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Robust Analysis of Nitrosamine in a Losartan Drug Substance using Strata™ Activated Carbon Extraction

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Introduction

The presence of nitrosamine as an impurity has been detected in drug substances and drug products, such as Losartan and other "-sartan" drugs. Losartan is an angiotensin II receptor blocker (ARB) and is used in the treatment of high blood pressure. N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) are Class 2A carcinogens that have been found to be present as impurities in several different generic "-sartan" drug substances and drug products. NDMA is highly toxic and is a known carcinogen in lab animals and, along with NDEA, has been classified as a probable human carcinogen. Accurate identification and quantification of these impurities is essential to assure quality and safety of the drug substance and drug product.

Here, a method that employs activated carbon for the extraction of polar analytes such as N-Nitrosodimethylamine is used. While dealing with polar molecules like nitrosamines, traditional sample preparation using Solid Phase Extraction (SPE) might not be efficient. Since the analyte is polar, a specialized extraction technique is needed. Activated carbon contains porous carbon with defined pore volume and high surface area that is an appropriate choice for polar analytes like nitrosamines.

In this technical note, we are providing the optimized extraction protocol for the extraction of nitrosamines from a Losartan drug substance using Strata Activated Carbon followed by GC-MS analysis. Traditional methods for analysis of nitrosamines require GC-MS/MS with a headspace capability to meet the low-level detection limits. With Strata Activated Carbon sample preparation and pseudo large volume injections, GC-MS was sufficient to achieve low-level detection of nitrosamines.

Sample Preparation

Condition:	Strata Activated Carbon, 400 mg cartridge (Part No. CS0-9210) with 2 washes of 3 mL Methylene Chloride, 3 washes of 3 mL Methanol
Equilibrate:	Cartridges with 6 washes of 3 mL Water
Load:	100 mL sample onto cartridges
Dry:	Cartridges for 10 min
Elute:	With 1 mL Methylene Chloride and repeat elution 3 more times (3 mL total volume), add 10 µL Ethylene Glycol
Water Removal:	Pass eluent through Sodium Sulfate tubes, 1 g/6 mL (Part No. 8B-S124-JCH) that is prewetted with Methylene Chloride and wash with 3 mL Methylene Chloride
Evaporate:	Solvent to approximately 1 mL in a water bath at 20-25 °C under a gentle stream of Nitrogen
Adjust:	Final volume to 1 mL with Methylene Chloride

GC-MS Conditions

Column:	Zebron™ ZB-WAX*PLUS*™
Dimension:	30 meter x 0.32 mm x 1.00 µm
Part No.:	7HM-G013-22
Injection:	Pulse Splitless (0.5 min @ 140 Kpa) @ 200 °C, 8 µL
Recommended Liner:	Zebron PLUS Liner for Shimadzu® 2010, 3.4 mm ID Single Taper
Liner Part No.:	AG2-4B10-05
Carrier Gas:	Helium @ 1.8 mL/min (Constant Flow)

Oven Program:	Ramp(°C/min)	Temp (°C)	Time(min)
	-	45	3.0
	10	130	3.0
	15	190	0.0
	40	230	10.0

Detector Mode:	SIM
Transferline Temperature:	200 °C
Solvent Delay:	5.0 min

Table 1. GC-MS SIM Parameters

Peak No.	Analyte Name	m/z
1	N-Nitrosodimethylamine-d6 (NDMA-d6)	80
2	N-Nitrosodimethylamine (NDMA)	74
3	N-Nitrosomethylethylamine (NMEA)	88
4	N-Nitrosodiethylamine (NDEA)	102
5	N-Nitrosodipropylamine (NDPA)	70
6	N-Nitrosodibutylamine (NDBA)	84
7	N-Nitrosopiperidine (NPIP)	114
8	N-Nitrosopyrrolidine (NPYR)	100

Ramkumar Dhandapani, PhD
Ramkumar has earned a PhD in Analytical Chemistry and has over 17 years troubleshooting and hands-on experience in chromatography. He loves to write poems, watch, and read Shakespeare's plays.

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Results and Discussion

Figure 1 is a representation of the Strata™ Activated Carbon extraction cartridges. The porous carbon is optimized for reproducible extraction of polar analytes. Although the 400 mg format was employed in this application, there is also a 2 g/6 mL format available for larger sample sizes.

All nitrosamines tested in this study were extracted and reconstituted with Dichloromethane (DCM), so a DCM blank was run to demonstrate there were no nitrosamines in the interface (**Figure 2**). As presented in **Figures 3**, **4**, and **5** the individual standards, including the internal standard (IS) N-Nitrosodimethylamine-d6 (NDMA-d6), were identified after extraction using the Strata Activated Carbon extraction cartridges at 0.5, 1, and 5 µg/mL respectively. This shows accuracy to low level concentrations of nitrosamines. The analytical method proved to be linear for the extraction and analysis at this concentration range.

Losartan and other members of the "-sartan" family of drugs are known to contain nitrosamine impurities or produce nitrosamines as they are broken down. **Figure 6** is a representative chromatogram of a Losartan sample after extraction using the Strata Activated Carbon extraction cartridge. For analysis, the Zebron™ ZB-WAX*PLUS*™ was utilized. The high polar selectivity, extensive cross-linkage in the stationary phase, and the highly deactivated fused silica in this column provide MS compatibility and an inert environment for active analytes. This results in low baseline noise and symmetric peaks for even the most challenging analytes like nitrosamines.

To establish accuracy of analysis, Losartan sample was spiked with nitrosamines at 0.5 µg/mL (**Figure 7**). Repeatability of the analysis was demonstrated using multiple injections of the sample spike. Finally, to clearly identify nitrosamines, and to show low detection in real samples, the Losartan sample was overlapped with nitrosamine standard at 5.0 µg/mL concentration (**Figure 8**).
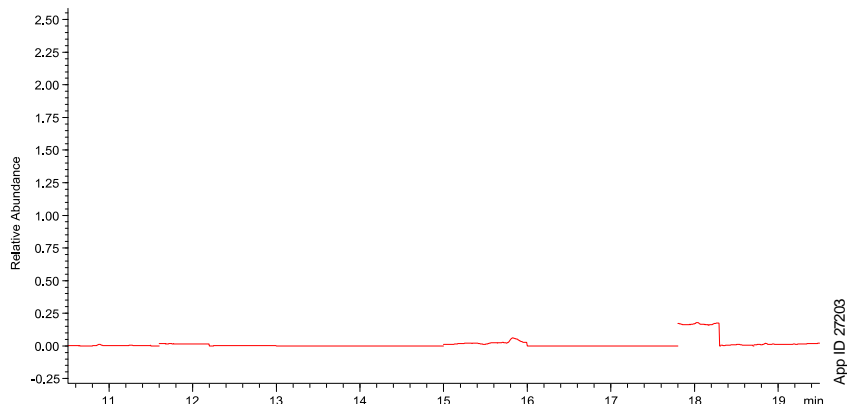
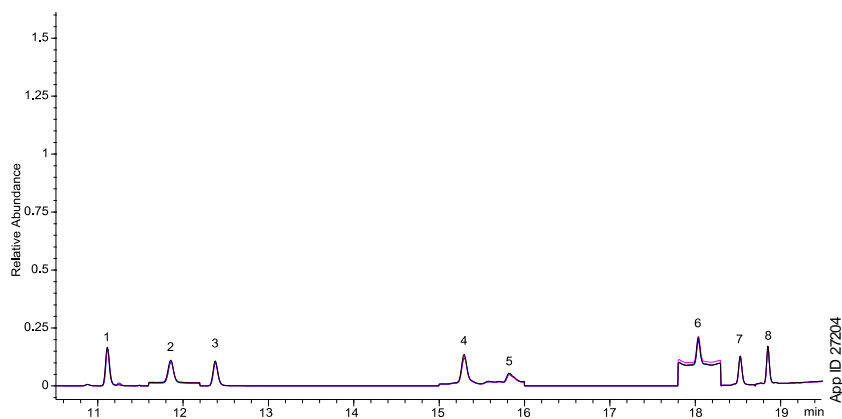**Figure 1.** Strata Activated Carbon Extraction Cartridges

400 mg   2 g
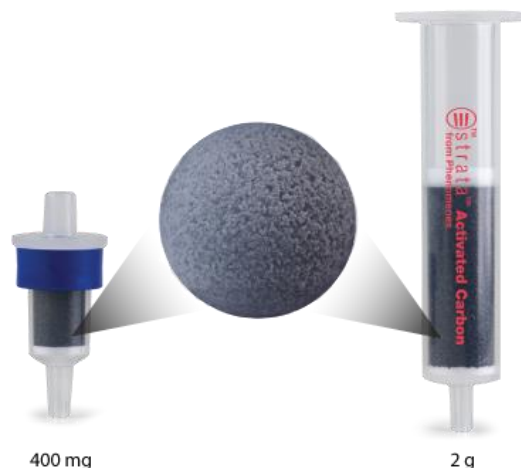
Table 2. % Recovery for Extracted Losartan Sample

Analyte Name	Conc (µg/mL)	% Recovery
NDMA-d6	0.9	-
NDMA	0.525	70
NMEA	0.516	103
NDEA	0.529	106
NDPA	0.498	7
NDBA	0.548	83
NPIP	0.514	68
NPYR	0.536	77

Figure 2. DCM Blank
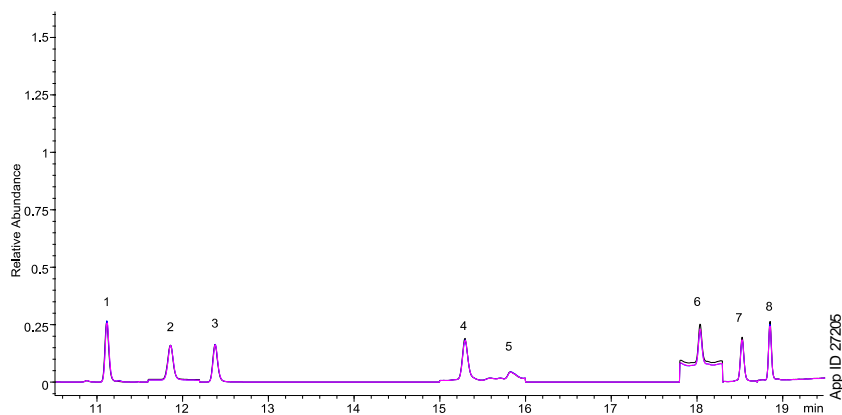
**Figure 3.** Nitrosamine Standard at 0.5 µg/mL after Strata™ Activated Carbon Extraction

Peak No.	Analyte	RT (min)	% RSD
1	NDMA-d6 (IS)	11.10	-
2	NDMA	11.11	0.3
3	NMEA	11.86	0.0
4	NDEA	12.38	0.5
5	NDPA	15.29	2.7
6	NDBA	18.03	6.4
7	NPIP	18.53	3.5
8	NPYR	18.85	3.5
N = 3 Injections			

App ID 27204

Figure 4. Nitrosamine Standard at 1.0 µg/mL after Strata Activated Carbon Extraction

Peak No.	Analyte	RT (min)	% RSD
1	NDMA-d6 (IS)	11.10	-
2	NDMA	11.11	0.4
3	NMEA	11.86	0.3
4	NDEA	12.38	0.7
5	NDPA	15.29	3.2
6	NDBA	18.03	6.4
7	NPIP	18.53	4.3
8	NPYR	18.85	4.0
N = 3 Injections			

App ID 27205

Figure 5. Nitrosamine Standard at 5.0 µg/mL after Strata Activated Carbon Extraction
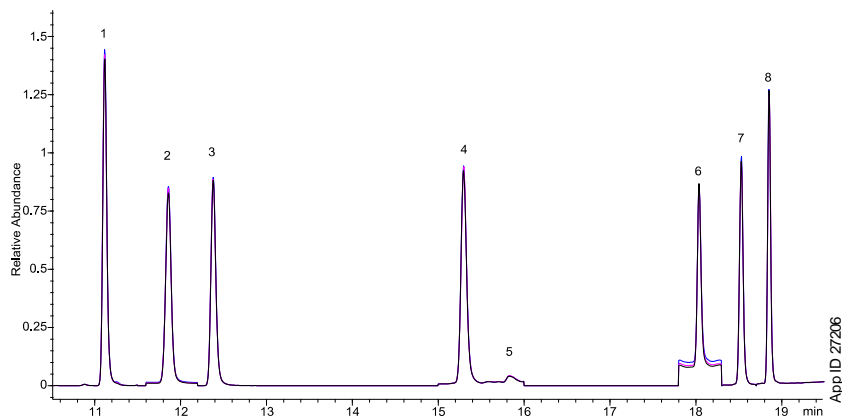
Peak No.	Analyte	RT (min)	% RSD
1	NDMA-d6 (IS)	11.11	-
2	NDMA	11.12	0.1
3	NMEA	11.86	0.1
4	NDEA	12.38	0.2
5	NDPA	15.29	0.3
6	NDBA	18.04	2.6
7	NPIP	18.53	0.9
8	NPYR	18.85	1.2
N = 3 Injections			

App ID 27206

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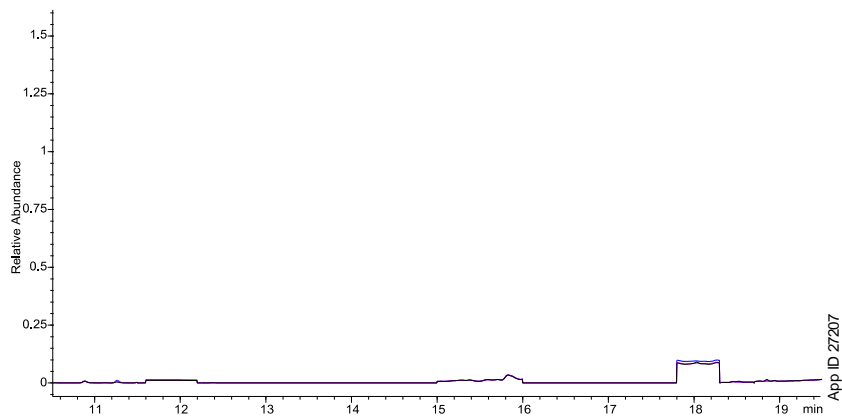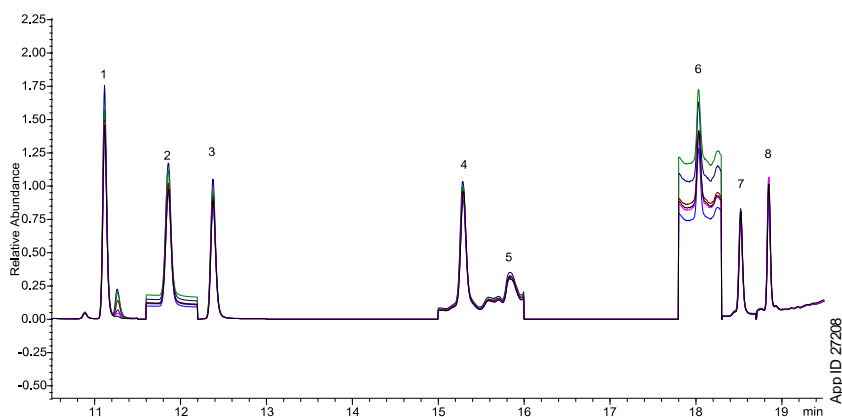**Figure 6.** Losartan Sample after Strata™ Activated Carbon Extraction

Figure 7. Chromatogram of 0.5 µg/mL Nitrosamine Spiked Losartan Sample after Strata Activated Carbon Extraction

Peak No.	Analyte	RT (min)	% RSD
1	NDMA-d6 (IS)	11.10	-
2	NDMA	11.12	0.8
3	NMEA	11.86	0.6
4	NDEA	12.38	0.9
5	NDPA	15.29	4.2
6	NDBA	18.03	9.4
7	NPIP	18.52	6.3
8	NPYR	18.85	6.1
N = 6 Injections			

Figure 8. Overlaid Chromatograms of Losartan Sample (**Figure 6**) and Nitrosamine Standard at 5.0 µg/mL (**Figure 5**)
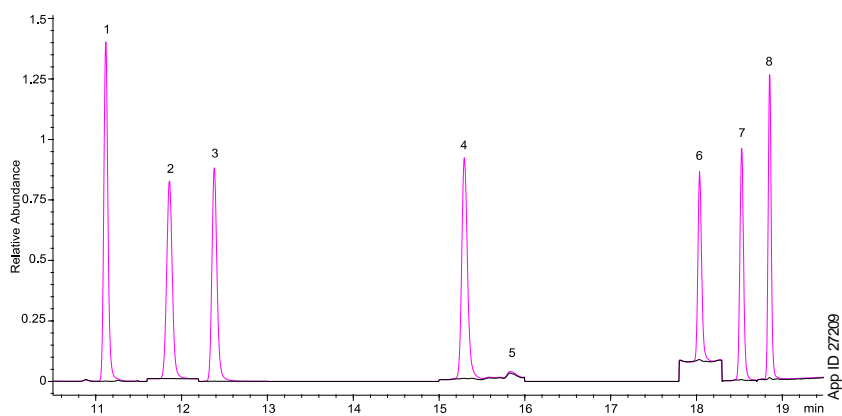
Peak No.	Analyte	RT (min)
1	NDMA-d6 (IS)	11.11
2	NDMA	11.12
3	NMEA	11.86
4	NDEA	12.38
5	NDPA	15.29
6	NDBA	18.04
7	NPIP	18.53
8	NPYR	18.85

Conclusions

Strata™ Activated Carbon extraction provided reproducible extraction of several nitrosamines in a Losartan sample matrix. The results demonstrated the range of detection of nitrosamines after extraction using the Strata Activated Carbon extraction cartridge and the ability to identify the presence or absence of nitrosamine in a Losartan sample. The combination of sample prep extraction using Strata Activated Carbon and pseudo large volume injection with pressure pulse helped achieve lower detection limits with simple GC-MS detection.

Strata Tubes and Cartridges Ordering Information

Strata Activated Carbon				
Part	**Sorbent Mass**	**Volume**	**Format**	**Units/pk**
CS0-9209	2 g	6 mL	Tube	30
CS0-9210	400 mg	Pass through	Cartridge	50

Zebron™ ZB-WAX*PLUS*™ GC Columns Ordering Information

ID(mm)	df(μm)	Temp. Limits °C	Part No.
10-Meter			
0.10	0.10	20 to 250/260	7CB-G013-02
15-Meter			
0.25	0.25	20 to 250/260	7EG-G013-11
0.53	1.00	20 to 230/240	7EG-G013-22
20-Meter			
0.18	0.18	20 to 250/260	7FD-G013-08
30-Meter			
0.25	0.25	20 to 250/260	7HG-G013-11
0.25	0.50	20 to 250/260	7HG-G013-17
0.32	0.25	20 to 250/260	7HM-G013-11
0.32	0.50	20 to 250/260	7HM-G013-17
0.32	1.00	20 to 230/240	7HM-G013-22
0.53	0.25	20 to 250/260	7HK-G013-11
0.53	3.00	20 to 230/240	7HK-G013-22
60-Meter			
0.25	0.15	20 to 250/260	7KG-G013-05
0.25	0.25	20 to 250/260	7KG-G013-11
0.25	0.50	20 to 250/260	7KG-G013-17
0.32	0.25	20 to 250/260	7KM-G013-11
0.32	0.50	20 to 250/260	7KM-G013-17
0.53	1.00	20 to 230/240	7KK-G013-22

Note: If you need a 5 in. cage, contact Technical support via Phenomenex.com/chat or simply reach out to your Technical consultant. Conditions may apply. Agilent 6850, some SRI and process GC systems use only 5 in. cages.

Liners Compatible with Shimadzu® GC Systems Ordering Information

Description	Application	Inlet Style	Dimensions	Deactivation	Part No.	Unit
Zebron™ PLUS Liners for 17A, 2014 and 2025 Models						
Single Taper Z-Liner™	Pesticides	S/SL	3.4 x 95	PLUS Inert	AG2-3B13-01 AG2-3B13-05 AG2-3B13-25	ea 5/pk 25/pk
Straight Z-Liner	Dirty samples, Volatiles, High initial oven temperatures	S/SL	3.4 x 95	PLUS Inert	AG2-3B03-01 AG2-3B03-05 AG2-3B03-25	ea 5/pk 25/pk
Zebron PLUS Liners for2010 Models						
Single Taper	Dirty samples, Volatiles, High initial oven temperatures	S/SL	3.4 x 95	PLUS Inert	AG2-4B10-01 AG2-4B10-05 AG2-4B10-25	ea 5/pk 25/pk
Single Taper Z-Liner	Pesticides	S/SL	3.4 x 95	PLUS Inert	AG2-4B13-01 AG2-4B13-05 AG2-4B13-25	ea 5/pk 25/pk
Straight	Volatiles	S/SL	3.4 x 95	PLUS Inert	AG2-4B00-01 AG2-4B00-05 AG2-4B00-25	ea 5/pk 25/pk
Straight Z-Liner	Dirty samples, Volatiles, High initial oven temperatures	S/SL	3.4 x 95	PLUS Inert	AG2-4B03-01 AG2-4B03-05 AG2-4B03-25	ea 5/pk 25/pk

Sodium Sulfate Tubes Ordering Information

Strata™			
Format	**Sorbent Mass**	**Part Number**	**Unit**
Tube	1 g	8B-S124-JCH	6 mL (30/box)
Giga™ Tube	5 g	8B-S124-LEG	20 mL (20/box)

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