would also be easy to transfer this to LC-MS if required.

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