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Highly Sensitive LC-MS/MS Method for Simultaneous and Trace Level Quantification of Ten Nitrosamine Impurities in Olmesartan Medoxomil

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Introduction

In 2018, the first incident of potential genotoxic Nitrosamine impurity N-Nitroso Dimethyl amine (NDMA) contamination reported in Angiotensin II Receptor Blocker (ARB) drug Valsartan. Later it expanded to addition of more nitrosamine impurities in various ARB category drug substances and products which lead to multiple product recalls by USFDA and EMA agencies. Stringent regulatory limit requirements for the control of nitrosamines and addition of multiple nitrosamines to the list necessitate the need of highly sensitive and multianalyte method for the quantification of nitrosamines.LC-MS/MS is the widely used technique for multiple nitrosamine quantification.

In the present work we have developed simple and rapid LC-MS/MS method for the simultaneous determination of ten nitrosamines namely NDMA, NDEA, NMBA, NEIPA, NDIPA, NDIPA, NMPA, NMEA, NPIP and NPyr in Olmesartan medoxomil which also comes under the category of ARB-II drugs.

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: 6470 triple quadrupole LC/MS

Experimental

Sample Preparation

1. Weigh 100mg(± 1mg) Olmesartan drug substance sample in a 15 mL centrifuge tube.
2. Add 0.25 mL of 100%Methanol and sonicate for 15min and then add 4.75ml of 100%water followed by 30 min sonication. 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 14.5 90 14.6 5 18.0 5
Stop time	18 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	+
NMBA	147	117	60	4	2	+
NMEA	89.1	61	75	10	4	+
NPIP	115.1	69.1	96	16	4	+
NPYR	101.1	55.1	90	20	4	+
NEIPA	117	74.9	82	8	8	+
NDIPA	131	89.1	80	5	4	+
NMPA	137	66	70	20	5	+
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 critical part of this method is achieving the sensitivity of 0.1ng/ml (5ppb wrt test) for all the ten Nitrosamine impurities and Chromatographic separation of Olmesartan from nitrosamine impurities to establish method selectivity and sensitivity which was successfully achieved. Sample preparation is also critical to establish the recovery of the method for all the ten impurities. Which we could establish using extraction protocol.

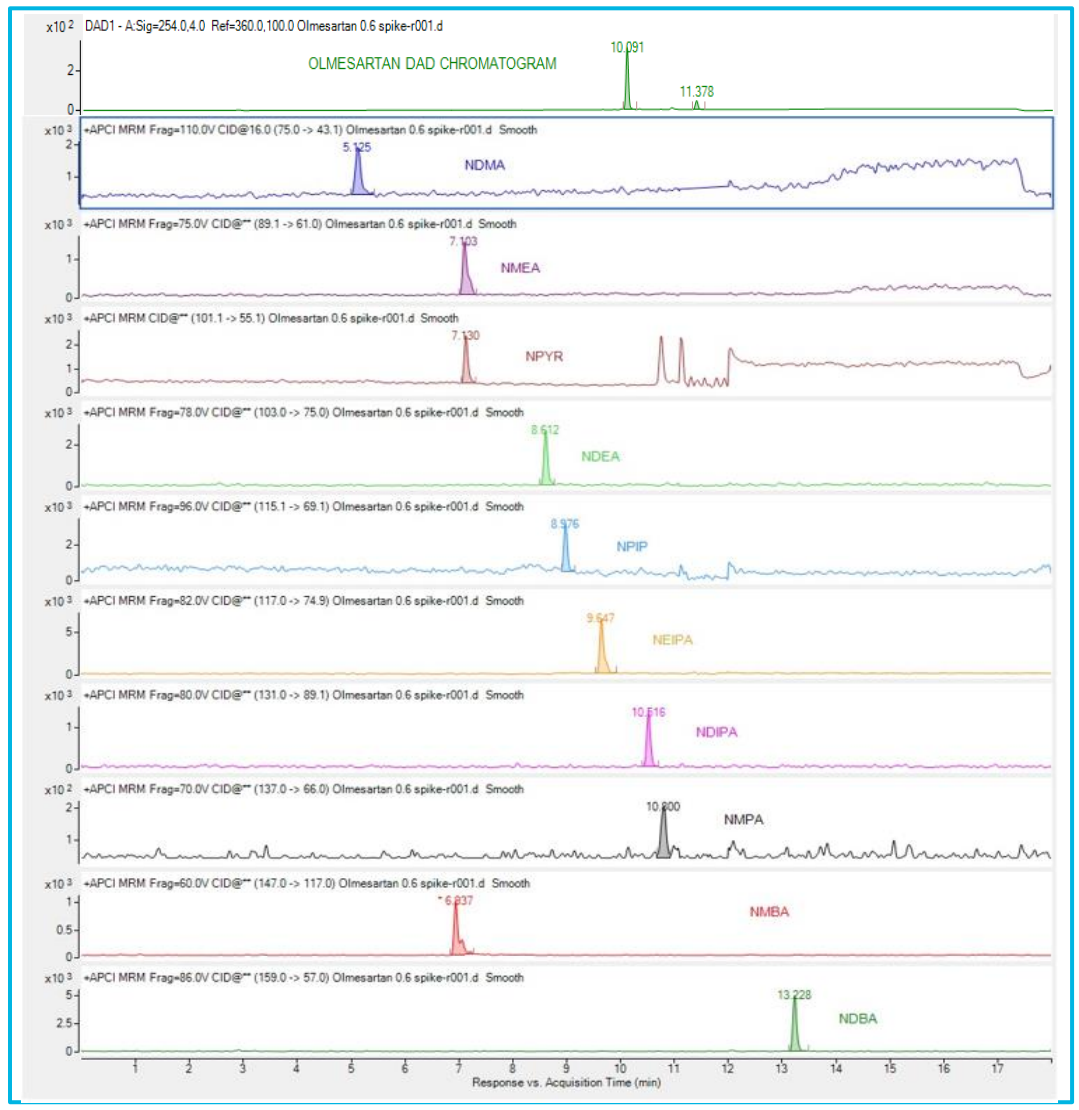

Figure2: Representative EIC of ten nitrosamine impurities at 0.6ng/ml (0.03ppm) wrt 20mg/mL of Olmesartan API and DAD Chromatogram of Olmesartan

Area and %RSD at 0.6ng/mL

S.NO	NDMA	NMBA	NMEA	NPYR	NDEA
1	13605	5466	6101	6550	9360
2	12009	5578	6655	6970	8602
3	14140	5091	6205	6794	8842
4	13180	5072	5459	7299	9305
5	13111	4974	6139	6444	8269
6	11967	5549	6275	7431	9336
7	13308	5592	6769	6412	8950
Average	13045.7	5331.7	6229.0	6842.9	8952.0
STD DEV	800.0	272.9	426.8	408.9	416.3
%RSD	6.1	5.1	6.9	6.0	4.6

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

Results and Discussion

Area and %RSD at 0.6ng/ml cont'd

S.NO	NPIP	NEIPA	NDIPA	NMPA	NDBA
1	10618	26014	6704	2070	24657
2	11651	25478	5976	2149	24986
3	11145	25631	6683	2163	24552
4	10541	27487	6164	2440	23949
5	10244	27060	6534	2471	24263
6	10877	26023	6403	2188	23534
7	10934	26812	6291	2297	26078
Average	10858.6	26357.9	6393.6	2254.0	24574.1
STD DEV	456.9	764.5	270.0	153.3	817.3
%RSD	4.2	2.9	4.2	6.8	3.3

Table 6: Peak areas and %RSD for 7 replicates at 0.6 ng/mL

Method Performance Characterization

Figure 3 shows the overlaid chromatograms of calibrations standards of NDMA, NDEA, NMBA, NMEA, NPIP, NPYR, NEIPA, NDIPA, NMPA and NDBA from 0.1ng/mL to 10ng/mL. The coefficient of regression achieved for each nitrosamine is > 0.990.

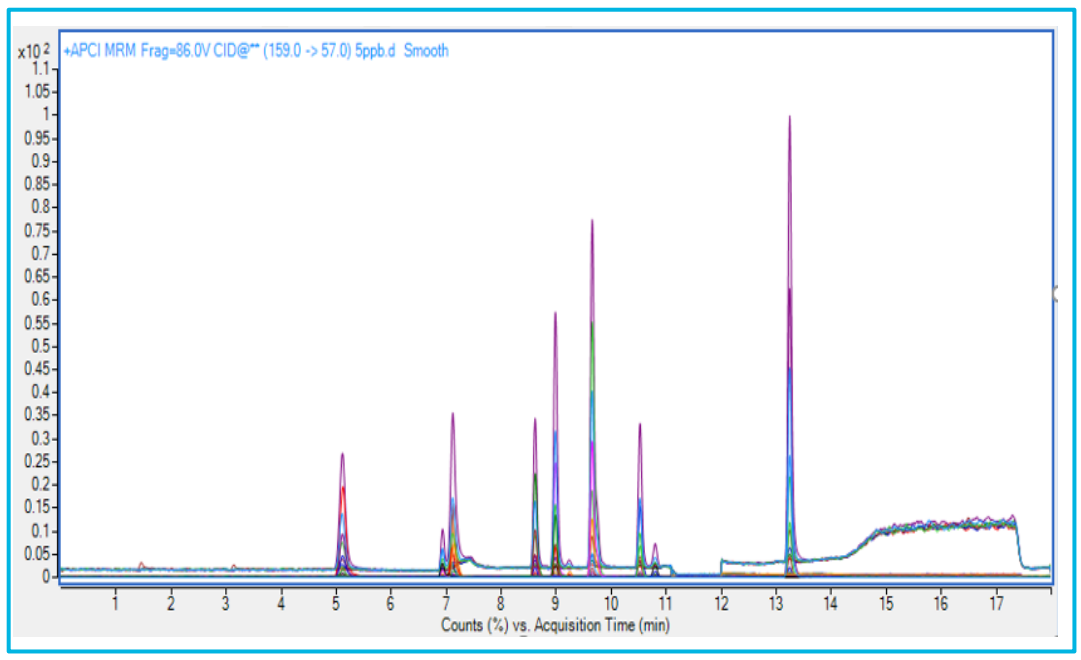

Figure 3: Overlaid chromatogram of ten Nitrosamine impurities from 0.1-10ng/ml

Recovery Study

The recovery experiment shows excellent recovery of ± 20 % of the spiked concentrations. For all the ten nitrosamine impurities In this experiment recovery study was performed at 2 different concentration levels. This recovery data makes the method ready for batch analysis of Olmesartan medoxomil drug substance.

S.No	Name of the Nitrosamine Impurity	Recovery at 0.6ng/ml (0.03ppm)	Recovery at 0.1ng/ml (LOQ)
1	NDMA	109.0	110.8
2	NMBA	98.6	83.2
3	NMEA	99.0	95.6
4	NPYR	99.1	105.8
5	NDEA	107.7	116.8
6	NPIP	95.3	99.5
7	NEIPA	97.7	107.0
8	NDIPA	96.0	101.2
9	NMPA	94.4	83.0
10	NDBA	82.4	90.9

Table 7: 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.
- High throughput method developed for the Simultaneous determination of ten nitrosamine impurities in Olmesartan.
- The method has all the critical performance parameters established which shows the robustness and efficiency to use for routine batch analysis of Olmesartan medoxomil drug substance.

References

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