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

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Introduction

The announcement for the recall of ARB medicines 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. Not only ARB drugs but there are other medicines like Pregabalin known as an anti-epileptic drug where the synthetic route or the manufacturing processes may cause the formation of some nitrosamine impurities at trace levels.

There seems to have a clear need for screening of such pharmaceuticals drugs as well for nitrosamine impurities. LCMS-based method presented here is carried out on 6470 triple quadrupole LC/MS (LC/TQ) and provides comprehensive analysis of 5 nitrosamine impurities at low detection limits. These nitrosamine impurities include: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitroso-methyl-4-aminopyridine (NMAP), N-nitrosopiperidine (NPIP) 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) Pregabalin 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 LCMS/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 3 x 150mm 4µm (P/N 693970-502T)
Column temperature	40 °C
Mobile phase A	0.2 % formic acid in water
Mobile phase B	Methanol
Flow rate	0.5 mL/min
Gradient	Time (min) %B 0.0 5 5.0 30 6.2 33.5 8 95 11 95 11.1 5 14 5
Stop time	14 minutes
Post time	1 minute

Table 2: 1290 UHPLC conditions

Method Optimization

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

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	+
NPIP	115.1	69.1	90	12	3	+
NPIP	115.1	41.2	90	24	3	+
NMAP	138.1	108	60	6	5	+
NMAP	138.1	79.2	60	42	5	+
NDBA	159.1	57.2	90	12	3	+
NDBA	159.1	41.1	90	22	3	+

MS Conditions

Equipment	6470 LC/TQ Parameters
Gas Temperature	300^{0}C
Gas Flow	6L/min
Capillary Voltage	3000V
Nebulizer Pressure	55psi
APCI Heater	350^{0}C
APCI Needle Positive	4 µA

Table 4: MS conditions

The most critical part of this method is chromatographic separation of Pregabalin from nitrosamine impurities. In this method Pregabalin peak (monitored at 200nm wavelength) is separated well from all five intended nitrosamine impurities and hence making it a very robust method in terms of avoiding high concentration drug substance contamination to mass spectrometer with the help of the diverter valve program.

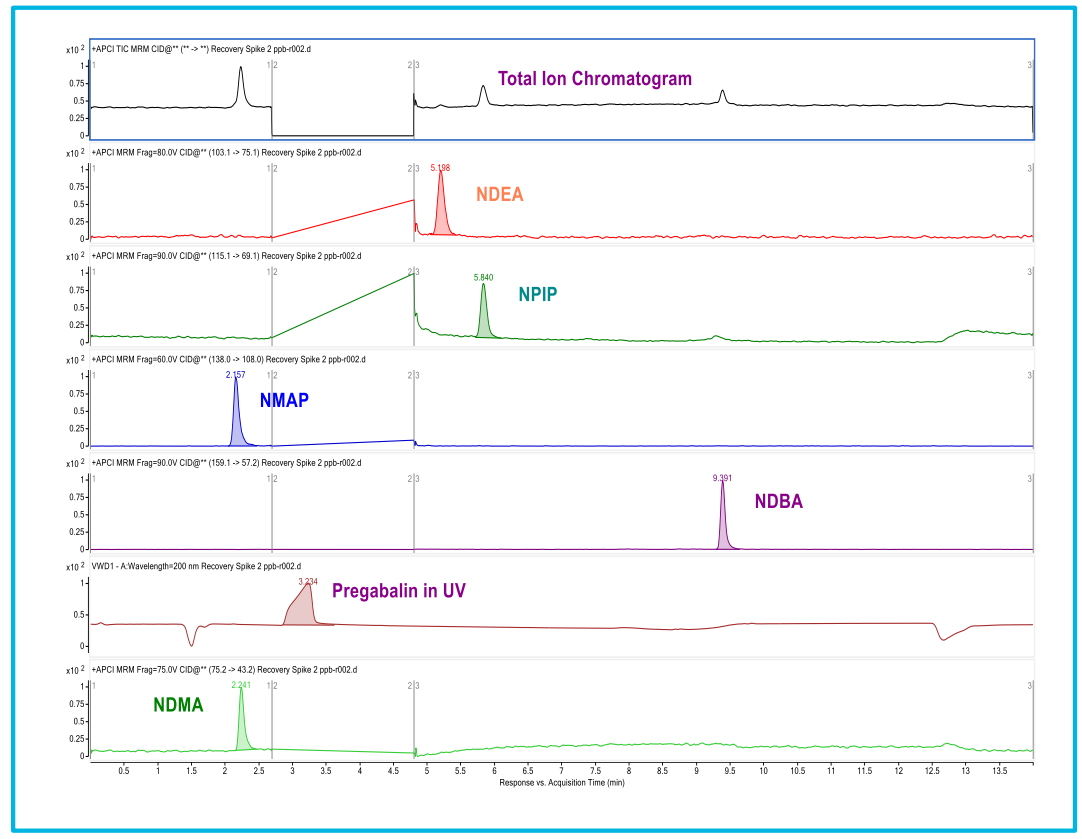

Figure2: Representative EIC of NDEA, NPIP, NMAP, NDBA and NDMA at 0.1 ppm conc. using 20mg/mL of Pregabalin API.

The table below presents the reproducibility data at 1ng/mL standard concentration for 7 replicates including bracketing standard (# 7) showing excellent area RSD % of < 2 % for each 5 nitrosamine impurities.

Area % RSD at 1ng/mL

#	NDMA	NMAP	NDEA	NPIP	NDBA
1	102233	5515	7590	42752	23307
2	101469	5388	7720	42832	23278
3	102858	5372	7701	42798	23269
4	102147	5577	7832	42969	23224
5	103343	5382	7784	43041	23133
6	102921	5347	7705	43029	23957
7	102268	5301	7692	43226	24152
Average	102462.71	5411.71	7717.71	42949.57	23474.29
SD	621.69	97.86	76.24	166.98	404.16
RSD (%)	0.61	1.81	0.99	0.39	1.72

Table 5: Peak area % RSD for 7 replicates at 1ng/mL

Results and Discussion

Method Performance Characterization

Figure 3 shows the calibration curves for the standard calibration of all 5 nitrosamines. The relevant calibration range for NDEA, NPIP, NMAP, NDBA and NDMA is from 0.1ng/mL to 100ng/mL. The coefficient of regression achieved for each nitrosamine is > 0.990.

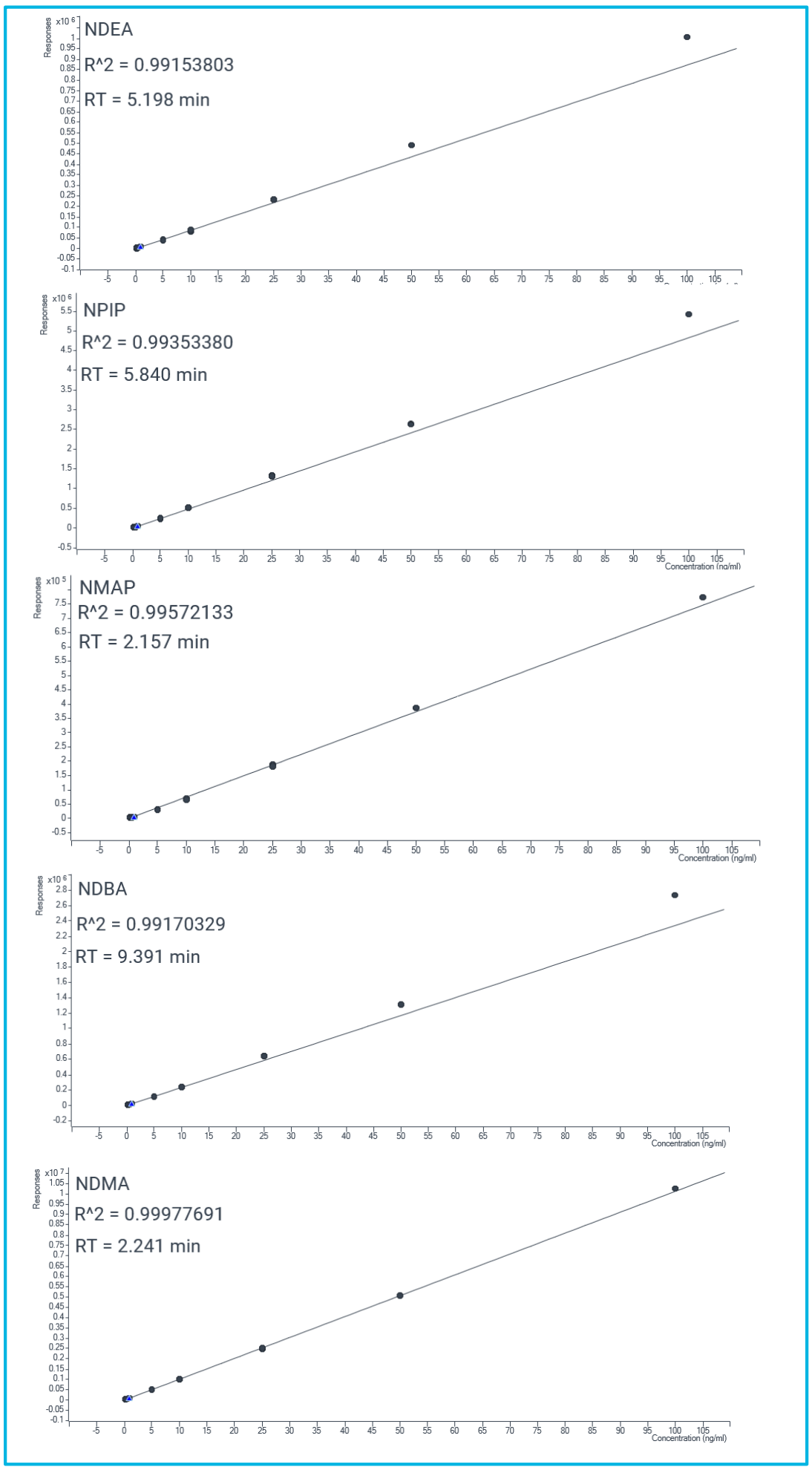

Figure 3: Calibration curves of all 5 nitrosamines

Recovery Study

The recovery experiment shows excellent recovery of ± 20 % of the spiked concentrations. In this experiment recovery study was performed at 5 different concentration levels. This recovery data makes the method ready for batch analysis of Pregabalin drug substance.

Spike Conc. (ng/mL)	Recovery %				
	NDEA	NPIP	NMAP	NDBA	NDMA
0.5	102.2	91.1	94.99	102.96	102.7
1	98.86	93.3	115	107.45	94.7
2	88.7	96.9	100.5	94.62	105.8
5	93.11	95.89	100.3	104.12	103
10	86.1	96.11	105.4	97.99	97.6

Table 6: Recovery data in Pregabalin drug substance

Conclusions

- The method provides excellent reproducibility at USFDA defined LOQ concentrations levels as it shows excellent reproducibility of < 2% with bracketing standard included in the calculations.
- The method is a ready to use method for analysis of Pregabalin drug substance batches as the method shows excellent recovery.
- As Pregabalin drug substance peak is chromatographically well separated from nitrosamine peaks so there is no contamination to mass spectrometer due to high concentration API.

References

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