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Determination of Nitrosamine impurities in Losartan Potassium drug substance using Triple Quadrupole Liquid Chromatography Mass Spectrometry

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Introduction

The announcement for the recall of ARB medicines Valsartan, Losartan and Irbesartan made N-Nitroso impurities a focus for regulatory agencies including the FDA and the European Medicines Agency (EMA). Nitrosamine impurities are byproducts produced in trace amounts during the manufacturing processes of these medicines. These impurities/compounds are classified as probable carcinogens (i.e. potentially genotoxic impurities).

The liquid chromatography mass spectrometry-based method described in this poster was carried out on the 6470 Triple Quadrupole LC/MS (LC/TQ), presenting a comprehensive analysis of 6 nitrosamine impurities in Losartan Potassium drug substance at very low detection limits. All nitrosamine impurities are of very small molecular weight. These nitrosamine impurities include: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitroso-4-methyl-4-aminobutyric acid (NMBA), N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA) and N-nitrosodibutylamine (NDBA).

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 (G6470A)

Table 1: Instrumentation detail

Figure 1: 6470 triple quadrupole LC/MS

Experimental

Sample Preparation

The sample preparation procedure was optimized using the following steps.

1.Weigh 100mg(± 2mg) Losartan Potassium drug substance sample in a 15 mL centrifuge tube.

2. Add 5 mL sample diluent and vortex for 2minute.

3. Now put the sample in shaker at 450rpm for 40 minutes.

4. Centrifuge the sample at 5000 rpm for 10 minutes.

5. Filter the supernatant using 0.2µm nylon syringe filter into an LCMS vial.

6. Inject the sample into LC/TQ.

LC Conditions			
Needle wash	Methanol: Water/ 80:20		
Sample diluent	Water: Methanol 95:5		
Multisampler temperature	6 °C		
Injection volume	20 µL		
Analytical column	Zorbax Eclipse Plus Phenyl-Hexyl, RRHD 2.1 x 100mm 1.8µm (P/N 959758-912)		
Column temperature	40 °C		
Mobile phase A	0.2 % formic acid in water		
Mobile phase B	Methanol		
Flow rate	0.25 mL/min		
Gradient	Time (min)	%B	Flow(mL/min)
	0.0	5	0.25
	5.0	25	0.25
	13.0	55	0.40
	20.0	55	0.40
	20.1	95	0.25
	23.0	95	0.25
	23.1	5	0.25
	25.0	5	0.25
Stop time	25 minutes		
Post time	2 minutes		

Table 2: LC conditions

Method Optimization

The 6470 LC/TQ was used for detecting the mass conditions for nitrosamine impurities in positive mode where M+H ion were found to be predominant precursor ions. The method was optimized using atmospheric pressure chemical ionization (APCI) source as most of the nitrosamines give better response and low noise background using APCI source. MRM method was converted into a dynamic MRM method.

MRM Transitions and Conditions

Compound	Prec. Ion (*m/z*)	Product Ion (*m/z*)	Frag. (V)	CE (V)	CAV (V)	±
NDEA	103.1	75.1	80	9	3	+
NDEA	103.1	47.1	80	17	3	+
NDMA	75.1	58	60	12	3	+
NDMA	75.1	43.1	60	18	3	+
NMBA	147.1	44.2	60	16	3	+
NMBA	147.1	87.2	60	10	3	+
NEIPA	117.1	75.1	75	8	3	+
NEIPA	117.1	47.1	75	18	8	+
NDIPA	131.1	89.1	75	6	3	+
NDIPA	131.1	43.1	75	12	8	+
NDBA	159.1	57.2	90	12	3	+
NDBA	159.1	41.1	90	22	3	+

Table 3: MRM transitions and conditions

MS Conditions	
Gas Temperature	300 ^{0}C
Gas Flow	6 L/min
Capillary Voltage	3000V
Nebulizer Pressure	55 psi
APCI Heater	350 ^{0}C
APCI Needle Positive	4 µA

Table 4: MS conditions

The chromatographic separation of Losartan Potassium drug substance and nitrosamine impurities was best achieved using Zorbax Eclipse Plus Phenyl-Hexyl column and diverter valve was programmed such that Losartan Potassium peak was diverted to waste and monitored using variable wavelength detector.

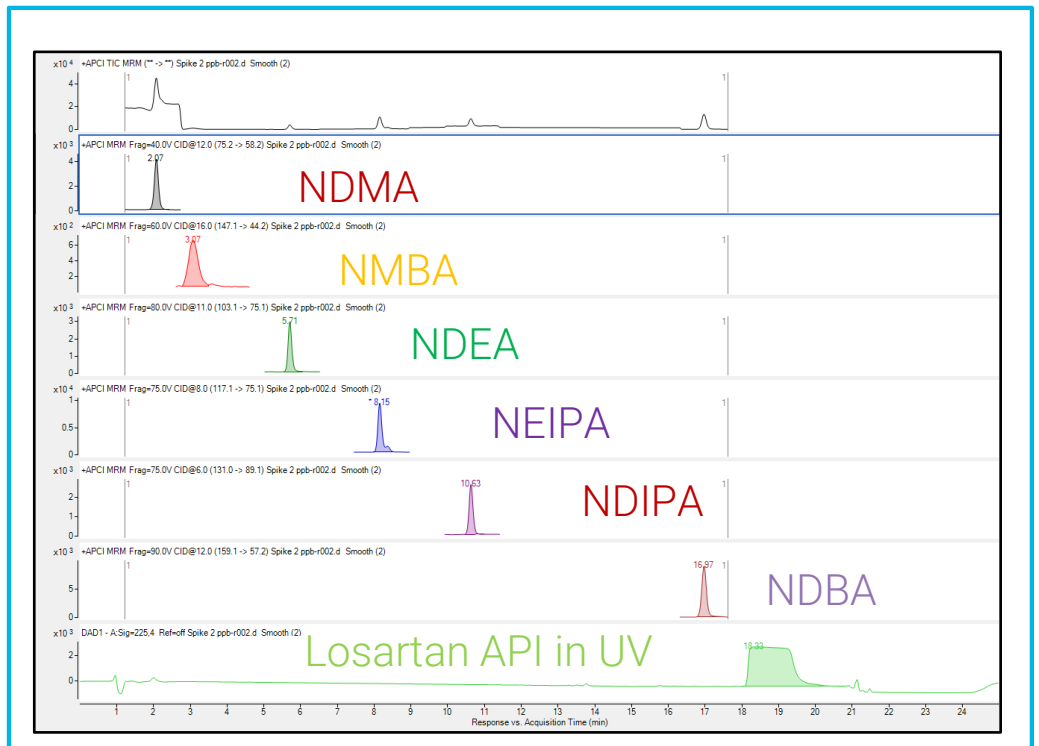

Figure 2: Representative EIC of NDMA, NMBA, NDEA, NEIPA, NDIPA and NDBA at 0.1 ppm conc. using 20mg/mL of Losartan Potassium API.

Below is presented the reproducibility data at 1ng/mL standard concentration for 8 replicates including bracketing standards (# 7 and 8) showing excellent peak area RSD % of < 6 % for each 6 nitrosamine impurities.

Area % RSD at 1ng/mL

#	NDMA	NMBA	NDEA	NEIPA	NDIPA	NDBA
1	2556	5484	10530	36010	14023	18686
2	2409	5609	10727	36593	13478	18853
3	2436	4844	9962	34563	13899	16452
4	2442	4937	10067	32146	13871	16342
5	2435	4827	10066	32805	14375	16942
6	2578	4996	10182	32838	13822	16670
7	2442	4987	10145	33254	14335	16706
8	2434	4966	10193	33108	13868	16691
Avg	2467	5081	10234	33915	13959	17168
SD	63.16	295.66	259.96	1629.64	289.90	1005.59
RSD (%)	2.56	5.82	2.54	4.81	2.08	5.86

Table 5: Peak area % RSD for 8 replicates at 1ng/mL

Results and Discussion

Method Performance Characterization

Figure 3 shows the calibration curves for the standard calibration of all 6 nitrosamines. The relevant calibration range for NDMA, NMBA and NDEA is from 0.05ng/mL to 25ng/mL and for NEIPA, NDIPA and NDBA is from 0.1 ng/mL to 25ng/mL.

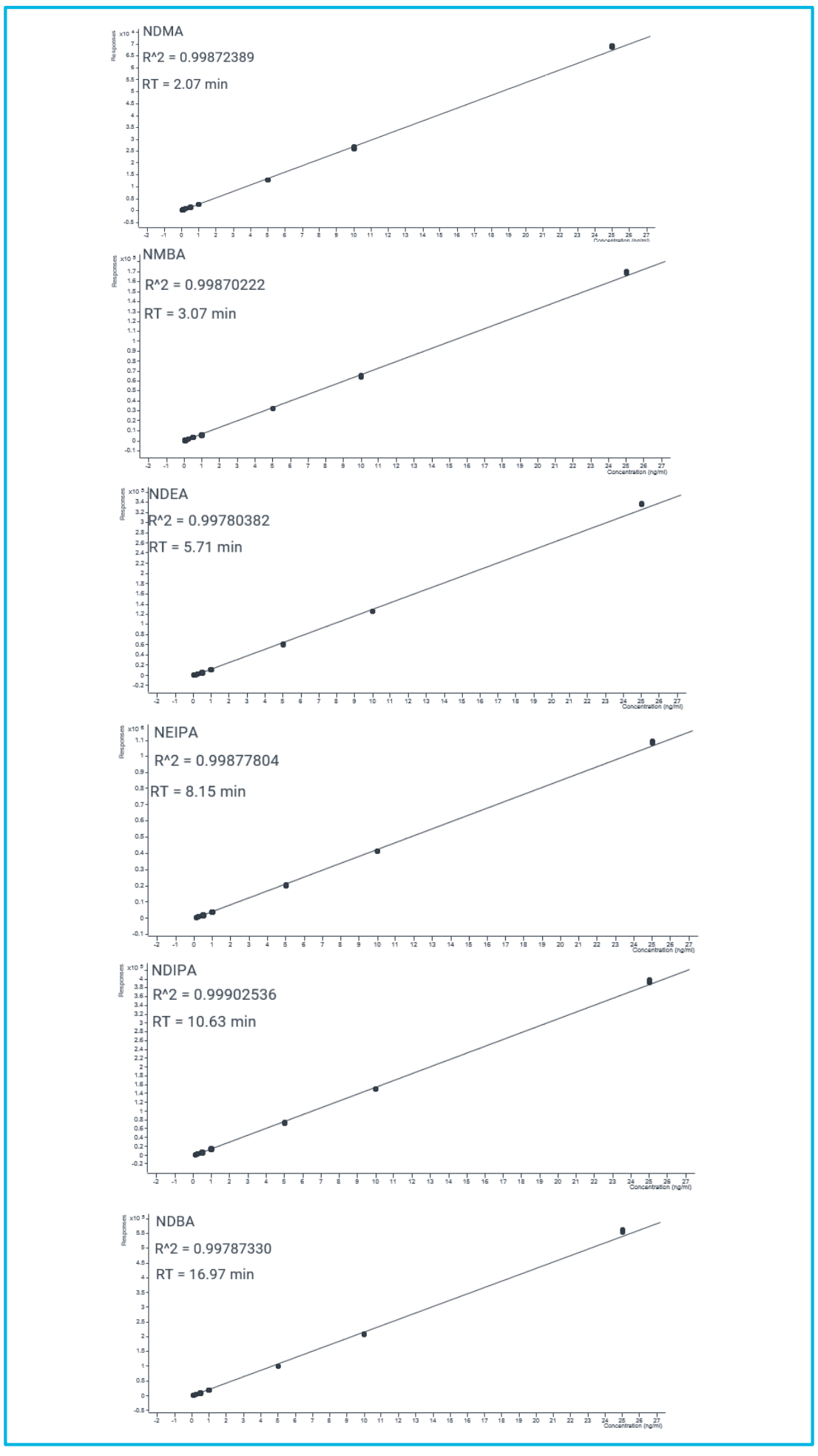

Figure 3: Calibration curves of all 6 nitrosamines with $r^2 > 0.997$

Recovery Study

The recovery experiment shows excellent recovery of ± 20 % of the spiked concentrations.

Nitrosamine Impurity	Concentration (ng/mL)	Recovery %
NDMA	2	110
NMBA	1	113
NDEA	1	103
NEIPA	1	100
NDIPA	1	98
NDBA	2	91

Table 6: Recovery data in Losartan API

Conclusions

- The method provides excellent reproducibility at USFDA defined LOQ concentrations levels as it shows area RSDs of < 6% with bracketing standards included in the calculations.
- The method is a ready to use method for analysis of Losartan Potassium drug substance batches as the method shows excellent recovery.
- The Losartan Potassium drug substance peak is chromatographically well separated from nitrosamine peaks so it can easily be diverted from the MS. Therefore, there is no contamination to the mass spectrometer due to a high concentration of API.

References

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