Test Report

Test item: <u>Field verification of N-nitrosodimethylamine in metformin sustained-release tablets</u>

Analytical instrument: <u>LC-MS/MS 3521</u>

Entrusted by: Padma

Report date: <u>June 8, 2023</u>

Basic information:

Sample name	Metformin sustained-release tablets	Test content	Field verification of N-nitrosodimethylamine in metformin sustained-release tablets
Sample properties	Solid	Test date	June 7, 2023
Tester		Contact information	support@hplctechnologies.com

1 Test purpose

The content of N-nitrosodimethylamine in metformin sustained-release tablets is analyzed and determined.

2 Instruments and reagents

2.1 Instruments

LC-MS/MS 3521 triple quadrupole tandem mass spectrometer; vortex mixer.

2.2 Experimental materials

Metformin sustained-release tablets are provided by the customer, and N-nitrosodimethylamine standard is purchased from TCI (TSI Chemicals Industry Development Co., Ltd.).

Ultrapure water, methanol and formic acid are all HPLC pure.

Other materials: pipette (1-10uL, 100-1000µL), pipette head (10uL, 1000µL).

2.3 Customer samples

Metformin sustained-release tablets are provided by the customer.

3 Experimental methods

3.1 LC-MS/MS conditions

LC conditions	Mobile phase	Phase A: 0.1% formic acid water Phase B: methanol
	Velocity of flow	0.5 mL/min
	Column temperature	40 °C
	Chromatographic column	Poroshell 120 SB-C18 (2.7µm, 4.6*150mm)
	Injection volume	5µL, partial loop injection
	Gradient elution	<table><tr><th>Time (min)</th><th>Mobile phase A (%)</th><th>Mobile phase B (%)</th></tr><tr><td>0.0</td><td>70</td><td>30</td></tr><tr><td>2.0</td><td>10</td><td>90</td></tr><tr><td>6.0</td><td>10</td><td>90</td></tr><tr><td>6.5</td><td>70</td><td>30</td></tr><tr><td>10</td><td>70</td><td>30</td></tr></table>
MS/MS conditions	Ion source	APCI+
	Scan mode	Positive ion scan mode
	Corona pin current	2.5 µA
	Vacuum interface temperature	110 ℃
	Desolvation gas temperature	270 ℃
	Atomizing gas flow	2.0 L/min
	Blowback flow	2.5 L/min
	Desolvation gas flow	14.0 L/min
	Acquisition mode	MRM
	Acquisition time	3.6-4.5 min

The MRM monitoring mode is adopted. Parameters such as the monitoring mass number and dwell time of the target compound and the post-column switching valve procedure are shown in Figure 1 below, where 75>58.1 is the quantitative ion, and 75>43.1 is the qualitative ion.
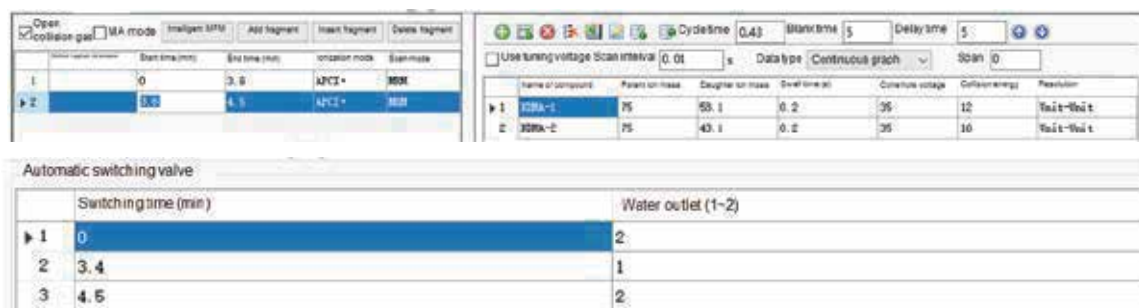

Figure 1 Instrument Method

3.2 Sample analysis flow chart

4 Test results

4.1 Spectra of blank and spiked samples

In order to eliminate instrument interference, 3 injections of blank solvent are tested in parallel, as shown in Figure 2 below. The 2.4ng/mL standard solution is shown in Figure 3 below, and the retention time of NDMA is 3.74 min.
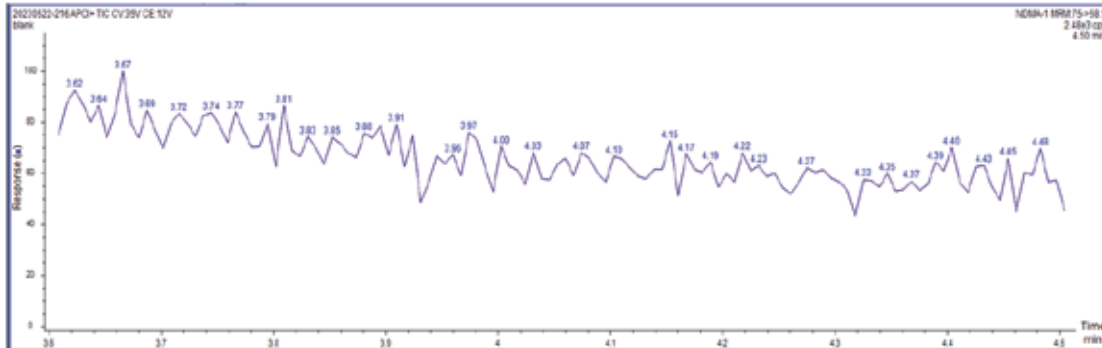

Figure 2 Test Diagram of Pure Solvent

Figure 3 Test Diagram of Standard

4.2 Linearity and LOQ

Methanol was used to prepare a series of gradient NDMA mixed standard sample solutions (0.8ng/mL, 2.4ng/mL, 6.0ng/mL, 12.0ng/mL, 60.0ng/mL and 100.0ng/mL). The quantitative ion chromatography peak area of each target was used as the ordinate, the mass concentration (ng/mL) of the target standard solution was used as the abscissa, and the weight was 1/X. Thereby, a standard curve was established. The fitted standard curve is shown in Figure 4. In the linear range of 0.8~100ng/mL, the square R^2 of the linear coefficients of NDMA quantitative ions (75>58.1) and qualitative ions (75>43.1) were both 0.9997, and the accuracy was 93.40~103.60% and 94.20~103.60%, respectively, showing good linearity.

The LOQ and LOD were calculated according to S/N=10 and S/N=3, taking 0.8ng/mL standard solution as sample injected. As shown in Figure 5, the signal-to-noise ratio of NDMA at the concentration level of 10ng/mL is 18, indicating good sensitivity.
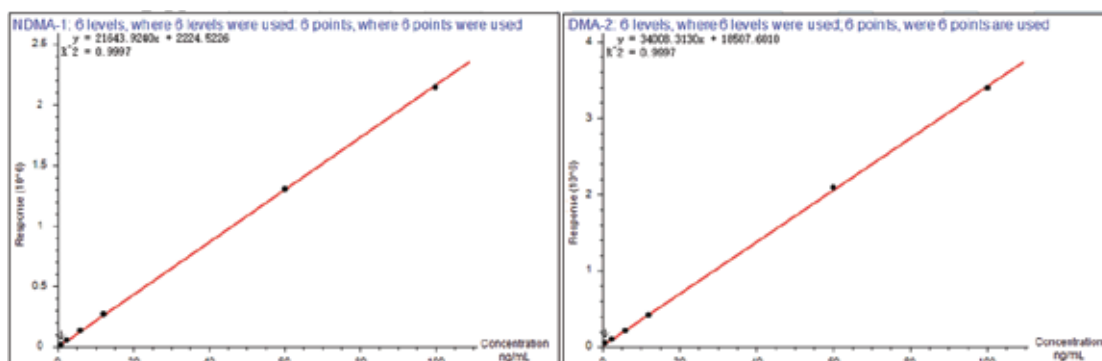
	Whether to use	Level	Expected concentration	Calculated concentration	Accuracy (%)	Qualitative ratio (%)	Peak area		Whether to use	Grade	Expected concentration	Calculated concentration	Accuracy (%)	Qualitative ratio (%)	Peak area
►	✓	1	0.80	0.75	93.40		18388.23	►	✓	1	0.80	0.83	103.60		46686.15
	✓	2	2.40	2.37	98.70		53495.08		✓	2	2.40	2.45	102.00		101744.90
	✓	3	6.00	6.29	104.80		138380.40		✓	3	6.00	5.65	94.20		210772.30
	✓	4	12.00	12.43	103.60		271196.70		✓	4	12.00	11.90	99.10		423108.40
	✓	5	60.00	60.27	100.40		1306626.00		✓	5	60.00	61.06	101.80		2095017.00
	✓	6	100.00	99.10	99.10		2147140.00		✓	6	100.00	99.31	99.30		3396023.00

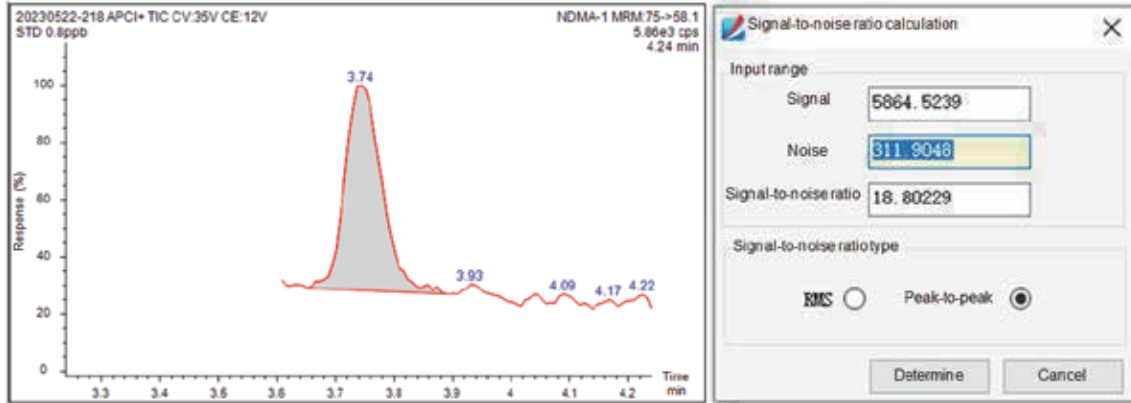Figure 4 Quantitative and Qualitative Ion Linear Curves and Accuracy of N-nitrosodimethylamine

Figure 5 Signal-to-noise ratio of N-nitrosodimethylamine at the Concentration of 0.8ng/mL

4.3 Within-day precision

For 0.2ng/mL, 0.8ng/mL and 2.4ng/mL NDMA standard solution, the relative standard deviation (RSD) values of retention time and peak area were calculated for 6 continuous injections. The precision results are shown in Figure 6-8 below. For 6 continuous injections, the RSD of retention time was less than 0.23% and that of peak area was less than 4.15%, indicating good precision.

	!	▼		Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Signal-to-noise ratio	Calculated concentration
1		▼ ✓		20230522-248	2023-06-07 18:15	STD 0.2ppb-1	20230607NDMA-Standard Curve	3.75	6053.66	8.8	0.18
2		▼ ✓		20230522-249	2023-06-07 18:25	STD 0.2ppb-2	20230607NDMA-Standard Curve	3.75	5896.07	9.6	0.17
3		▼ ✓		20230522-250	2023-06-07 18:35	STD 0.2ppb-3	20230607NDMA-Standard Curve	3.74	6462.05	9.4	0.20
4		▼ ✓		20230522-251	2023-06-07 18:46	STD 0.2ppb-4	20230607NDMA-Standard Curve	3.74	5826.64	8.8	0.17
5		▼ ✓		20230522-252	2023-06-07 18:56	STD 0.2ppb-5	20230607NDMA-Standard Curve	3.73	5771.63	9.1	0.16
► 6		▼ ✓		20230522-253	2023-06-07 19:06	STD 0.2ppb-6	20230607NDMA-Standard Curve	3.75	6044.57	9.3	0.18
Min								3.73	5771.63	8.8	0.16
Max								3.75	6462.05	9.6	0.20
AVG								3.75	6009.10	9.2	0.17
SD								0.01	249.34	0.3	0.01
RSD								0.23	4.15	3.4	6.59
IDL								0.00	0.00	0.0	0.00

Figure 6 Precision Test Results of 0.2ng/mL NDMA

	!	▼		Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Signal-to-noise ratio	Calculated concentration
► 1		▼ ✓		20230522-254	2023-06-07 19:17	STD 0.8ppb-1	20230607NDMA-Standard Curve	3.74	18372.11	19.6	0.75
2		▼ ✓		20230522-255	2023-06-07 19:27	STD 0.8ppb-2	20230607NDMA-Standard Curve	3.75	17538.32	18.3	0.71
3		▼ ✓		20230522-256	2023-06-07 19:37	STD 0.8ppb-3	20230607NDMA-Standard Curve	3.75	19080.85	19.9	0.78
4		▼ ✓		20230522-257	2023-06-07 19:48	STD 0.8ppb-4	20230607NDMA-Standard Curve	3.74	17885.90	20.6	0.72
5		▼ ✓		20230522-258	2023-06-07 19:58	STD 0.8ppb-5	20230607NDMA-Standard Curve	3.75	18526.55	19.8	0.75
6		▼ ✓		20230522-259	2023-06-07 20:08	STD 0.8ppb-6	20230607NDMA-Standard Curve	3.75	18145.85	21.4	0.74
Min								3.74	17538.32	18.3	0.71
Max								3.75	19080.85	21.4	0.78
AVG								3.74	18258.26	19.9	0.74
SD								0.00	535.01	1.0	0.02
RSD								0.09	2.93	5.2	3.34
IDL								0.00	0.00	0.0	0.00

Figure 7 Precision Test Results of 0.8ng/mL NDMA

	!	▼	Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Signal-to-noise ratio	Calculated concentration
							NDMA-1			
1		✔	20230522-228	2023-06-07 13:44	STD 2.4ppb-1	20230607NDMA-Standard Curve	3.74	52040.23		2.30
2		✔	20230522-229	2023-06-07 13:55	STD 2.4ppb-2	20230607NDMA-Standard Curve	3.74	53420.03		2.37
3		✔	20230522-230	2023-06-07 14:05	STD 2.4ppb-3	20230607NDMA-Standard Curve	3.74	53540.90		2.37
4		✔	20230522-231	2023-06-07 14:15	STD 2.4ppb-4	20230607NDMA-Standard Curve	3.75	54655.78		2.42
5		✔	20230522-232	2023-06-07 14:26	STD 2.4ppb-5	20230607NDMA-Standard Curve	3.75	53375.81		2.36
▶ 6		✔	20230522-233	2023-06-07 14:36	STD 2.4ppb-6	20230607NDMA-Standard Curve	3.75	52498.30		2.32
Min							3.74	52040.23	0.0	2.30
Max							3.75	54655.78	0.0	2.42
AVG							3.74	53255.18	0.0	2.36
SD							0.00	909.29	0.0	0.04
RSD							0.11	1.71		1.78
IDL							0.00	0.00		0.00

Figure 8 Precision Test Results of 2.4ng/mL NDMA

4.4 Between-day precision

The 2.4ng/mL NDMA standard solution and the sample with a spike concentration of 2.4ng/mL were injected for 6 times in parallel for 2 consecutive days, and the relative standard deviation RSD value of the retention time and peak area was calculated for a total of 12 injections for 2 consecutive days. The precision results are shown in Figure 9-10 below. The RSDs of between-day precision and retention time were both less than 0.12% for 2 consecutive days, and the RSDs of peak area were both less than 3.15% for 2 consecutive days, indicating good precision.

	!	▼	Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Calculated concentration
							NDMA-1		
1		✔	20230522-228	2023-06-07 13:44	STD 2.4ppb-1	20230607NDMA-Standard Curve	3.74	52040.23	2.30
2		✔	20230522-229	2023-06-07 13:55	STD 2.4ppb-2	20230607NDMA-Standard Curve	3.74	53420.03	2.37
3		✔	20230522-230	2023-06-07 14:05	STD 2.4ppb-3	20230607NDMA-Standard Curve	3.74	53540.90	2.37
4		✔	20230522-231	2023-06-07 14:15	STD 2.4ppb-4	20230607NDMA-Standard Curve	3.75	54655.78	2.42
5		✔	20230522-232	2023-06-07 14:26	STD 2.4ppb-5	20230607NDMA-Standard Curve	3.75	53375.81	2.36
6		✔	20230522-233	2023-06-07 14:36	STD 2.4ppb-6	20230607NDMA-Standard Curve	3.75	52498.30	2.32
7		✔	20230522-282	2023-06-08 13:20	Day2-STD 2.4ppb-1	20230607NDMA-Standard Curve	3.74	49978.12	2.21
8		✔	20230522-283	2023-06-08 13:31	Day2-STD 2.4ppb-2	20230607NDMA-Standard Curve	3.75	52132.91	2.31
9		✔	20230522-284	2023-06-08 13:41	Day2-STD 2.4ppb-3	20230607NDMA-Standard Curve	3.75	53996.23	2.39
10		✔	20230522-285	2023-06-08 13:51	Day2-STD 2.4ppb-4	20230607NDMA-Standard Curve	3.74	52008.61	2.30
11		✔	20230522-286	2023-06-08 14:02	Day2-STD 2.4ppb-5	20230607NDMA-Standard Curve	3.75	54318.59	2.41
▶ 12		✔	20230522-287	2023-06-08 14:12	Day2-STD 2.4ppb-6	20230607NDMA-Standard Curve	3.75	52851.21	2.34
Min							3.74	49978.12	2.21
Max							3.75	54655.78	2.42
AVG							3.75	52901.39	2.34
SD							0.00	1280.19	0.06
RSD							0.10	2.42	2.53
IDL							0.00	0.00	0.00

Figure 9 Between-day Precision Test Results of 2.4ng/mL NDMA Calibration Point

	!	▼		Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Calculated concentration
► 1		✓		20230522-237	2023-06-07 16:22	R-2.4ppb-1	20230607NDMA-Standard Curve	3.73	51503.87	2.28
2		✓		20230522-238	2023-06-07 16:32	R-2.4ppb-2	20230607NDMA-Standard Curve	3.74	52725.29	2.33
3		✓		20230522-239	2023-06-07 16:42	R-2.4ppb-3	20230607NDMA-Standard Curve	3.75	49025.24	2.16
4		✓		20230522-240	2023-06-07 16:53	R-2.4ppb-4	20230607NDMA-Standard Curve	3.75	50632.63	2.24
5		✓		20230522-241	2023-06-07 17:03	R-2.4ppb-5	20230607NDMA-Standard Curve	3.74	48136.97	2.12
6		✓		20230522-242	2023-06-07 17:13	R-2.4ppb-6	20230607NDMA-Standard Curve	3.74	51190.43	2.26
7		✓		20230522-289	2023-06-08 14:32	Day2-R-2.4ppb-1	20230607NDMA-Standard Curve	3.75	48971.09	2.16
8		✓		20230522-290	2023-06-08 14:43	Day2-R-2.4ppb-2	20230607NDMA-Standard Curve	3.74	51194.27	2.26
9		✓		20230522-291	2023-06-08 14:53	Day2-R-2.4ppb-3	20230607NDMA-Standard Curve	3.74	50027.54	2.21
10		✓		20230522-292	2023-06-08 15:03	Day2-R-2.4ppb-4	20230607NDMA-Standard Curve	3.75	48866.14	2.15
11		✓		20230522-293	2023-06-08 15:14	Day2-R-2.4ppb-5	20230607NDMA-Standard Curve	3.75	47493.21	2.09
12		✓		20230522-294	2023-06-08 15:24	Day2-R-2.4ppb-6	20230607NDMA-Standard Curve	3.74	48845.39	2.15
Min								3.73	47493.21	2.09
Max								3.75	52725.29	2.33
AVG								3.74	49884.34	2.20
SD								0.00	1571.19	0.07
RSD								0.12	3.15	3.30
IDL								0.00	0.00	0.00

Figure 10 Between-day Precision Test Results of 2.4ng/mL NDMA Spike Recovery

4.5 Recovery

The spike recoveries of blank metformin sustained-release tablets were tested: Parallel tests were performed for 6 times at the spike concentration of 0.8ng/mL and 2.4ng/mL of LOQ; and parallel tests were performed for 3 times at the spike concentration of 6ng/mL. The recovery results of the target compound are shown in Figure 11-13 below. At different spike concentrations of 0.8ng/mL, 2.4ng/mL and 6ng/mL, the average recovery rates of NDMA were 92.35%, 93.00% and 87.97%, respectively, showing good recovery test results.

	!	▼		Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Signal-to-noise ratio	Calculated concentration
1	▼	✓		20230522-261	2023-06-07 20:29	R-LOQ-0.8ppb-1	20230607NDMA-Standard Curve	3.74	18029.93	0.73	91.28
2	▼	✓		20230522-262	2023-06-07 20:39	R-LOQ-0.8ppb-2	20230607NDMA-Standard Curve	3.74	18317.55	0.74	92.94
3	▼	✓		20230522-263	2023-06-07 20:49	R-LOQ-0.8ppb-3	20230607NDMA-Standard Curve	3.74	17982.29	0.73	91.01
4	▼	✓		20230522-264	2023-06-07 21:00	R-LOQ-0.8ppb-4	20230607NDMA-Standard Curve	3.74	18699.12	0.76	95.15
5	▼	✓		20230522-265	2023-06-07 21:10	R-LOQ-0.8ppb-5	20230607NDMA-Standard Curve	3.74	18623.36	0.76	94.71
► 6	▼	✓		20230522-266	2023-06-07 21:20	R-LOQ-0.8ppb-6	20230607NDMA-Standard Curve	3.74	17641.92	0.71	89.04
Min								3.74	17641.92	0.71	89.04
Max								3.74	18699.12	0.76	95.15
AVG								3.74	18215.70	0.74	92.35
SD								0.00	407.06	0.02	2.35
RSD								0.08	2.23	2.55	2.55
IDL								0.00	0.06	0.07	0.07

Figure 11 Test Results at Spike Concentration of 0.8ng/mL (LOQ)

	!	▼		Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Signal-to-noise ratio	Calculated concentration
► 1		✓		20230522-237	2023-06-07 16:22	R-2.4ppb-1	20230607NDMA-Standard Curve	3.73	51503.87	2.28	94.87
2		✓		20230522-238	2023-06-07 16:32	R-2.4ppb-2	20230607NDMA-Standard Curve	3.74	52725.29	2.33	97.22
3		✓		20230522-239	2023-06-07 16:42	R-2.4ppb-3	20230607NDMA-Standard Curve	3.75	49025.24	2.16	90.10
4		✓		20230522-240	2023-06-07 16:53	R-2.4ppb-4	20230607NDMA-Standard Curve	3.75	50632.63	2.24	93.19
5		✓		20230522-241	2023-06-07 17:03	R-2.4ppb-5	20230607NDMA-Standard Curve	3.74	48136.97	2.12	88.39
6		✓		20230522-242	2023-06-07 17:13	R-2.4ppb-6	20230607NDMA-Standard Curve	3.74	51190.43	2.26	94.26
Min								3.73	48136.97	2.12	88.39
Max								3.75	52725.29	2.33	97.22
AVG								3.74	50535.74	2.23	93.00
SD								0.01	1685.53	0.08	3.24
RSD								0.14	3.34	3.49	3.49
IDL								0.01	0.27	0.28	0.28

Figure 12 Test Results at Spike Concentration of 2.4ng/mL

			Sample information				NDMA-1			
	?	⚐	Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Signal-to-noise ratio	Calculated concentration
1		✓	20230522-244	2023-06-07 17:34	R-6ppb-1	20230607NDMA-Standard Curve	3.75	117728.00	5.34	88.94
2		✓	20230522-245	2023-06-07 17:44	R-6ppb-2	20230607NDMA-Standard Curve	3.74	115794.40	5.25	87.45
▸ 3		✓	20230522-246	2023-06-07 17:54	R-6ppb-3	20230607NDMA-Standard Curve	3.75	115866.90	5.25	87.51
Min							3.74	115794.40	5.25	87.45
Max							3.75	117728.00	5.34	88.94
AVG							3.75	116463.10	5.28	87.97
SD							0.00	1096.04	0.05	0.84
RSD							0.09	0.94	0.96	0.96
IDL							0.04	0.39	0.40	0.40

Figure 13 Test Results at Spike Concentration of 6ng/mL

4.6 Sample testing

To test the actual samples of metformin sustained-release tablets, the metformin sustained-release tablet powder was first extracted with methanol to obtain the metformin sustained-release tablet solution with a matrix concentration of 50mg/mL. The test results show that no NDMA was detected in the actual sample, and the ion flow diagram of quantitative and qualitative ion pairs extracted in the actual sample is shown in Figure 14 below.
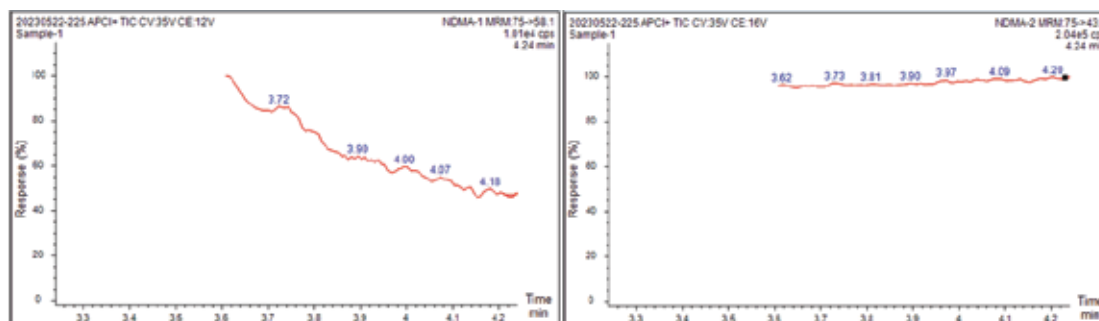

Figure 14 Ion Flow Diagram of Target Compound NDMA Extraction in Sample

4.7 Stability

The standard curve point of 2.4ng/mL and the sample with the spike concentration of 2.4ng/mL were tested at the 0th, 18th and 24th hours respectively, with 2 injections per time, and the stability was investigated by the variation trend of the mean peak area of each sample. The results show that the peak area of 2.4 g/mL standard curve points at the 18th and 24th hours changed by -2.58% and -0.06% respectively compared with the peak area at the 0th hour; the peak area of 2.4ng/mL spike recovery at the 18th and 24th hours changed by -4.15% and 0.83% respectively compared with the peak area at the 0th hour. The peak area changes of 2.4ng/mL standard curve points and 2.4ng/mL spike recovery at the 16th and 24th hours were less than 10%, indicating that the stability was good.

Time	Mean peak area (2.4ng/mL standard curve point)	Variation trend	Mean peak area (2.4ng/mL spike recovery)	Variation trend
The 0th hour	52730.13	0	52114.58	0
The 18th hour	51369.92	-2.58%	49953.77	-4.15%
The 24th hour	52699.20	-0.06%	50370.17	0.83%

			Sample information				NDMA-1		
	!	▼		Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Calculated concentration
1			✓	20230522-228	2023-06-07 13:44	STD 2.4ppb-1	20230607NDMA-Standard Curve	3.74	52040.23	2.30
2			✓	20230522-229	2023-06-07 13:55	STD 2.4ppb-2	20230607NDMA-Standard Curve	3.74	53420.03	2.37
3			✓	20230522-274	2023-06-08 08:07	18h STD 2.4ppb-1	20230607NDMA-Standard Curve	3.75	52078.75	2.30
4			✓	20230522-275	2023-06-08 08:17	18h STD 2.4ppb-2	20230607NDMA-Standard Curve	3.75	50661.08	2.24
5			✓	20230522-280	2023-06-08 13:00	24h STD 2.4ppb-1	20230607NDMA-Standard Curve	3.75	51718.52	2.29
▶ 6			✓	20230522-281	2023-06-08 13:10	24h STD 2.4ppb-2	20230607NDMA-Standard Curve	3.75	53679.88	2.38
Min								3.74	50661.08	2.24
Max								3.75	53679.88	2.38
AVG								3.75	52266.41	2.31
SD								0.00	1121.82	0.05
RSD								0.12	2.15	2.24
IDL								0.00	0.00	0.00

Figure 15 Peak Area at the 0th, 18th and 24th hours for 2.4ng/mL Standard Curve Points

			Sample information				NDMA-1		
	!	▼		Data name	Acquisition time	Sample name	Quantitative method	Retention time	Peak area	Calculated concentration
1			✓	20230522-237	2023-06-07 16:22	R-2.4ppb-1	20230607NDMA-Standard Curve	3.73	51503.87	2.28
2			✓	20230522-238	2023-06-07 16:32	R-2.4ppb-2	20230607NDMA-Standard Curve	3.74	52725.29	2.33
3			✓	20230522-276	2023-06-08 08:27	18h R-2.4ppb-1	20230607NDMA-Standard Curve	3.74	48119.25	2.12
4			✓	20230522-277	2023-06-08 08:37	18h R-2.4ppb-2	20230607NDMA-Standard Curve	3.74	51788.29	2.29
▶ 5			✓	20230522-295	2023-06-08 15:34	24h R-2.4ppb-1	20230607NDMA-Standard Curve	3.74	51265.01	2.27
6			✓	20230522-296	2023-06-08 15:44	24h R-2.4ppb-2	20230607NDMA-Standard Curve	3.74	49475.32	2.18
Min								3.73	48119.25	2.12
Max								3.74	52725.29	2.33
AVG								3.74	50812.84	2.24
SD								0.00	1693.24	0.08
RSD								0.10	3.33	3.48
IDL								0.00	0.00	0.00

Figure 16 Peak Area at the 0th, 18th and 24th hours after 2.4ng/mL Spike Recovery

5 Conclusions

In this test, the content of NDMA in metformin sustained-release tablets was analyzed. In the linear range of 0.8~100ng/mL, the R^2 of the target compound was 0.9997, the accuracy was 93.40~103.60%, and the linearity was good; the signal-to-noise ratio at the lowest point of linearity 0.8ng/mL was 18, showing good sensitivity; the RSD values of retention time and peak area were less than 0.23% and 4.15% for 0.2, 0.8 and 2.4ng/mL NDMA standard solution, respectively, showing good within-day precision; the retention time RSD of between-day precision was less than 0.12% for continuous 2 days, and the peak area RSD was less than 3.15%, showing good between-day precision; the average recovery of NDMA was 87.97%~93.00% at different concentration of 0.8ng/mL, 2.4ng/mL and 6ng/mL, showing good accuracy; the peak area of 2.4ng/mL standard curve point and 2.4ng/mL spike recovery point changed less than 10% at the 18th and 24th hours, showing good stability. No NDMA was detected in metformin sustained-release tablets.