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Method Development of NDMA and NDEA Quantification in Sartan Drug by LC-MS/MS

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Introduction

The issue of genotoxic impurities(GI) in drug substance and drug product has drawn lots of attentions in China. One of the examples is the quantification of NDMA and NDEA (Figure 1) in Sartan drug substance. A fast, sensitive and robust method is required extensively from reginal customers. For quantitative measurements of lower levels of NDMA and NDEA, the combination of chromatography and mass spectrometry provides high selectivity and sensitivity, and is the most appropriate approach.

Here, a highly efficient, sensitive, accurate and reliable LC-MS/MS method for quantification of NDMA and NDEA in Sartan drug is demonstrated using Agilent Ultivo LC/TQ (Figure 2), the smallest LC/TQ with good performance.

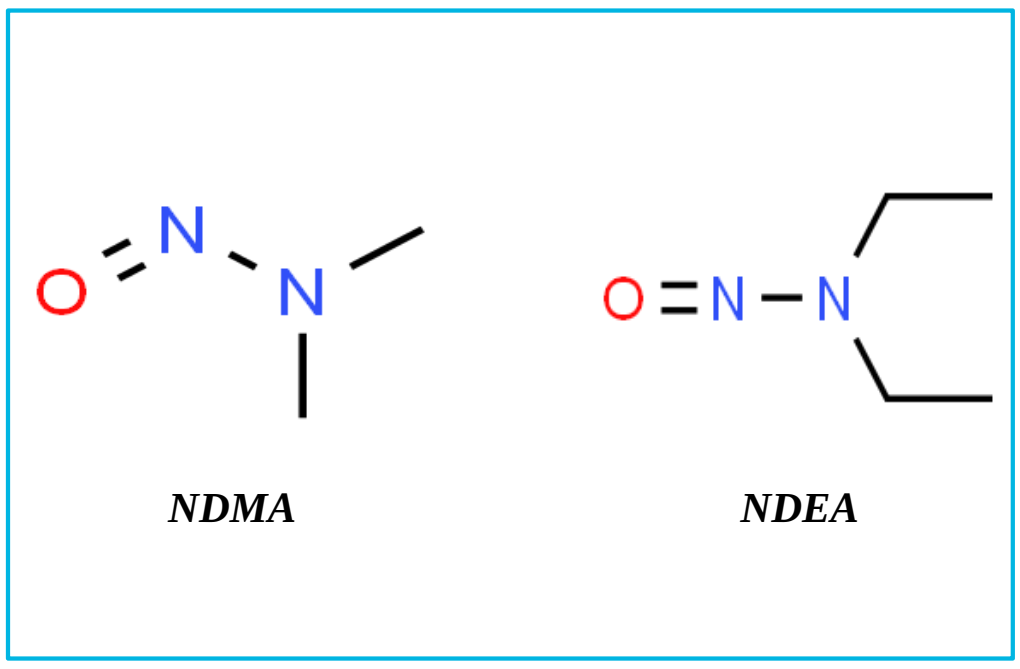

Figure 1. Structure of NDMA and NDEA

Figure 2. The smallest LC/TQ in the world—Agilent Ultivo LC/TQ

Experimental

Equipment and conditions

Sample preparation workflow

Weigh the sartan drug 100±1mg into a 50 mL centrifuge tube

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Add 200 µL ISTD solution prepared in methanol

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Add 300 µL methanol and 9.5 mL deionized water following vortex and ultrasonic extract, respectively

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High-speed centrifugation and filter membrane for Ultivo LC/TQ analysis

LC conditions

HPLC system	Agilent 1290 Infinity II Binary Pump UHPLC
LC Column	ZORBAX Eclipse Plus C18 (3.0x100mm,1.8µm)
Mobile phase	Phase A: 0.1% Formic Acid in H2O Phase B: Methanol
Gradient program	0min, 5%B; 3min, 5%B; 8min, 60%B; 9min, 95%B; 15min, 95%B
Injection volumn	20 µL
Flow rate	0.5 mL/min

MS conditions

Mass system	Agilent Ultivo LC/TQ
Ion source & Polarity	APCI, Positive mode
Gas Temp.	300℃
Gas Flow	6 L/min
Nebulizer	45 psi
APCI Heater	350℃
Corona	8 µA
Capillary	2000 V

MRM conditions

Compound Name	Prec.Ion [m/z]	Prod. Ion [m/z]	Frag.(V)	CE(V)	Polarity
NDMA	75	58.1	80	4	+
	75	44	80	4	+
	75	**43***	80	8	+
NDMA-d6	81.1	64.1	90	4	+
	81.1	**46.1***	90	10	+
NDEA	103.1	**75.1***	80	2	+
	103.1	47.1	80	8	+
	103.1	28.9	80	1	+
NDEA-d10	113	81	90	2	+
	113	**34***	90	4	+

*=Quantifier. Note: in case that 75>43 has a higher background (noise), 75>58.1 can be used as quantifier.

Chromatographic retention and separation

NDMA and NDEA, especially NDMA, are hydrophilic compounds. For accurate quantitative analysis, a good chromatographic retention is necessary. Besides this, the high concentration of API may result in inaccurate quantitative results due to the matrix effect. So, the good chromatographic separation between target GIs and API is required. In this method, the two kinds of Sartan drugs substance, valsartan and Irbesartan, are used as the test samples. For chromatographic condition optimization, the NDMA、NDEA and API were prepared as a mixture of 100 ng/mL with 5% methanol/water (V/V). As illustrated in Figure 3, we have achieved excellent retention and separation between the target GIs and API with symmetric peak shapes in the optimized method. To reduce or eliminate interference and protect instrument, the API was switched to waste under the action of a switch valve in the final method.

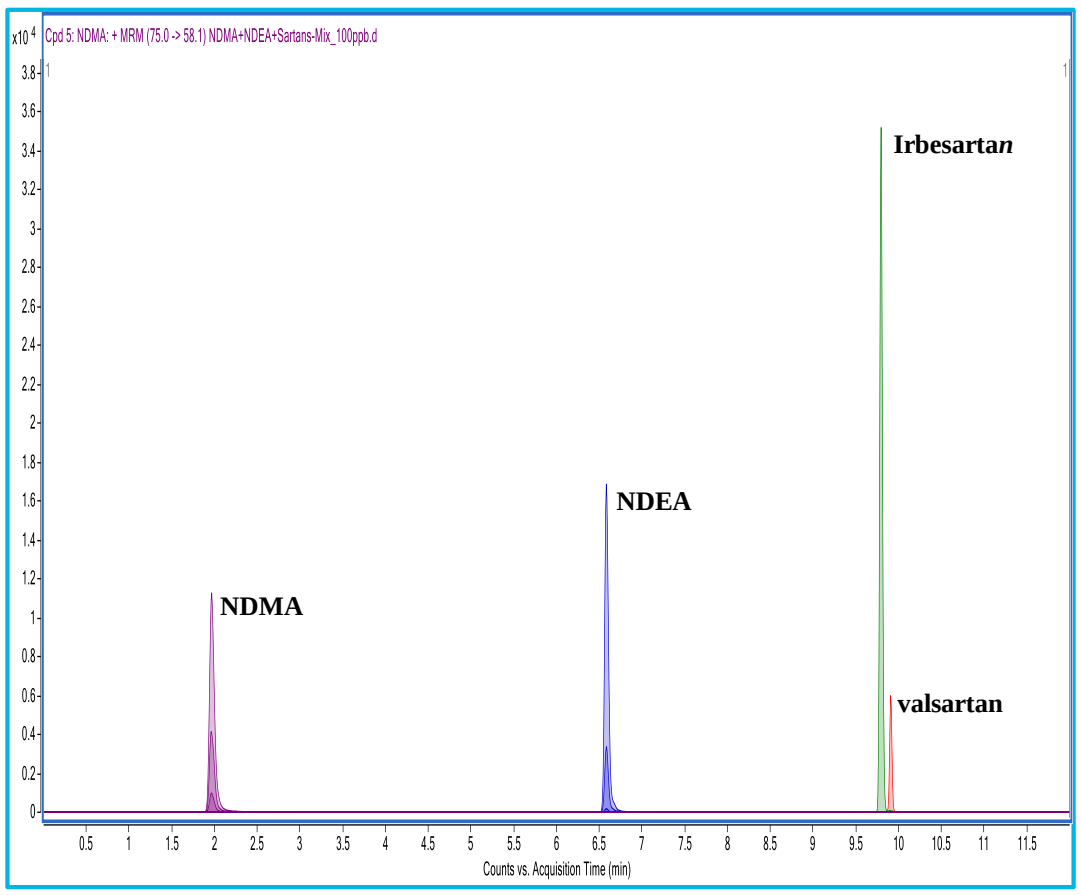

Figure 3. Chromatographic separation of NDMA、NDEA and API at 100 ng/mL

Method sensitivity and precision

The sensitivity and precision of Ultivo LC/TQ were assessed by analyzing NDMA and NDEA standards prepared in 5% methanol/water (V/V). Figure 4 showed that both analytes exhibited excellent sensitivity with ng/L level as LOD and LOQ. Figure 5 showed that both analytes had good precision with RSD% less than 10% at four levels, from low, medium, to high for consecutive 10 injections.

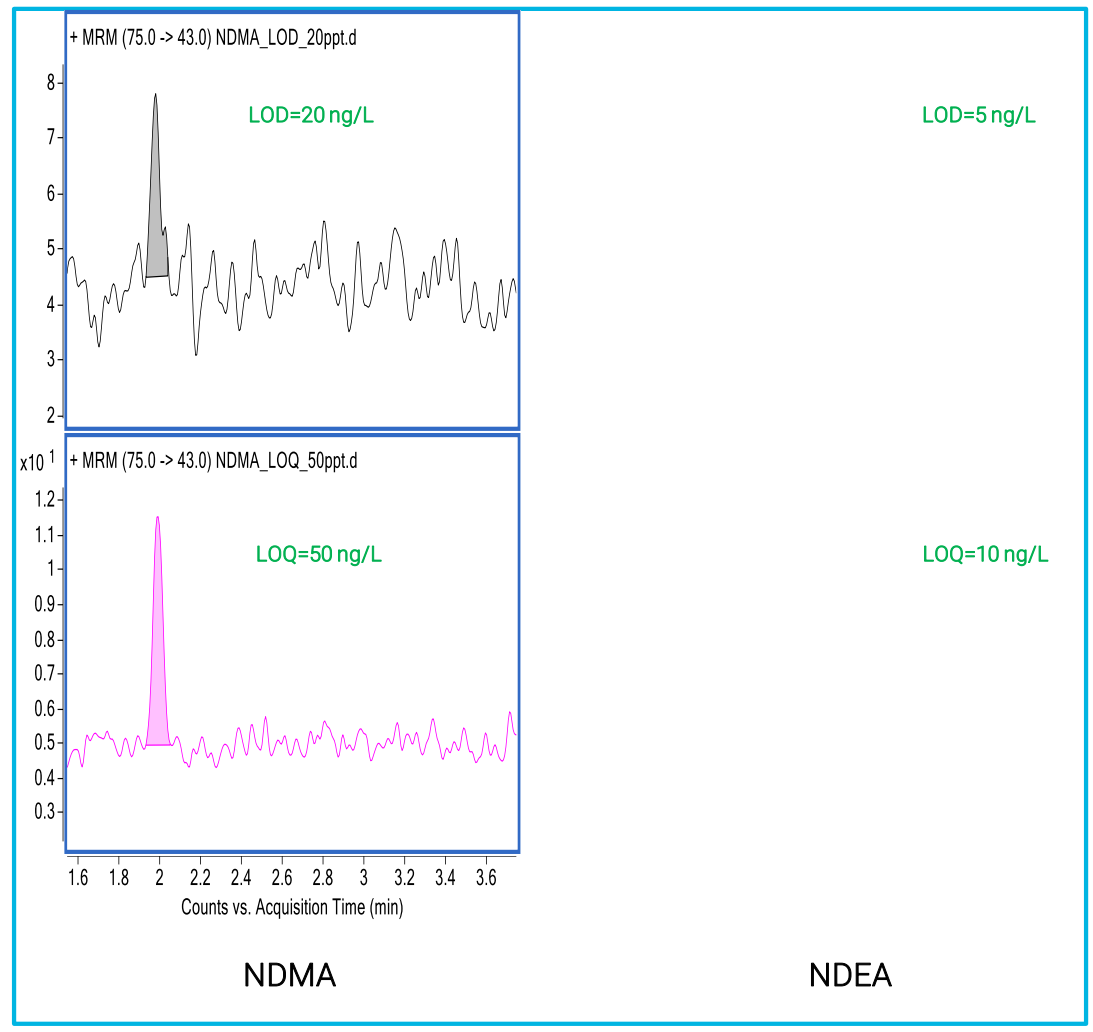

Figure 4. Chromatogram of NDMA and NDEA at LOD and LOQ

Figure 5. The results of RSD% of quantifier peak for 10 replicate injections of both analytes

Results and Discussion

Method linearity

Both analytes showed good linearity with $1/x^2$ weighting, and the R^2 values of both calibration curves are 0.998 and 0.997 with 90%~110% accuracy of curve readback for each level. Calibration levels ranged from 0.1 ng/mL to 300 ng/mL for both analytes, which are equivalent to NDMA and NDEA content in Sartan drug from 0.01mg/kg to 30 mg/kg, which is lower than the method LOQ requirement of certain international official standard methods and regulations such as U.S. FDA method[1]、Council of Europe EDQM method[2] and China Pharmacopoeia method[3]. Figure 6 showed the good results of calibration curves.

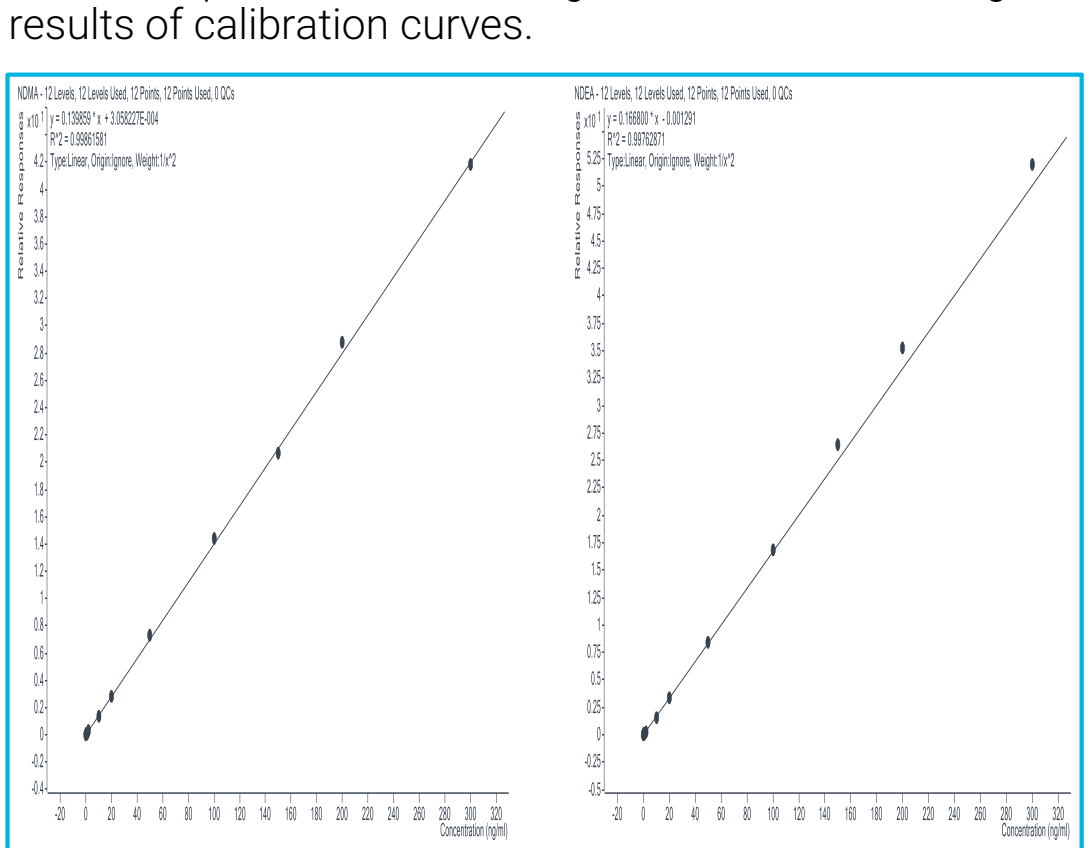

Figure 6. Calibration curves from 0.1 ng/mL to 300 ng/mL for NDMA and NDEA demonstrating the sensitivity and linearity for the quantitative analysis for both analytes

Method recovery

The recovery of NDMA and NDEA was evaluated in Valsartan and Irbesartan drug samples, where the detections of both analytes at the spiking level of 1 mg/kg were conducted. This level was close to the analyte content in both positive samples. Six replicates of the spiking were measured in the recovery study. For both analytes, the average recovery was between 91% and 97% with 3%~4% standard deviation (%RSD). Our results well met the required 80% to 120% acceptable recovery with high precision, as outlined in Figure 7.

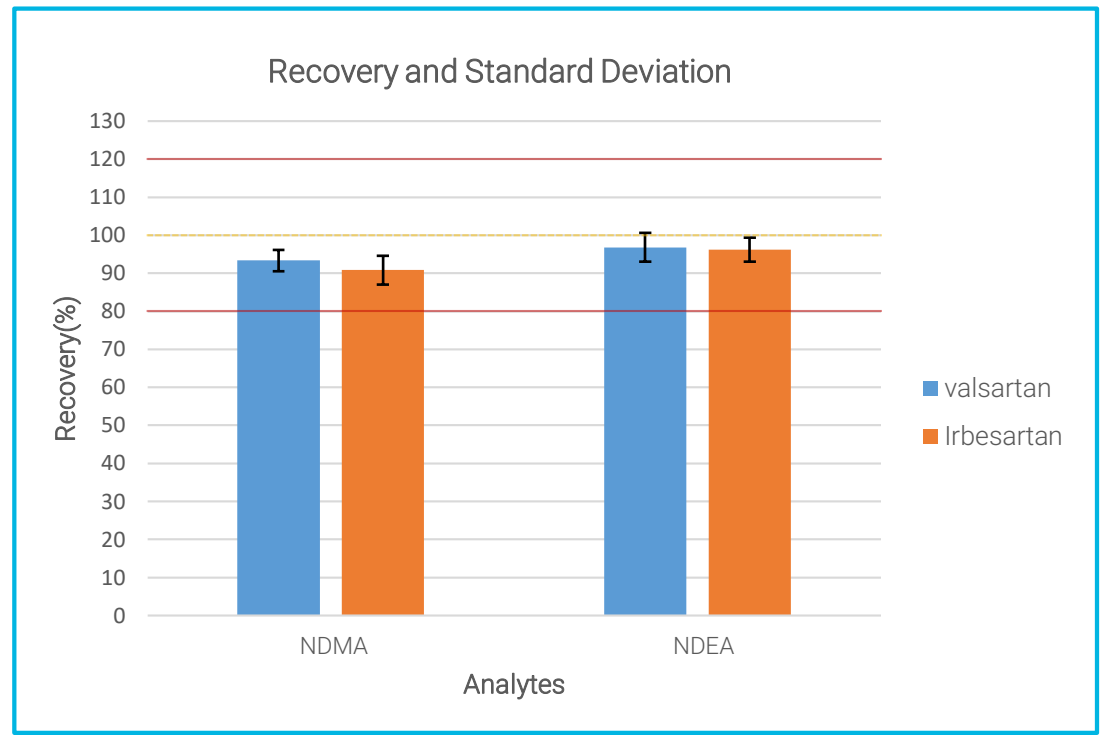

Figure 7. Recovery of NDMA and NDME in valsartan and Irbesartan at 1 mg/kg spiking level. Error bars denote the standard deviation of six replicates.

Conclusions

We have demonstrated a rapid, highly sensitive and accurate UHPLC-MS/MS method for quantitative analysis of NDMA and NDEA in Sartan drugs with Agilent Ultivo LC/TQ.

- Good chromatographic separation of NDMA ,NDEA and API has been achieved.
- The ng/L level instrument LOD & LOQ with high precision (%RSD < 10%) have been achieved.
- The linear dynamic range for NDMA and NDEA is 0.1 ng/mL to 300 ng/mL, which can quantitate 0.01mg/kg to 30mg/kg NDMA and NDEA content in Sartan drug.
- 86%~102% recovery with high precision indicated that our method can provide accurate and reliable quantification for real sample analysis.

References

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