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Qualitative Analysis of 15 Traditional Nitrosamines in Thalassemia drug at Ultra-trace Levels Using LC/TQ

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Introduction

Abstract

Mutagenic impurities in APIs and drug products pose a significant risk to health and safety— even in small quantities—and thus are a major concern for drug makers. The current developed method on traditional nitrosamines is a robust method with UV absorbance at 280nm for deferiprone while showcasing 80-120 % recovery at 0.001ppm among tablet sample (figure 1).

Introduction

Deferiprone is an iron chelating drug useful across disorders such as thalassemia. A dMRM based LCMS method was developed for 15 impurities (NDELA, NDMA, NMOR, NDIPLA, NMBA, NPYR, NDEA, NTHP, NPIP, NEIPA, NDIPA, NMPA, NMPEA, NDBA, and NDPH) whereas the 100mg powdered sample was dissolved in 1mL of ultrapure water for quantitative analysis of the impurities in tablet sample. Recovery analysis of NDELA was conducted by spiking 1ng in 100mg of sample whereas for the remaining analytes a 0.1 ng quantity was spiked. The spiked sample was vortexed for 5min followed by centrifugation at 10000g for 5min. The supernatant was filtered with 0.2µm filter before injecting. The recovery calculations include comparison of area of impurity in spiked and standard. Method repeatability study was done by injecting six replicates of standard at 0.05 ng/mL and suitability to higher concentration was established by having calibration plot across 0.05 to 5 ng/mL concentration of impurities.

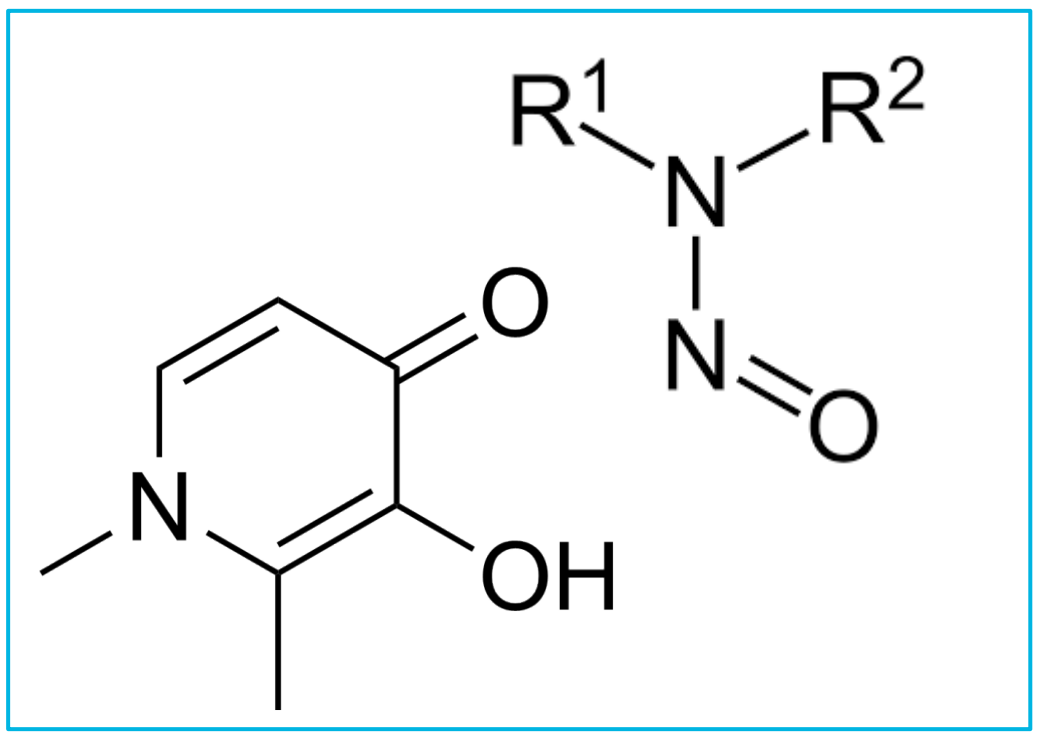

Figure 1: Deferiprone and Nitrosamines

Experimental

Mass Spectrometry Parameters

The LC/TQ parameters were optimized and are seen below in Table 1.

Parameter	Value
Multi sampler	15 °C
Injection Volume	20 µL
Column	40 °C, Poroshell 120 EC-C18,
Drying gas	290 °C at 11 L/min
Nebulizer gas	55 psi
Capillary Voltage	2000 V
Corona current	4 (µA)
Vaporizer temp	350 °C
Scan mode	Dynamic MRM
Diverter Valve	diverted to waste between 5.25 to 8 min
Ionization	APCI +ve

Time	Mob Phase A	Mob Phase B
0.0	100	0
5.0	100	0
6.0	90	10
10.0	55	45
13.0	40	60
17.0	30	70
18.0	10	90
20.0	100	0
30.0	100	0

Table 1: LC/TQ method parameters

Figure 2: 1290 UHPLC, 6495D LC/TQ and APCI source

Chromatographic Separation:

A good separation was achieved for 15 nitrosamine impurities using developed chromatography while the main drug was diverted to waste (figure 3) by making usage of built-in diverter valve, preventing probable contamination of Agilent 6495 LC/TQ from higher concentration of deferiprone. The chromatogram of impurities and deferiprone is seen in figure 3 and 4.

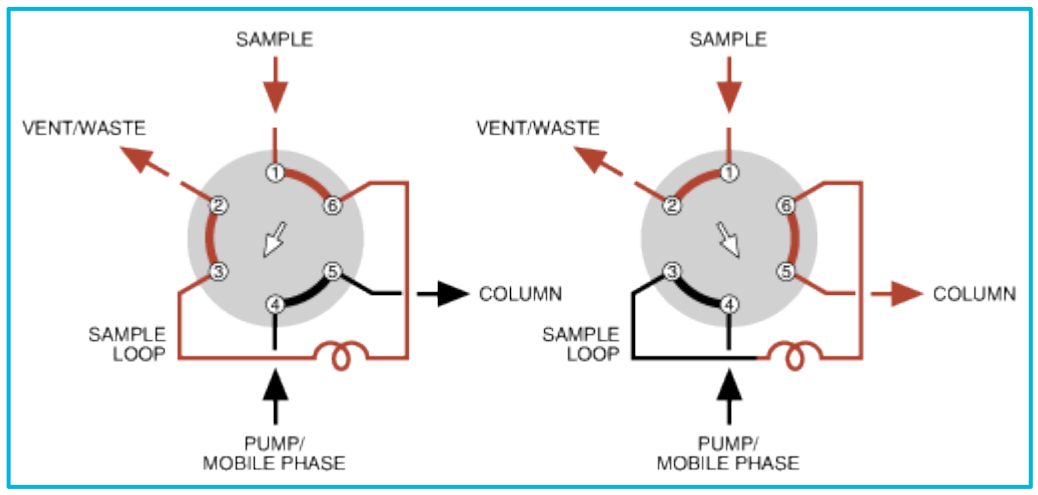

Figure 3: Built-in Flow diverter valve program

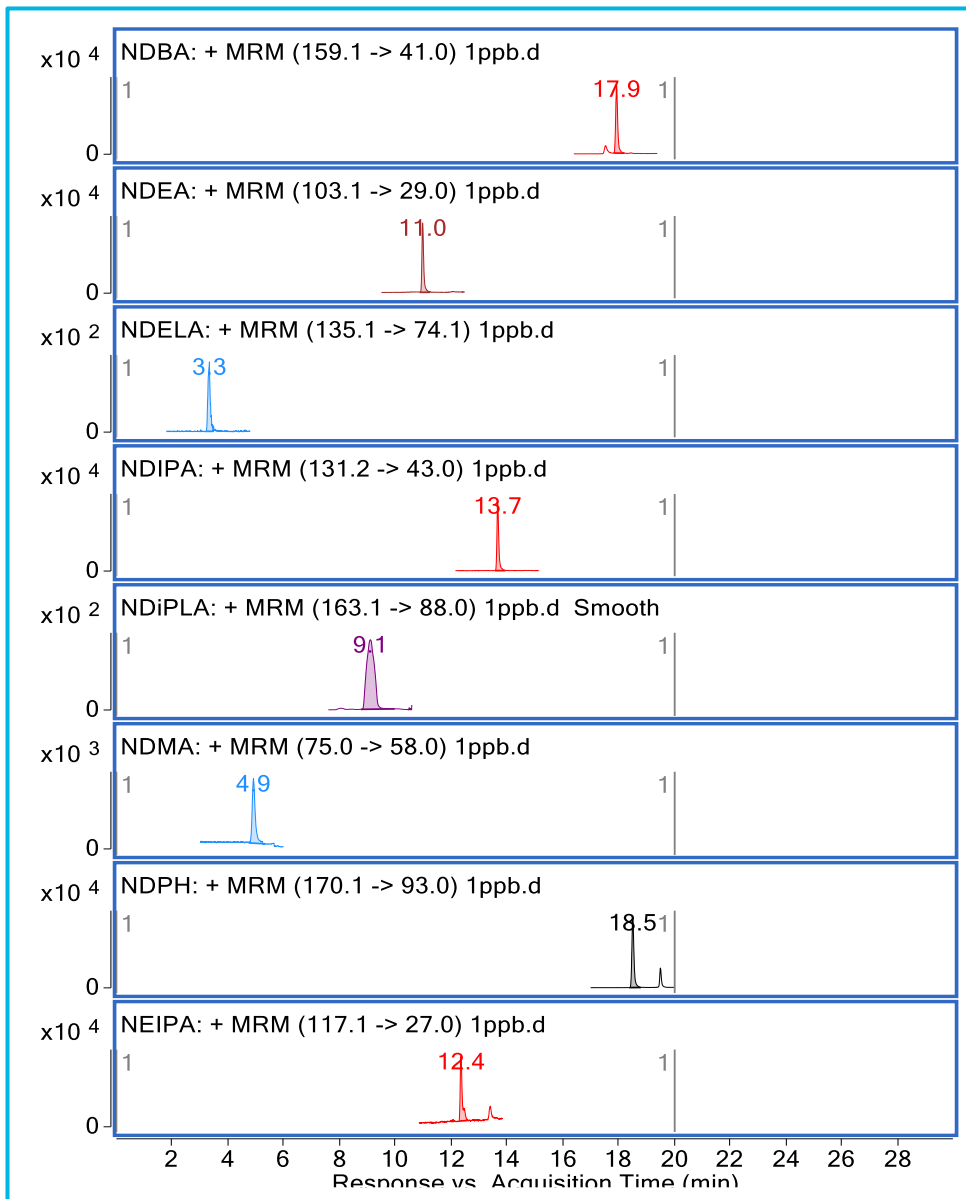

Figure 4: MRM plot of NDBA, NDEA, NDELA, NDIPA, NDIPLA, NDMA, NDPH and NEIPA.

Figure 5: MRM plot of NMBA, NMOR, NMPA, NMPEA, NPIP, NPYR, NTHP and Retention time of deferiprone at 280nm

Calibration, RSD and Recovery data:

A calibration plot for all impurities had R2 values ≥0.998 across 0.05 ng/mL to 5 ng/mL concentrations (figure 6). The RSD obtained, for six replicates, between 2.5% to 9% reflected a good method reproducibility. The recovery calculations done at 1ng/100mg for NDELA and at 0.1 ng/100mg for all other impurities had values within ±20%, reflecting that the method is sensitive by three times to more than 1000 time better than desired LOQ values. The table 2 showcases, acceptable limits, targeted spike levels and obtained LOQ against drug.

Results and Discussion

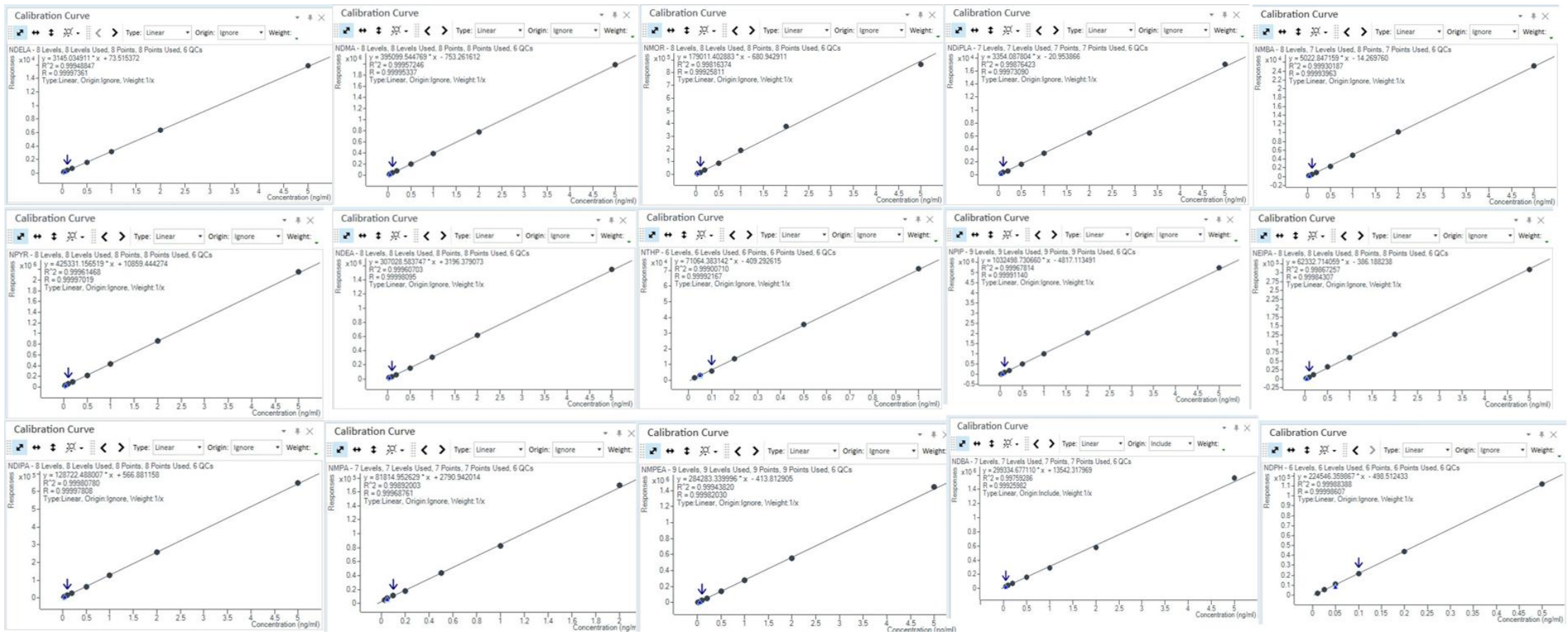

Figure 5: Calibration plot of nitrosamines across 0.05 ng/mL to 5 ng/mL with R2 values ≥ 0.998

S. No	Analyte	Acceptable Limit of drug	Spike (0.1 ng/100 mg = 1 ng/g)	Obtained LOQ vs acceptable Limit	Recovery at LOQ	RSD at 0.05 ng/mL
1	NDELA	0.211 ppm = 211 ng/g	1 ng/100mg = 10ng/g	>20 times better	99.04	6.38 %
2	NDMA	0.011 ppm = 11 ng/g	0.1 ng/ 100 mg	>10 times better	102.2	5.36 %
3	NMOR	0.014 ppm = 14 ng/g	0.1 ng/ 100 mg	>10 times better	92.9	3.14 %
4	NDIPLA	0.044 ppm = 44 ng/g	0.1 ng/ 100 mg	>40 times better	115.7	5.96 %
5	NMBA	0.011 ppm = 11 ng/g	0.1 ng/ 100 mg	>10 times better	118.5	4.42 %
6	NPYR	0.189 ppm = 189 ng/g	0.1 ng/ 100 mg	>175 times better	85.5	4.12 %
7	NDEA	0.003 ppm = 3 ng/g	0.1 ng/ 100 mg	>3 times better	90.8	4.53 %
8	NTHP	0.004 ppm = 4 ng/g	0.1 ng/ 100 mg	>4 times better	99.13	8.76%
9	NPIP	0.144 ppm = 144 ng/g	0.1 ng/ 100 mg	>125 times better	97.32	2.72%
10	NEIPA	0.003 ppm = 3 ng/g	0.1 ng/ 100 mg	>3 times better	107.8	8.15%
11	NDIPA	0.003 ppm = 3 ng/g	0.1 ng/ 100 mg	>3 times better	89.71	4.48 %
12	NMPA	0.003 ppm = 3 ng/g	0.1 ng/ 100 mg	>3 times better	93.4	4.6%
13	NMPEA	0.001ppm = 1 ng/g	0.1 ng/ 100 mg	>3 times better	81.84	5.04%
14	NDBA	0.003 ppm = 3 ng/g	0.1 ng/ 100 mg	>3 times better	94.7	2.62 %
15	NDPH	8.667 ppm = 8667 ng/g	0.1 ng/ 100 mg	8000 times better	82.1	3.7 %

References

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