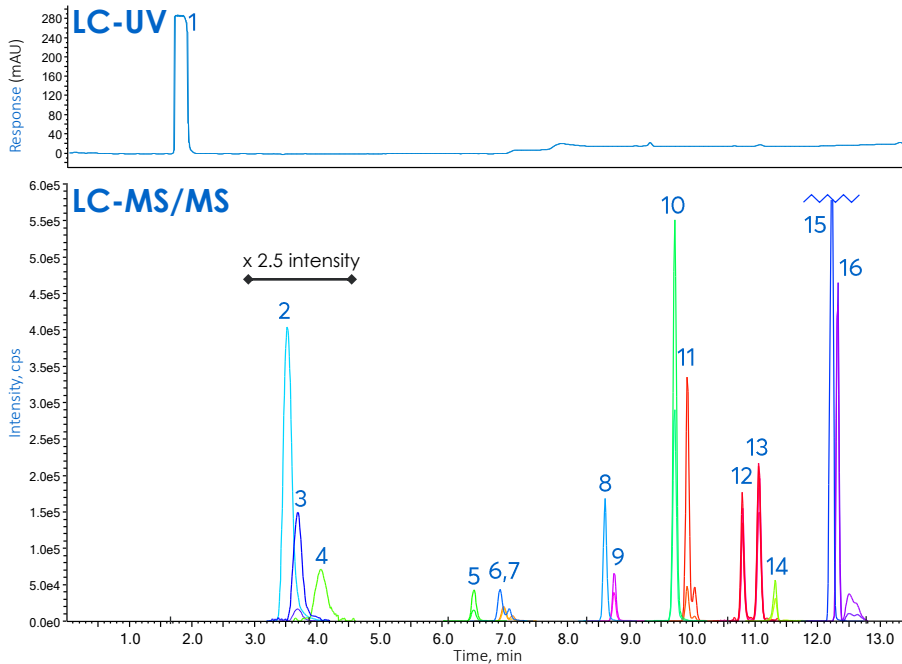


Determination of 10 nitrosamines and DMF in metformin drug substance



1. Metformin
2. NDMA-d6 (IS)
3. N-nitroso-dimethylamine (NDMA)
4. N,N-dimethyl formamide (DMF)
5. N-nitroso-morpholine (NMOR)
6. NMBA-d3 (IS)
7. N-nitroso-N-methyl-4-aminobutyric acid (NMBA)
8. NDEA-d10 (IS)
9. N-nitroso-diethylamine (NDEA)
10. N-nitroso-piperidine (NPIP)
11. N-ethyl-N-nitroso-2-propanamine (NEIPA)
12. N-nitroso-di-isopropylamine (NDIPA)
13. N-nitroso-di-n-propylamine (NDPA)
14. N-nitroso-methylphenylamine (NMPA)
15. NDBA-d18 (IS)
16. N-nitroso-di-n-butylamine (NDBA)

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 H₂O
B: 0.1% formic acid in MeOH

Time (mins)	% B
0	2
3.0	2
13.0	100
14.0	100
14.1	2
18.0*	2

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

Flow Rate: 0.6 ml/min

Temperature: 40 °C

Injection volume: 4 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

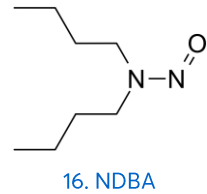
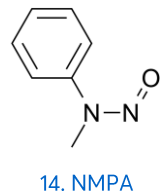
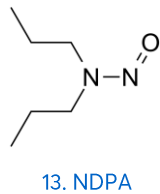
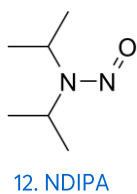
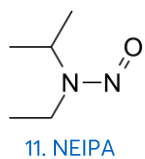
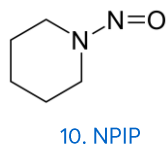
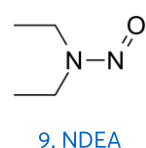
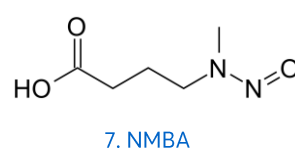
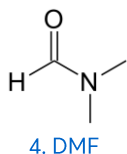
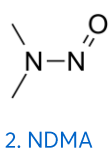
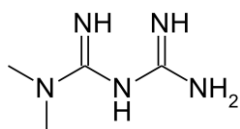
Integrated valve: Divert to waste from 0 to 3.1 mins

Sample: Metformin in MeOH spiked with nitrosamines at 10 ng/ml and filtered through 0.22 µm, 25 mm PVDF syringe filter. NDMA-d6, NMBA-d3, NDEA-d10 and NDBA-d18 were used as internal standards.

Note: An interfering background contaminant was observed in the NDBA MRM chromatogram during blank runs. An Avantor® ACE® PFAS Delay column (PFASD-0503) was installed prior to the injector and was found to successfully remove the interference allowing accurate quantification.

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
NDMA-d6	+81.2 amu → +46.0 amu	40	22	11
	+81.2 amu → +64.1 amu	40	17	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
NMBA-d3	+150.1 amu → +120.2 amu	16	11	8
	+150.1 amu → +47.1 amu	21	17	8
NDEA	+103.1 amu → +75.1 amu	16	21	10
	+103.1 amu → +47.1 amu	16	23	22
NDEA-d10	+113.2 amu → +34.2 amu	21	33	6
	+113.2 amu → +49.1 amu	6	23	6
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
NDBA-d18	+177.3 amu → +66.2 amu	46	23	8
	+177.3 amu → +46.2 amu	41	37	22



ACCURACY AND PRECISION

Calibration curves were generated using standards and isotopically labelled internal standards between 0.5 to 50.0 ng/ml (Figure 1). Table 1 shows accuracy values calculated for triplicate injections of the calibration standards. Good precision from multiple mid-level QC samples (n=8) was obtained (Table 2).

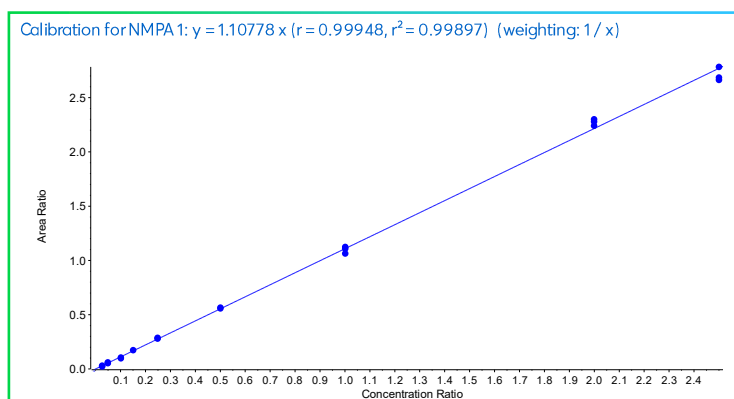


Figure 1: Example calibration curve for ADONA, fitted to linear calibration line forced through zero (weighted 1/x).

Table 1: Example accuracy data for calibration curves between 0.5 to 50.0 ng/ml. All curves were fitted to linear calibration lines forced through zero (weighted 1/x).

Concentration (ng/ml)	NDMA		NMOR		NMBA		NDEA		NPIP		NEIPA		NDIPA		NMPA		NDPA		NDBA	
	Accuracy %	%CV	Accuracy %	%CV	Accuracy %	%CV	Accuracy %	%CV	Accuracy %	%CV	Accuracy %	%CV	Accuracy %	%CV	Accuracy %	%CV	Accuracy %	%CV	Accuracy %	%CV
0.5	N/A	N/A	102.0	2.67	96.0	11.40	96.0	7.89	90.0	0.93	90.0	1.09	110.0	2.79	94.0	3.21	88.0	1.24	102.0	7.21
1	106.0	1.64	110.0	2.47	114.0	2.33	108.0	4.41	105.0	2.44	97.0	3.12	108.0	2.68	98.0	6.02	94.0	1.46	106.0	1.98
2	102.0	1.82	101.0	1.54	100.5	3.85	99.0	1.84	96.5	0.48	92.5	0.60	95.0	0.93	93.0	7.32	91.0	0.51	94.5	3.12
3	111.3	0.34	114.3	0.72	103.0	1.47	109.3	0.84	108.0	0.33	103.0	1.19	105.7	0.47	104.3	1.03	101.7	0.39	107.3	0.76
5	103.6	1.89	107.6	3.12	102.2	2.26	106.8	1.60	102.6	0.62	99.4	0.35	99.6	0.76	100.6	1.63	99.2	0.97	102.4	0.60
10	105.4	1.03	107.9	3.26	102.8	1.48	105.0	2.55	104.1	1.14	99.8	0.31	99.0	0.93	102.1	0.85	99.6	0.42	103.2	0.64
20	103.2	0.40	103.6	1.62	103.5	1.44	104.2	0.40	103.8	0.58	100.5	1.02	98.8	0.19	99.1	3.05	100.7	1.82	102.1	0.86
40	101.2	1.24	100.9	1.12	100.7	0.56	100.3	0.57	101.5	0.50	102.7	0.54	102.7	0.89	102.6	1.37	103.3	1.11	101.4	0.33
50	95.4	1.16	94.4	2.31	96.7	0.86	95.8	0.18	95.9	1.01	98.0	0.38	98.1	0.62	97.9	2.35	97.9	1.44	96.9	0.40
r ²	0.9983		0.9990		0.9989		0.9991		0.9984		0.9994		0.9997		0.9990		0.9996		0.9995	

Table 2: Accuracy and precision data for QC sample (8 replicate injections).

Analyte	Concentration (ng/ml)	Mean calculated (ng/ml)	% Precision	% Accuracy
NDMA	10	9.91	1.42	99.1
NMOR	10	9.89	2.13	98.9
NMBA	10	10.09	1.64	100.9
NDEA	10	10.09	1.40	100.9
NPIP	10	9.98	0.88	99.8
NEIPA	10	10.03	0.73	100.3
NDIPA	10	10.03	0.42	100.3
NMPA	10	9.82	1.77	98.2
NDPA	10	9.90	0.68	99.0
NDBA	10	9.91	0.34	99.1

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