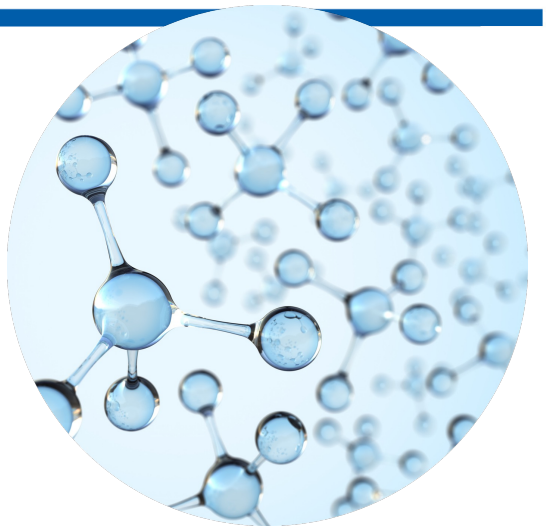

TN-1375

N-Nitroso Desmethyl Doxepin Analysis using a SCIEX 5500+ System

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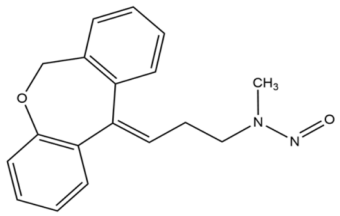
Introduction

Desmethyl Doxepin is a tricyclic antidepressant that acts as a norepinephrine reuptake inhibitor. It has been determined to be the major active metabolite of Doxepin and is more potent than Doxepin alone. Due to the fact that it is a secondary amine, it is possible that an N-nitroso Desmethyl Doxepin impurity (NDSRI – nitroso drug substance related impurity) can form during synthesis or storage. As NDSRIs have the potential to be carcinogenic it is important to monitor the active pharmaceutical compound or formulations for the presence of the NDSRI. In this technical note, a method is described where N-nitroso Desmethyl Doxepin is separated from the parent drug, allowing for sensitive detection by LC-MS/MS.

Sample Preparation

Standard preparation (Impurity). 5 mg of the impurity was dissolved in 5 mL of Acetonitrile to prepare a stock solution of 1000 PPM concentration.

Drug product sample preparation (Doxepin HCl 5% cream). 20 mg of cream base corresponds to ~1 mg of doxepin hydrochloride and was dissolved in 1.0 mL of Methanol. The sample was vortexed for 10-15 min. The sample was later centrifuged at 14000 rpm at 5°C and the supernatant filtered using a 0.22 µm PVDF syringe filter. The sample was then transferred into an autosampler glass vial for analysis



N-Nitroso desmethyl doxepin

Molecular Formula: $C_{18}H_{18}N_2O_2$
Monoisotopic m/z: 294.14

LC Conditions

Column: Kinetex Biphenyl 2.6 µ
Dimensions: 150 x 3.0 mm
Part No.: 00F-4622-Y0
Mobile Phase: A: 1 mM Ammonium formate with 0.1% formic acid in Water
B: 0.1% formic acid in Methanol

Gradient:

Time (min)	% B
0.00	40
4.00	40
6.00	65
9.00	65
11.00	70
19.00	70
20.00	95
22.00	95
22.10	40
25.00	40

Valve Method: 0.0 – 0.5 & 11.0-19.0 min (Flow diverted to MS)
0.5-11 & 19.0-24.0 min (Flow diverted to Waste)
Flow Rate: 0.5 mL/min
Injection Volume: 10 µL
Temperature: 40° C
LC System: EXION LC 30 AD
Detection: MS/MS
Detector: SCIEX 5500+

MS/MS Conditions

Ion Source: ESI
Polarity: Positive
Source Temperature: 550° C
GS1: 85
GS2: 80
CUR: 40
IS: 5500
CUR: 40
CAD: 8

Table 1. MS Transitions.

Analyte	Q1 Mass (Da)	Q3 Mass (Da)
N-Nitroso Desmethyl Doxepin-01	295.1	265.2
N-Nitroso Desmethyl Doxepin-02	295.1	250.2
N-Nitroso Desmethyl Doxepin-03	295.1	236.2
N-Nitroso Desmethyl Doxepin-04	295.1	174.2

Have questions or want more details on implementing this method? We would love to help!
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Table 2. Recovery Results in Doxepin Sample.

Average are observed in control samples (N=3)					ND			
	Area count observed for standard sample				Area count observed for spiked API samples			
	Limit of Quantitation	STD-2	STD-3	Specification Standard	Limit of Quantitation	STD-2	STD-3	2x Higher Specification
Inj. No.	(0.0024 PPM)	(0.006 PPM)	(0.015 PPM)	(0.030 PPM)	(0.0053 PPM)	(0.006 PPM)	(0.015 PPM)	(0.133 PPM)
	0.0024 ng/mL	0.006 ng/mL	0.015 ng/mL	0.030 ng/mL	0.0024 ng/mL	0.006 ng/mL	0.015 ng/mL	0.030 ng/mL
1	1977	4461	10679	21630	1478	4908	10360	18973
2	2191	4190	10780	21184	1402	4914	10252	19116
3	1674	4412	11028	20915	1473	4935	9991	19818
4	1457	-	-	20956	1537	-	-	19416
5	1864	-	-	22612	1275	-	-	20190
6	1889	-	-	20193	1501	-	-	19352
Average	1842.0	4354.3	10829.0	21248.3	1444.3	4919.0	10201.0	19477.5
STDEV (%)	252.65	144.41	179.59	814.82	94.05	14.18	189.71	453.5
% CV	13.72	3.32	1.66	3.83	6.51	0.29	1.86	2.33
% Recovery	% Recovery = Area Observed in Rec Samples/Area Observed in Neat Standards				78.39	112.97	94.2	91.67

Figure 1. Representative Spectra for UV and XIC Data: Standard

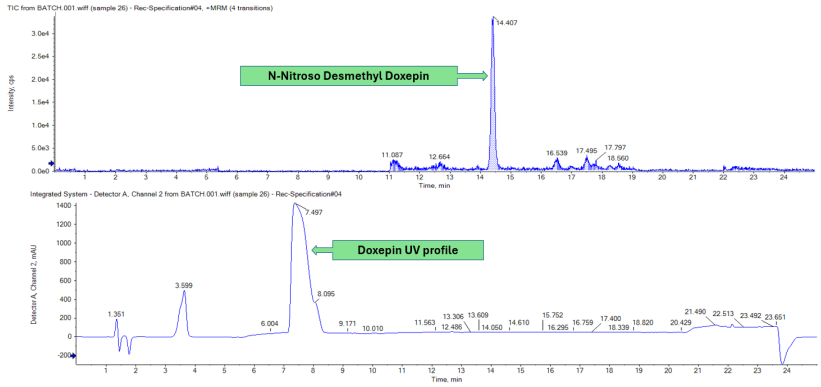
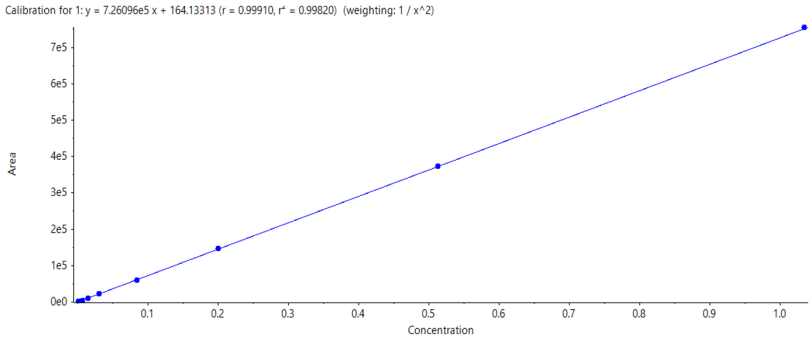


Figure 2. Linearity data: N-Nitroso Desmethyl Doxepin



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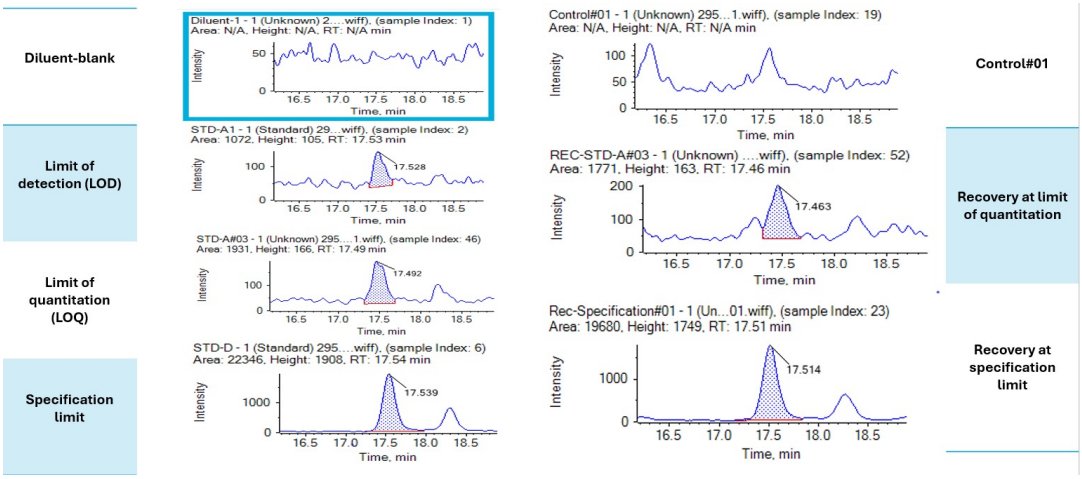
Results and Discussion

Kinetex Biphenyl was selected for the separation due to the polar aromatic nature of the stationary phase. Interactions with the N-nitroso group on the target analyte increase retention of the impurity, providing excellent resolution from the parent drug molecule. This allows the desmethyl doxepin, present in excess, to be directed to waste which aids in keeping the first quadrupole clean in the MS/MS system. As shown in Table 2, the Limit of Quantitation for N-Nitroso Desmethyl Doxepin was 0.0024 ng/mL and was maintained in API samples. Good linearity (Figure 2) and %CVs were maintained to produce a robust and reproducible method. As can be seen in Figure 1, additional specificity and sensitivity was achieved by utilizing LC-MS/MS. The core-shell morphology of the Kinetex Biphenyl column provided high efficiency meaning that the N-Nitroso Desmethyl Doxepin peak exhibited a narrow peak width, ensuring that peak height was maximized. This peak height, combined with the sensitive MS/MS detector, provided the excellent limit of quantitation 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.

Figure 3. Representative Chromatograms



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

for 3.0 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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