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N,N-DMF Selective and Highly Sensitive Quantification of NDMA and NDEA in Tetracycline Class Antibiotic Medication - A Case Study of Tigecycline

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Introduction

Nitrosamine Impurities are categorized under Cohort of Concern as per ICH M7 guideline. Since 2018 there has been multiple recalls of drug substances and drug products like Sartans, Ranitidine and Metformin by various regulatory agencies like USFDA and EMEA due to presence of Multiple Nitrosamine impurities. Recent identification of N,N-Dimethyl formamide interference with N-Nitroso Dimethyl amine (NDMA) in Metformin drug products raises concern for the accurate quantification due to the presence of possible isotopic interference from N,N-Dimethyl formamide(N,N-DMF). Methods published using High Resolution Mass spectrometry for the separation of NDMA and N,N-DMF as there is a coelution observed for NDMA and N,N-DMF.

Possibility of N,N-DMF interference can be applicable to analysis of NDMA in other therapeutic drug categories and shows a clear requirement for the chromatographic separation between NDMA and N,N-DMF when using LC-MS/MS triple quadrupole mass spectrometer due to the limitation of unit mass resolution capabilities. In the present work we have developed a novel, simple and highly sensitive quantification of NDMA in presence of N,N-DMF in tetracycline class antibiotic Tigecycline with a Limit of quantification of 5ppb with respect to Tigecycline test concentration.

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

The sample preparation procedure was optimized using the following steps.

1. Weigh 100mg(± 1mg) Tigecycline drug substance sample in a 15 mL centrifuge tube.
2. Add 5 mL sample diluent and vortex for 2minute to completely dissolve.
3. Filter 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	100% Water
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 2 6.0 2 7.0 20 10.0 75 11.0 90 13.0 90 13.1 2 16.0 2
Run time	16 minutes

Table 2: 1290 UHPLC conditions

Method Optimization

The 6470 LC/TQ was used for detecting the mass conditions for NDMA and NDEA 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	75	78	12	4	+
NDEA	103	47	78	20	4	+
NDMA	75	58	80	10	2	+
NDMA	75	43.1	90	16	3	+
N,N-DMF	74.1	46.3	90	16	4	+
N,N-DMF	74.1	44.2	90	20	4	+

Table 3: MRM transitions and conditions

MS Conditions

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

Table 4: MS conditions

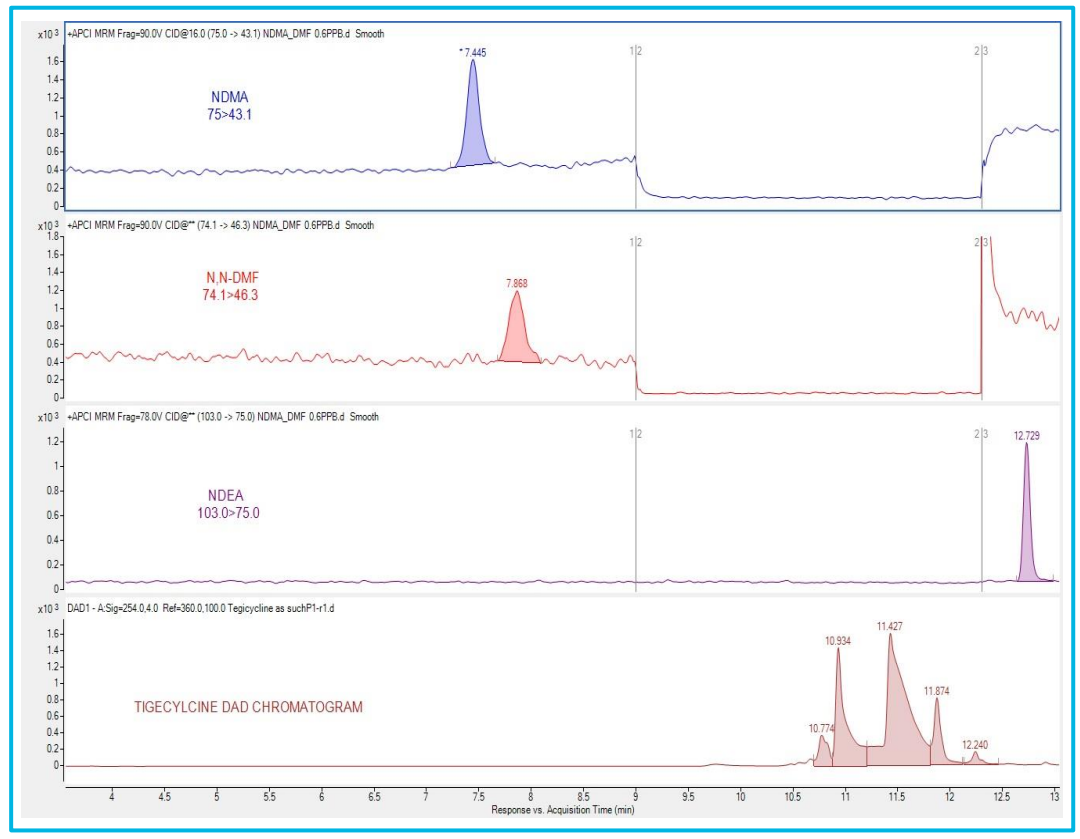

Figure2: Representative EIC of using NDMA, N,N-DMF and NDEA, at 0.6ng/ml (0.03ppm) and 20mg/mL of Tigecycline API and DAD Chromatogram of Tigecycline

Area % RSD at 0.6ng/mL

	S.NO	NDMA	NDEA
Initial Replicates	1	9210	4387
	2	8053	4947
	3	8851	4536
	4	9012	4618
	5	8918	4505
	6	9745	4898
Bracketing standard	7	8964	4268
	Average	8964.7	4594.1
	STD DEV	502.9	251.0
	%RSD	5.6	5.5

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

The most critical part of this method is chromatographic separation of NDMA and N,N-DMF for accurate quantification. Tigecycline (monitored at 254nm wavelength) separation with NDMA and NDEA also need be achieved 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.

Results and Discussion

Method reproducibility at LOQ of 0.1ng/ml (0.005ppm wrt test)

	S.NO	NDMA	NDEA
Initial Replicates	1	1683	713
	2	1697	789
	3	1429	768
	4	1556	759
	5	1462	817
	6	1532	663
	Average	1559.8	751.5
	STD DEV	110.9	55.4
	%RSD	7.1	7.4

Table 6: Peak area % RSD at LOQ at 0.1ng/mL

Method Linearity performance

Figure 3 shows the calibration curves for the standard calibration of NDMA and NDEA. Linearity range is from 0.1ng/mL to 25ng/mL(0.005ppm to 1.25ppm) . The coefficient of regression achieved for each nitrosamine is > 0.99.

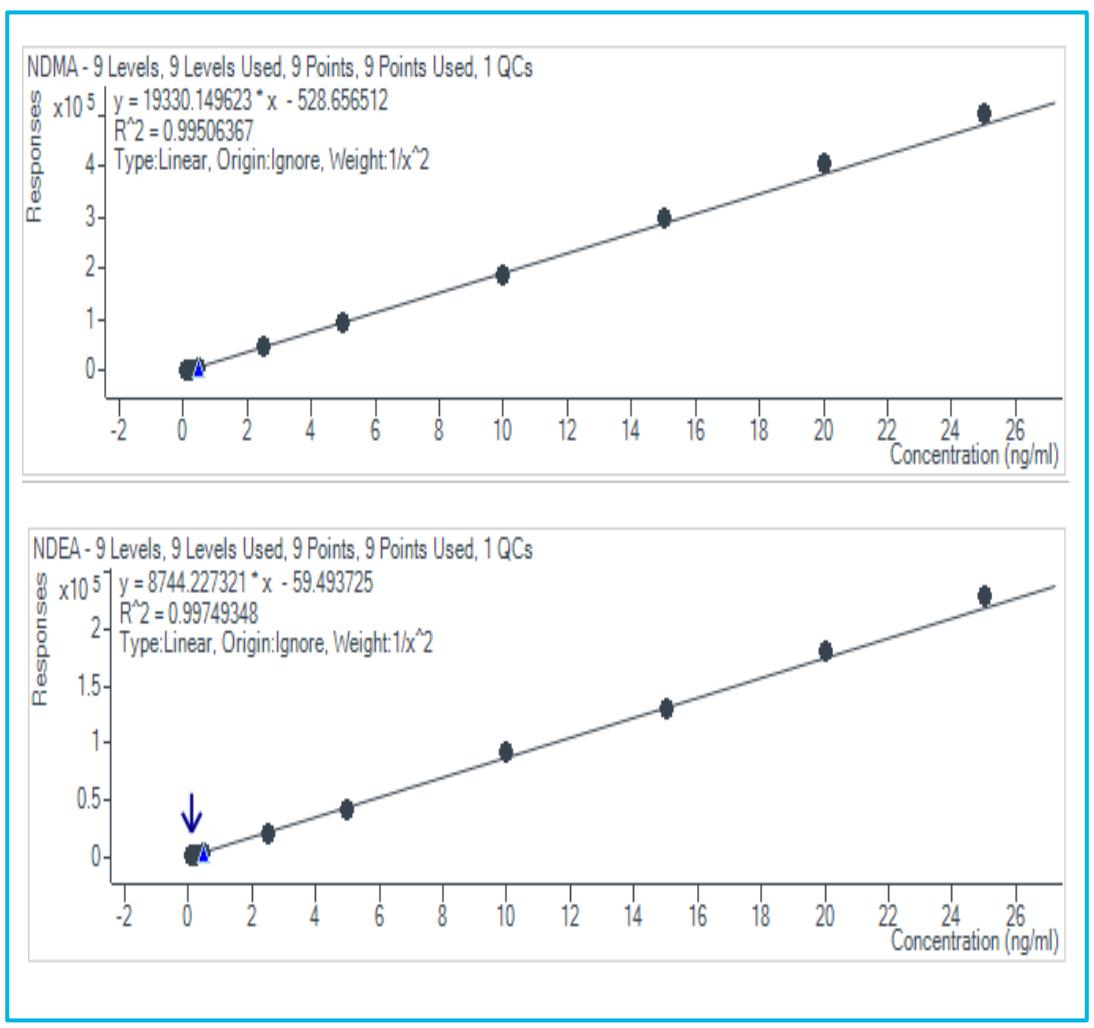

Figure3: Representative Calibration curves for NDMA and NDEA

Recovery Study

Recovery study performed for NDMA and NDEA at 0.03ppm to evaluate the method efficiency and we could observe the results which are well within the acceptance criteria of +/- 20% of the limit which shows the applicability of method for the routine batch analysis of Tigecycline drug substance.

Spike Conc. (ng/mL)	Recovery %	
	NDMA	NDEA
0.6 (0.03ppm)	91.7	94.1

Table7 : Recovery data in Pregabalin drug substance

Conclusions

- The method provides excellent sensitivity as per the latest USFDA guidance on control Nitrosamine in drug substances.
- The method developed is very selective in terms of N,N –DMF interference with NDMA and could provide accurate quantification of NDMA in presence of N,N-DMF as the N,N-DMF peak is chromatographically separated with NDMA.
- Method shows excellent reproducibility including bracketing standards which shows the applicability of method for easy transfer to routine batch analysis of Tigecycline.

References

1.FDA guidance document: Control of Nitrosamine Impurities in Human Drugs

2.USFDA News: Rigorous Detection of Nitrosamine Contaminants in Metformin Products: Balancing Product Safety and Product Accessibility

3. A Cautionary Tale: Quantitative LC-HRMS Analytical Procedures for the Analysis of N-Nitroso dimethylamine in Metformin

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