LC/MS/MS Method for Quantifying N-Nitrosamines on DIAMONDBOND®-C18

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Technical Bulletin # 295

This application note shows the separation of nine pharmaceutical packaging related N-nitrosamines using a DiamondBond®-C18 column. Detection is by a mass spectrometer using Multiple Reaction Monitoring (MRM) mode of the characteristic MRM transition of each individual compound. The nine N-nitrosamines are quantitated by comparison to a standard curve. The method is suitable for the analysis of N-nitrosamine extractable/leachable at sub-ppb level for pharmaceutical containers.

Introduction

PPD Development, Inc. (http://www.ppdi.com) provides complete bioanalytical and GMP services for drug development. Bioanalytical laboratories are located in Madison, WI and Richmond, VA. A GMP laboratory is located in Madison, WI. PPD method development experts came to ZirChrom looking for assistance in developing an approach to quantify nine structurally similar N-nitrosamines in pharmaceutical packaging. Collaborative efforts led to the development and validation of the following LC/MS/MS method.

Table 1: Method Detection/Quantification Limits.
ng/mL (ppb) in water

Compound	LOD	LOQ
N-Nitrosodimethylamine (NDMA)	0.3	1.0
N-Nitrosodiethylamine (NDEA)	0.2	0.6
N-Nitrosomethylethylamine (NMEA)	0.04	0.12
N-Nitrosodi-n-propylamine (NDPA)	0.1	0.3
N-Nitrosodi-n-butylamine (NDBA)	0.04	0.12
N-Nitrosodiphenylamine (NDFA)	0.3	1.0
N-Nitrosomorpholine (NMOR)	0.2	0.6
N-Nitrosopiperidine (NPIP)	0.2	0.6
N-Nitrosopyrrolidine (NPYR)	0.2	0.6

Experimental

A mixture of N-nitrosamines (see Table 1) was separated at 50 ºC using a DiamondBond®-C18 column and a Metalox™ 200-C column heater. The separation conditions were as follows:

Column: DiamondBond®-C18, 100 mm x 4.6 mm i.d., 5 micron (Part Number: DB01-1046-5)

Mobile Phase: Gradient elution

Time	% A	% B
0	97.5	2.5
10	10	90
15	10	90
15.1	97.5	2.5
25	97.5	2.5

A: 0.1% (v/v) formic acid
B: acetonitrile

Temperature: 50 ºC with Metalox™ 200-C column heater
Flow Rate: 0.5 ml/min.
Injection: 0.1 ng
Detection: LC/MS/MS

These chromatographic conditions capitalize on the unique temperature stability and surface chemistry of zirconia-based stationary phases to achieve baseline resolution of these compounds in less than 13 minutes.

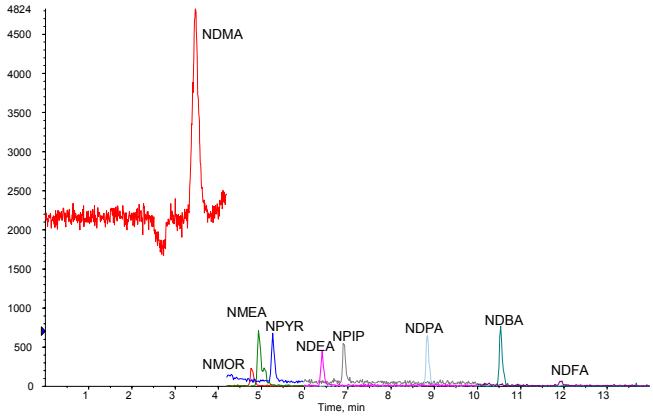

Figure 1: MRM chromatograms of nine N-nitrosamines at 0.1 ng injection.

This method can be tailored to your specific application needs. ZirChrom method developers can help to optimize and transfer this method to your site. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for details.

ZirChrom phases offer unique selectivity, high efficiency, and excellent chemical and thermal stability.

Acknowledgements

T. J. Deng, Prasanna Sunthankar and Aryo Nikopour,
PPD Development, Inc., (Madison, Wisconsin, USA)

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