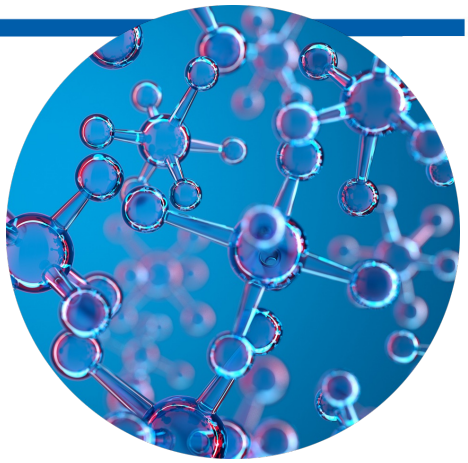

TN-1381

N-Nitroso Furosemide analysis using a SCIEX 5500+ system

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Introduction

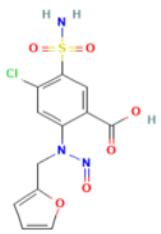
Furosemide is used to treat fluid retention caused by heart failure, liver scarring or kidney disease. It is also used in the treatment of high blood pressure. Furosemide works by reducing the amount of sodium reabsorption in the kidneys and can also result in the lowering of other plasma electrolytes such as potassium. As with any routinely prescribed medication, it is important to ensure purity of the active pharmaceutical ingredient together with any formulated product. As Furosemide is a secondary amine, there is the potential that an NDSRI (nitrosamine drug substance related impurity) can be formed. Such NDSRIs have the potential to be carcinogenic and therefore the presence of NDSRIs must be monitored. This technical note describes the separation and detection of Furosemide and it's associated NDSRI.

Sample Preparation

1.00 mg of N-Nitroso Furosemide was diluted in 1 mL of methanol in a 5 mL polypropylene tube. Subsequent dilutions and sample were prepared in 50:50 v/v of methanol:water.

20 mL of furosemide solution (10 mg/mL) corresponding to ~ 1 mg/mL of Furosemide was aliquoted and diluted with 1.0 mL of diluent 50:50 v/v of Methanol:water. The sample was vortexed for 10-15 min. Samples were then centrifuged at 14000 rpm at 5 °C and filtered with 0.22 µm PVDF syringe filters. The sample was then transferred to a glass autosampler vial for analysis.

Sr. No.	Standard details (as per 1 mg API Load)	Actual concentration (ng/mL)
1	Standard Solution (18.570 PPM)	18.570
2	LOQ Solution (1.857 PPM)	1.857
3	LOD Solution (0.370 PPM)	0.370



N-Nitroso Furosemide
Mol. For.: $C_{12}H_{10}ClN_3O_6S$

LC Conditions

Column: Kinetex™ Biphenyl 2.6 µm
Dimensions: 150 x 3.0 mm
Part No.: 00F-4622-Y0
Mobile Phase: A: 2 mM Ammonium Formate in water with 0.1 % Formic Acid
B: 50:50 methanol: Acetonitrile with 0.1% Formic Acid

Gradient:

Time (min)	% B
0.00	10
2.00	10
4.00	30
7.00	30
10.00	40
15.00	40
16.00	98
18.00	98
18.10	10
21.00	10

Flow Rate: 0.5 mL/min
Injection Volume: 5 µL
Temperature: 40 °C
LC System: EXION LC 30 AD
Detection: MS/MS
Detector: SCIEX 5500+

MS/MS Conditions

Ion Source: ESI
Polarity: Negative
Source Temperature: 600 °C
GS1: 55
GS2: 80
CUR: 40
IS: -4500
CUR: 40
CAD: 8

Table 1. MS Transitions.

Analyte	Q1 Mass (Da)	Q3 Mass (Da)
N-Nitroso Furosemide	358.0	284.0

Have questions or want more details on implementing this method? We would love to help!
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Table 2. Repeatability data (%RSD) for specification, LOQ & LOD

Samples

	Area count observed for			Signal to Noise Ratio$^{\$\$}$ for		
Inj. No.	**Specification (0.053 PPM)**	**LOQ (0.0053 PPM)**	**LOD (0.00159 PPM)**	**Specification (0.053 PPM)**	**LOQ (0.0053 PPM)**	**LOD (0.00159 PPM)**
1	152959	14014	2674	7608	915	217
2	152540	14297	2775	8314	904	185
3	153406	13879	2639	9424	847	200
4	153278	14096		8473	1044	
5	154176	14172		8993	869	
6	153994	14003		9725	925	
Average	**153392**	**14077**	**2696**	**8756**	**917**	**201**
SD (%)	**617**	**146**	**71**	**779**	**69**	**16**
Precision (%)	**0.40**	**1.04**	**2.62**	**8.9**	**7.48**	**7.98**

$^{\$\$}$**Note**: Tabulated S/N Ratio is from the Software Processed Data for the Quantifier MRM Transition.

Figure 2. Representative Specta for UV and XIC Data

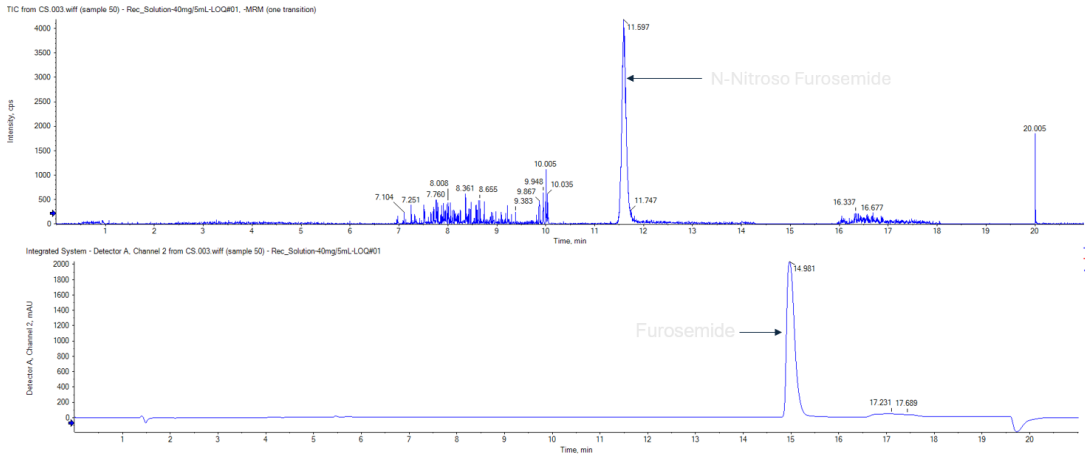

Figure 2. Representative Chromatograms.

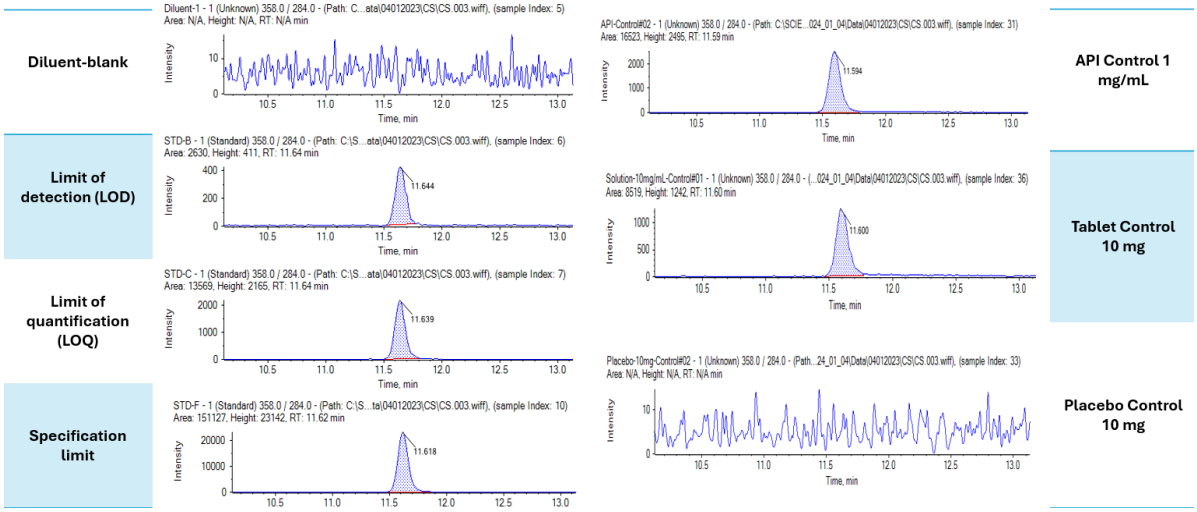

Table 3: Recovery in spiked samples at limit of quantitation level, specification limit and higher specification limit (2 x specification limit)

Mean Area ± S.D (% Recovery)				
	Blank/control samples	**Limit of quantitation (0.0053 ppm)**	**Specification Limit (0.053 ppm)**	**Higher specification Limit (0.106 ppm)**
Diluent	Not Detected	14137 ± 164	153172 ± 1153	352264 ± 1494
Solution 40 mg/5 mL	9034 ± 104	24728 ± 282 **(106.85%)**	171604 ± 2234 **(105.79%)**	379112 ± 1799 (104.93%)
Placebo 40 mg/ 5 mL	Not Detected	15890 ± 202 **(112.41%)**	161602 ± 718 **(105.5%)**	365651 ± 191 **(103.8%)**

Results and Discussion

Kinetex Biphenyl has successfully been employed in the separation of both nitrosamines and NDSRIs in previous experiments. It was selected for this application based on the interactions that it provides, it is weakly hydrophobic and provides van der Waals interactions, together with aromatic or π- π interactions. This combination of interactions allows for increased retention of analytes which have the N-nitroso group present in their structure. By selectivity retaining the NDSRI to a greater extent than the parent drug molecule this allows the drug molecule, present in excess in the sample, to be diverted to waste minimising the amount of sample entering the mass spectrometer which in turn assists in keeping the Q1 quadrupole zone clean. As shown in table 2, the Limit of Quantitation for N-nitroso Furosemide was 1.857 ng/L (0.0053 PPM) and this was maintained in API samples as highlighted in table 3. The method was found to be robust when %RSD data was analysed.

The Core-Shell nature of Kinetex Biphenyl provided high efficiency, ensuring that the N-nitroso Furosemide elutes with a narrow peak width which enhances the peak height. It is the combination of this peak height and the sensitive MS/MS detector which provides the excellent limit of quantitation we have reported

As regulatory agencies issue guidance recommending that manufactures of APIs and Drug Products take steps to detect and prevent unacceptable levels of N-Nitrosamine(s) impurities, as well as NDSRIs in drug product(s), increasingly selective and sensitive analytical methods will be necessary. In this Technical Note, we demonstrate such a method utilizing the chromatographic selectivity of Kinetex Biphenyl and the sensitivity of the SCIEX 5500+ system.

Kinetex™ Ordering Information

2.6 µm Midbore™ Columns (mm)						SecurityGuard™ ULTRA Cartridges (mm)‡
Phases	**30 x 3.0**	**50 x 3.0**	**75 x 3.0**	**100 x 3.0**	**150 x 3.0**	**3/pk**
EVO C18	00A-4725-Y0	00B-4725-Y0	—	00D-4725-Y0	00F-4725-Y0	AJ0-9297
PS C18	00A-4780-Y0	00B-4780-Y0	—	00D-4780-Y0	00F-4780-Y0	AJ0-8950
Polar C18	—	00B-4759-Y0	—	00D-4759-Y0	00F-4759-Y0	AJ0-9531
Biphenyl	—	00B-4622-Y0	—	00D-4622-Y0	00F-4622-Y0	AJ0-9208
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJ0-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	00F-4497-Y0	AJ0-8777
HILIC	00A-4461-Y0	—	—	00D-4461-Y0	00F-4461-Y0	AJ0-8779
Phenyl-Hexyl	—	00B-4495-Y0	—	00D-4495-Y0	00F-4495-Y0	AJ0-8781
F5	—	00B-4723-Y0	—	00D-4723-Y0	00F-4723-Y0	AJ0-9321

‡SecurityGuard Ultra Cartridges require holder, Part No.:AJ0-9000 For 3.0 mm ID

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