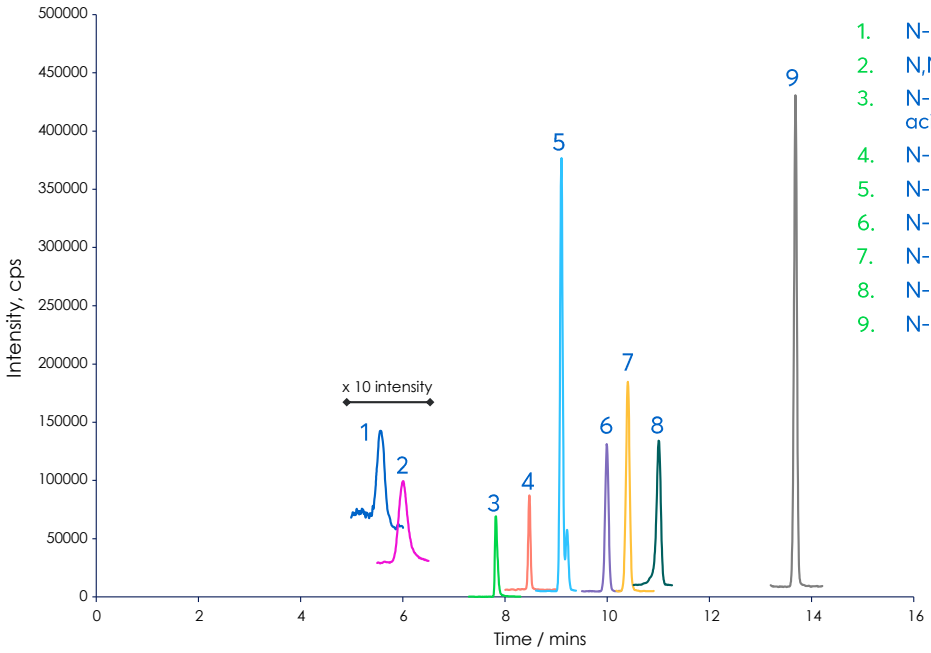
Chromatography Solutions

Application note #7890

LC-MS/MS determination of nitrosamine impurities and DMF in metformin drug substance using U.S. FDA methodology



1. N-nitroso-dimethylamine (NDMA)
2. N,N-dimethylformamide (DMF)
3. N-nitroso-N-methyl-4-aminobutyric acid (NMBA)
4. N-nitroso-diethylamine (NDEA)
5. N-nitroso-ethyl-isopropylamine (NEIPA)
6. N-nitroso-diisopropylamine (NDIPA)
7. N-nitroso-di-n-propylamine (NDPA)
8. N-nitroso-methylphenylamine (NMPA)
9. N-nitroso-di-n-butylamine (NDBA)

Avantor® ACE®

Method Details

CONDITIONS

Column: Avantor® ACE® UltraCore Biphenyl

Particle Size: 3.5 µm

Dimensions: 150 x 3.0 mm

Mobile Phases: A: 0.1% formic acid in H_2O

B: 0.1% formic acid in MeOH

Time (mins)	% B
0	2*
3.0	2
5.0	10
6.0	60
10.0	60
13.0	80
13.1	100
15.0	100
15.1	2
18**	2

*The initial organic composition was reduced from 5 %B to 2 %B in order to achieve chromatographic separation of NDMA and DMF

**The post column re-equilibration time may need to be adjusted for different LC systems

Flow Rate: 0.4 ml/min

Temperature: 40 °C

Injection volume: 3 µl

Detection: Sciex QTRAP® 6500+ LC-MS/MS system.

Ionisation mode: APCI, positive mode; Source temperature: 300 °C; Curtain gas: 28 psig; Collision gas: medium; Ionspray™; Ion source gas: 40 psig; Needle current: 2 µA

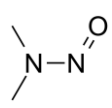
Integrated valve: Divert to waste from 0 to 3.5 mins

Sample: Metformin in MeOH spiked with nitrosamines at 10 ng/ml.

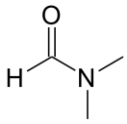
Reference method: FY20-106-DPA-S "Liquid Chromatography-Electrospray Ionization-High Resolution Mass Spectrometry (LC-ESI-HRMS) Method for the Determination of Nitrosamine Impurities in Metformin Drug Substance and Drug Product".
https://www.fda.gov/media/138617/download

MRM TRANSITIONS

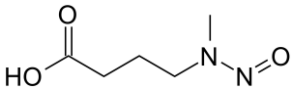
Analyte	MRM	Optimised MS Parameters		
		Declustering potential (V)	Collision energy (V)	Cell exit potential (V)
NDMA	+75.0 amu → +43.0 amu	16	21	18
	+75.0 amu → +58.0 amu	16	23	12
DMF	+74.0 amu → +42.0 amu	1	46	12
	+74.0 amu → +30.0 amu	1	33	14
NMOR	+117.0 amu → +87.0 amu	11	15	4
	+117.0 amu → +44.9 amu	11	25	4
NMBA	+147.1 amu → +117.1 amu	11	11	12
	+147.1 amu → +87.1 amu	11	17	10
NDEA	+103.1 amu → +75.1 amu	16	21	10
	+103.1 amu → +47.1 amu	16	23	22
NPIP	+115.0 amu → +69.1 amu	1	21	8
	+115.0 amu → +41.0 amu	1	31	10
NEIPA	+117.1 amu → +75.1 amu	26	17	10
	+117.1 amu → +47.1 amu	21	23	10
NDIPA	+131.1 amu → +89.1 amu	76	15	10
	+131.1 amu → +47.1 amu	71	23	10
NMPA	+137.1 amu →+66.0 amu	21	23	8
	+137.1 amu →+107.1 amu	16	21	12
NDPA	+131.1 amu → +89.1 amu	16	17	10
	+131.1 amu → +43.1 amu	16	21	10
NDBA	+159.2 amu → +57.1 amu	46	17	10
	+159.2 amu → +103.2 amu	51	15	10



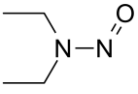
1. NDMA



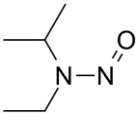
2. DMF



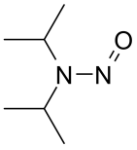
3. NMBA



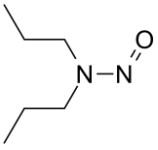
4. NDEA



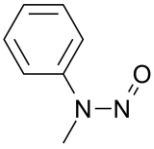
5. NEIPA



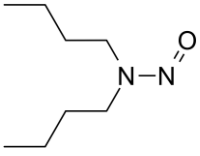
6. NDIPA



7. NDPA



8. NMPA



9. NDBA

ORDERING TABLE

Product	Details	Size	Part Number
Avantor® ACE® UltraCore Biphenyl	HPLC Column	150 x 3.0 mm	CORE-35D-1530
Methanol	VWR HiPerSolv CHROMANORM® for LC-MS	2.5 L	83638.320
Water	VWR HiPerSolv CHROMANORM® for LC-MS	2.5 L	83645.320
Formic acid	VWR HiPerSolv CHROMANORM® for LC-MS	10 x 1 mL	85048.001

ORDERING TABLE (US)

Product	Details	Size	Catalog Number
Avantor® ACE® UltraCore Biphenyl	HPLC Column	150 x 3.0 mm	76458-030
Methanol ≥99.9%	ULTRA for LC-MS, tested for UHPLC, J.T.Baker®	1 L	JT9863-2
Water	ULTRA for LC-MS, tested for UHPLC, J.T.Baker®	1 L	JT9823-1