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Highly Sensitive Quantification of Nitrosamines in Polymer-based Therapeutics-A Case Study of Sevelamer Hydrochloride 800mg Tablets

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Introduction

During recent years, the presence of Nitrosamine impurities in various drug substances and drug products lead to the multiple recalls by regulatory agencies like USFDA and EMEA. Initially nitrosamine impurities observed in Angiotensin II Receptor Blocker (ARB) drug categories and later expanded to other products like ranitidine and metformin. The possibility of nitrosamine contamination can be further extended to other APIs and drug products due to use of vulnerable process and material that can produce nitrosamines.

Sevelamer Hydrochloride is a polymeric amine that binds phosphate and used to control the serum phosphorous in patients with chronic kidney disease (CKD) on dialysis. Based on the maximum daily dosage of sevelamer hydrochloride, the limits of nitrosamines were decided, and it required highly sensitive LC-MS/MS method for quantification. In the present work we have developed method for the quantitative screening of six nitrosamine impurities NDMA, NDEA, NEIPA, NDIPA, NDPA and NDBA in Sevelamer hydrochloride 800mg tablets.

Instrumentation

1290 Infinity II high-speed pump (G7120A)
1290 Infinity II multisampler (G7167B)
1290 Infinity II multicolumn thermostat (G7116B)
1290 Infinity II variable wavelength detector (G7114B)
6470 triple quadrupole LC/MS (G6470B)

Table 1: Instrumentation detail

Figure 1: 6470B triple quadrupole LC/MS

Experimental

Sample Preparation

1. Weigh 5-6 tablets of Sevelamer HCl 800mg, crush the tablets and transfer equivalent to 100mg(± 1mg) of Sevelamer HCl API in a 15 mL centrifuge tube.
2. Add 5ml of diluent (95:5 Water: Methanol) and sonicate for 30min and then Centrifuge for 15min at 4500rpm
3. Filter the supernatant using 0.22µm PVDF syringe filter into an LCMS vial and inject into LC-MS/MS

LC Conditions		
Needle wash	Methanol: Water/ 80:20	
Sample diluent	Water: Methanol 95:5	
Multisampler temperature	6 °C	
Injection volume	20 µL	
Analytical column	Infinity Lab Poroshell HPH C18 4.6 x 150mm 2.7µm (P/N 693975-702T)	
Column temperature	40 °C	
Mobile phase A	0.1% formic acid in water	
Mobile phase B	0.1% formic acid in Methanol	
Flow rate	0.5 mL/min	
Gradient	Time (min)	%B
	0.0	5
	2.0	5
	7.0	60
	10.0	75
	11.0	90
	16.5	90
	16.6	5
	21.0	5
Stop time	21 minutes	

Table 2: 1290 UHPLC conditions

Method Optimization

The 6470 LC/TQ was used for optimizing the mass spectrometric conditions for nitrosamine impurities in positive mode where $[M+H]^+$ species were found to be predominant precursor ions using Atmospheric pressure chemical ionization (APCI) .

MRM Transitions and Conditions

Compound	Prec. Ion (*m/z*)	Product Ion (*m/z*)	Frag. (V)	CE (V)	CAV (V)	±
NDMA	75	43.1	90	16	3	+
NDEA	103	75	78	12	4	+
NEIPA	117	74.9	82	8	8	+
NDIPA	131	89.1	80	5	4	+
NDPA	131	89.1	80	5	4	+
NDBA	159	57	86	12	4	+

Table 3: MRM transitions and conditions

MS Conditions

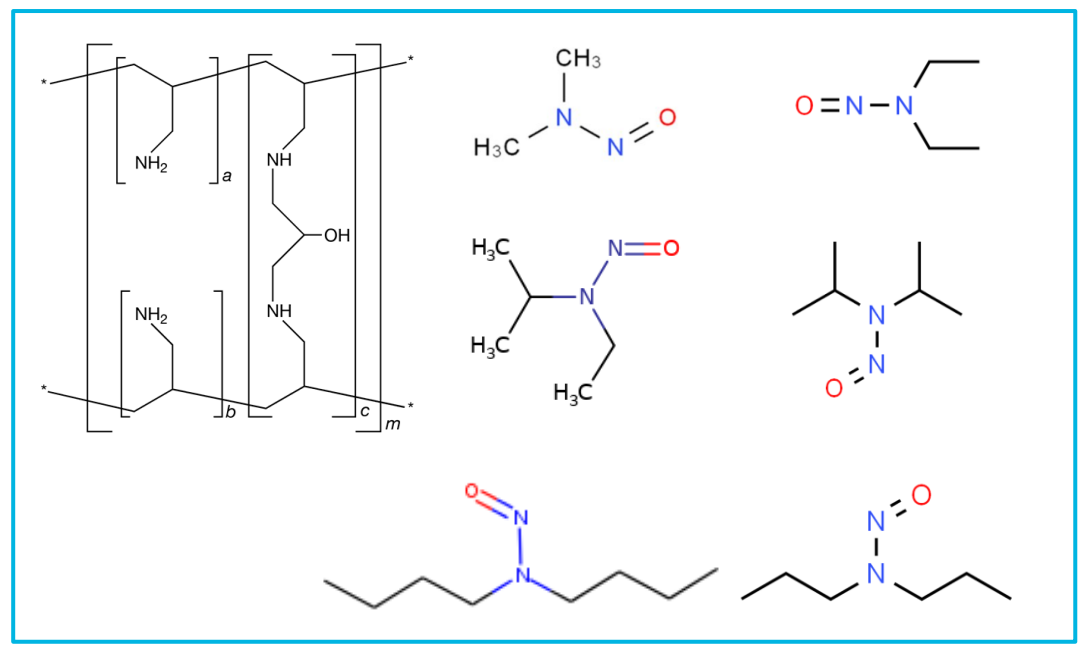
Equipment	6470 LC/TQ Parameters
Gas Temperature	300^{0}C
Gas Flow	7L/min
Capillary Voltage	4000V
Nebulizer Pressure	25psi
APCI Heater	350^{0}C
APCI Needle Positive	4 µa

Table 4: MS conditions

The most challenging part of the method is to establish the sensitivity and selectivity of Nitrosamines in polymer molecule Sevelamer HCl which does not have any chromophore to find out the UV wavelength which usually helps in observing the retention time of API followed by establishing the selectivity of impurities.

In this case we have run Sevelamer 800mg tablets in Total Ion Chromatogram(TIC) mode to understand the elution and ionization pattern of the polymer and the other excipients present. After performing different gradient and column conditions we have separated all the six nitrosamines from the most abundant peaks observed in TIC of Sevelamer Hcl 800mg tablets. We have established the recovery with the help of optimized sample preparation conditions mentioned earlier.

Chemical Structures of Sevelamer and Nitrosamines NDMA, NDEA, NEIPA, NDIPA, NDPA and NDBA



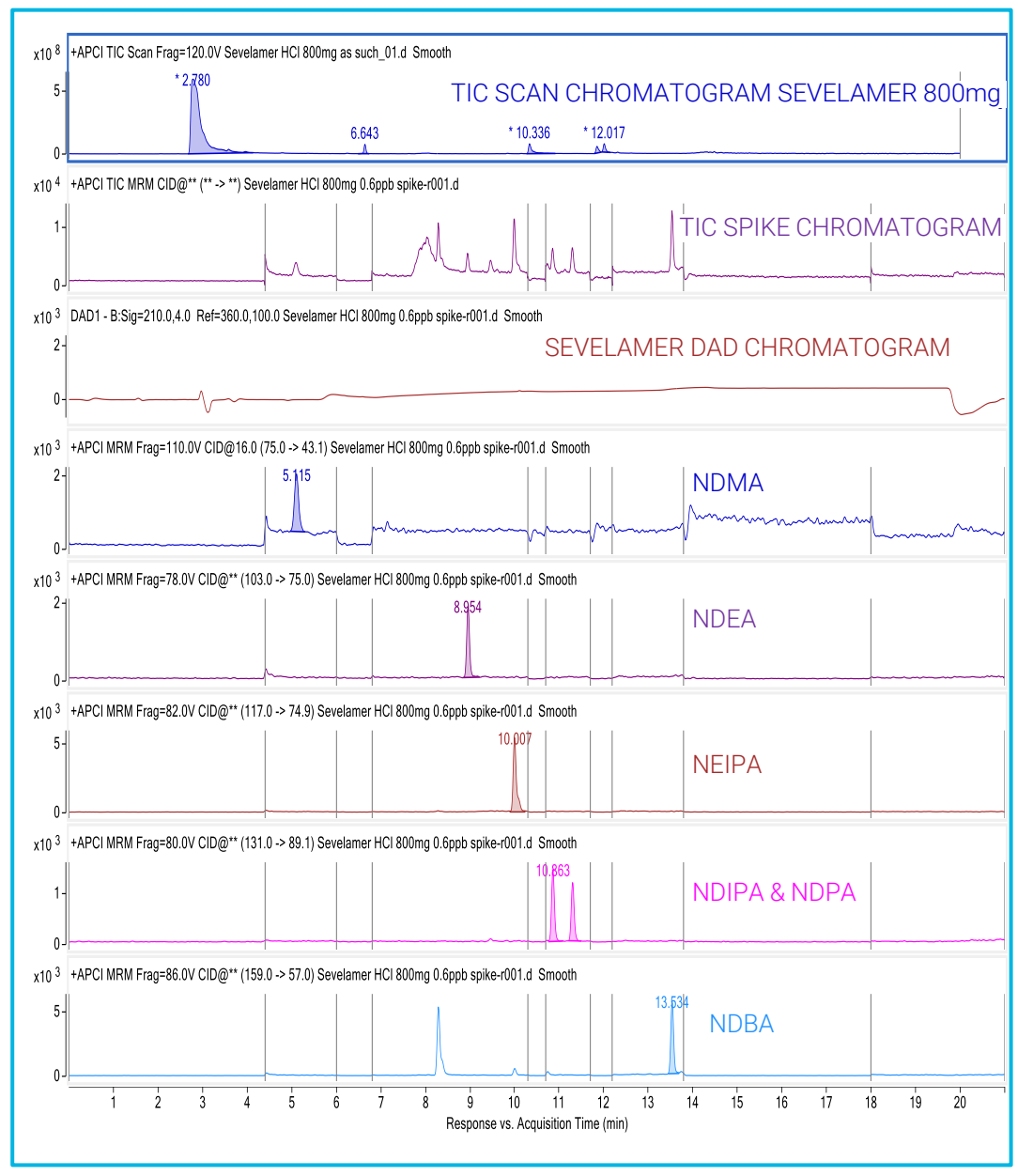


Figure 2: Representative EIC of Six nitrosamine impurities at 0.6ng/ml (0.03ppm) wrt 20mg/mL of Sevelamer 800mg tablets and TIC Scan and DAD chromatogram of Sevelamer 800mg tablets

Results and Discussion

Area % RSD at 0.6ng/mL

S.NO	NDMA	NDEA	NEIPA	NDIPA	NDBA	NDPA
1	11404	8312	28083	6476	30751	5592
2	12301	8773	30134	6298	29367	5482
3	11551	8825	30822	6539	31814	5889
4	11834	8759	31292	5989	30053	5330
5	11817	8686	29664	6680	30679	5473
6	11354	8524	30506	6812	30697	5744
7	10982	8087	28223	5957	31088	5955
Average	11606.1	8566.6	29817.7	6393.0	30635.6	5637.9
STD DEV	423.5	276.1	1247.1	328.8	770.4	232.1
%RSD	3.6	3.2	4.2	5.1	2.5	4.1

Table 5: Peak area % RSD for 7 replicates at 0.6 ng/mL

Recovery Study

The recovery experiment shows excellent recovery of ± 20 % of the spiked concentrations. For all the six nitrosamine impurities In this experiment recovery study was performed at 0.6ng/ml (0.03ppm wrt test 20mg/ml) and LOQ of 0.1ng/ml (0.005ppm wrt 20mg/ml) for NDMA and 0.04ng/ml (0.002ppm wrt 20mg/ml). This recovery data makes the method ready for batch analysis of Sevelamer 800mg tablets

S.No	Name of the Nitrosamine Impurity	Recovery at 0.6ng/ml (0.03ppm)	Recovery at 0.1 and 0.04 ng/ml (LOQ)
1	NDMA	94	88
2	NDEA	90	95
3	NEIPA	95	85
4	NDIPA	97	72
5	NDPA	94	106
6	NDBA	80	76

Table 6: Recovery data in Olmesartan medoxomil drug substance

Conclusions

- The method provides excellent sensitivity and reproducibility as per recent USFDA guidance on control of nitrosamine impurities in drug substances and drug products.
- High throughput method developed for the Simultaneous determination of six nitrosamine impurities in Sevelamer Hcl 800mg tablets.
- The method developed has all the critical performance parameters established which shows the robustness and efficiency to use for routine batch analysis of Sevelamer HCl 800 mg tablets.

References

1. FDA guidance document: Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs.
2. FDA guidance document: Control of Nitrosamine Impurities in Human Drugs
3. Determination of Nitrosamine Impurities Using the Ultivo Triple Quadrupole LC/MS. Agilent Technologies application note, publication number 5994-1383EN, 2019.
4. Simultaneous Determination of Eight Nitrosamine Impurities in Metformin Using the Agilent 6470 Triple Quadrupole LC/MS Agilent Technologies application note, publication number 5994-2286EN, 2020

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