Poster Reprint

ASMS 2025
Poster number ThP 727

Analysis of Carcinogenic Nitrosamines at Ultra-trace Levels Among Terbinafine Using LC/TQ

Vikrant Goel[1], Vivek Dhyani, Saikat Banerjee[1], David Stella[2],John Mehl[3]

[1]Agilent Technologies India Pvt. Ltd.,

[2]Agilent Technologies, Inc., Tallahassee, Florida,

[3]Agilent Technologies Inc., Wilmington, Delaware.

Introduction

Abstract

In recent years, the FDA has announced recalls of several medications due to the presence of genotoxic N-nitroso impurities in APIs posing a significant risk to health and safety, posing as a major concern for drug makers. The current experiment analyzing three Nitrosamine Drug Substance Related Impurities (NDSRIs) in terbinafine showcases 96-108% recovery at LOQ levels of among API and tablet samples.

Introduction

Terbinafine is an antifungal drug useful in nail and skin infections, however this nitrogen containing compound is likely to contain NDSRIs namely n-nitroso-desmethyl terbinafine (NDT), n-nitroso terbinafine impurity A (NTA) and n-nitroso terbinafine degradant (NTD). The recovery challenges in NTA, poor response of NTD and interference for NDT were addressed by efficient column chromatography, sensitivity and diverter valve programming of 1290-6495D LC/TQ. 37 mg powder was dissolved in 1 mL ultrapure water and vortexed for 10min followed by centrifugation at 10000 rpm. The recovery within ±10% at 0.007 ppm and %RSD less than 10% ensures robustness at low sensitivity and high reproducibility.

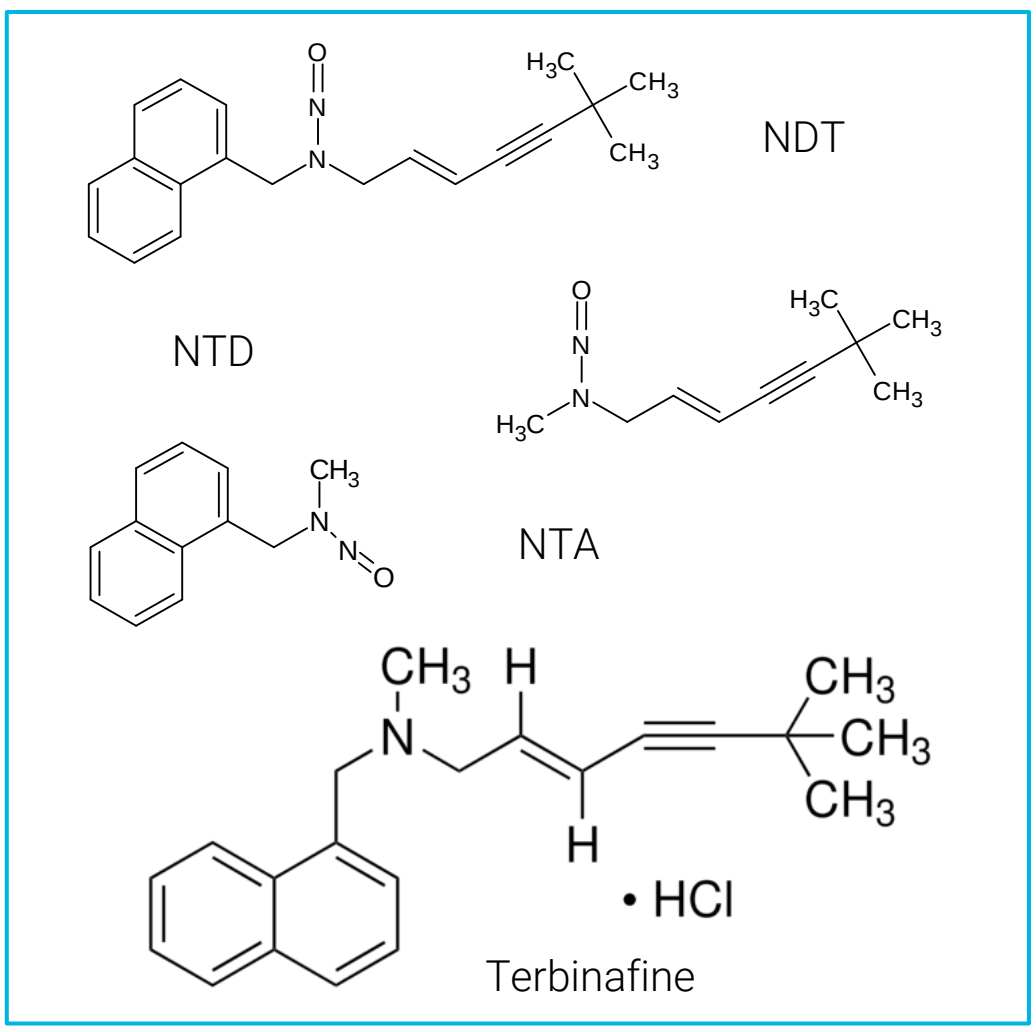

Figure 1: Terbinafine and Nitrosamines

Experimental

Mass Spectrometry Parameters

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

Parameter	Value
Multi sampler	10 °C
Injection Vol	20 µL
Column	30 °C, HPH-C18; 3 x 150mm x 2.7µm
Drying gas	250 °C at 15 L/min
Nebulizer gas	45 psi
Capillary Voltage	2000 V
Nozzle voltage	2000 V
Sheath gas	350 °C at 9 L/min
UV	220 nm
Scan mode	Dynamic MRM
Q1/Q3 Resolution	Narrow / Narrow
Gain	8
Diverter Valve	UV flow diverted to MS between 14.2 min to 20 min and 25.5 min to 30 min.

Time	5mM Amm Acetate in Water	Methanol
0.0	98	2
5.0	98	2
10.0	50	50
25.0	25	75
35.0	10	90
38.0	10	90
38.1	98	2
41.0	98	2

Table 1: LC/TQ method parameters

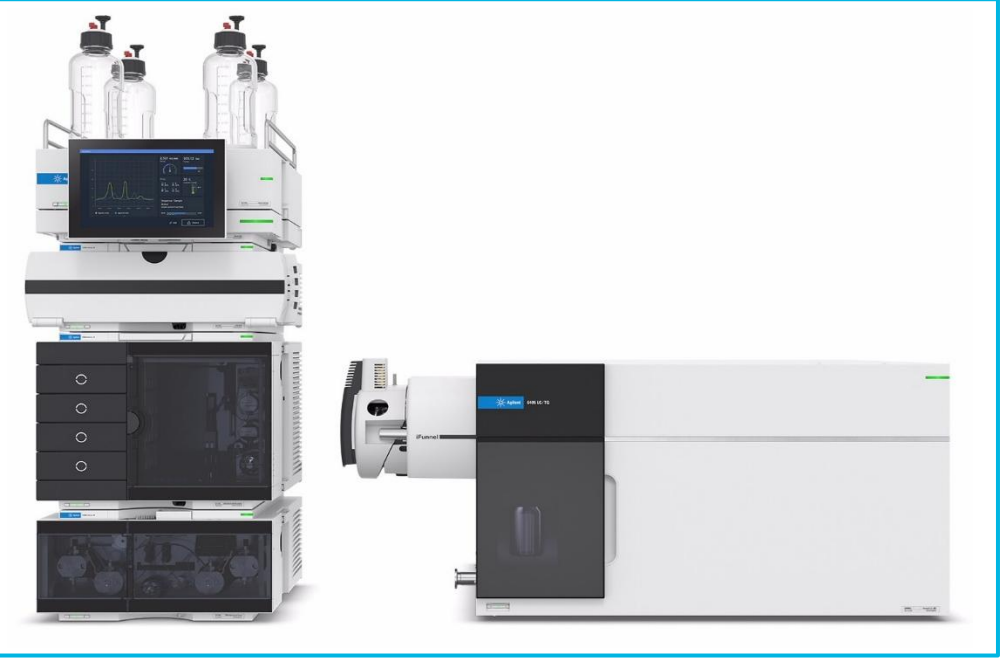

Figure 2: 1290 UHPLC, 6495D LC/TQ

Chromatographic Separation:

A good separation was achieved for the three NDSRIs 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 terbinafine. The UV data for drug at 220 nm is seen in figure 4 and chromatographic profile of 3 NDSRI are seen in figure 5.

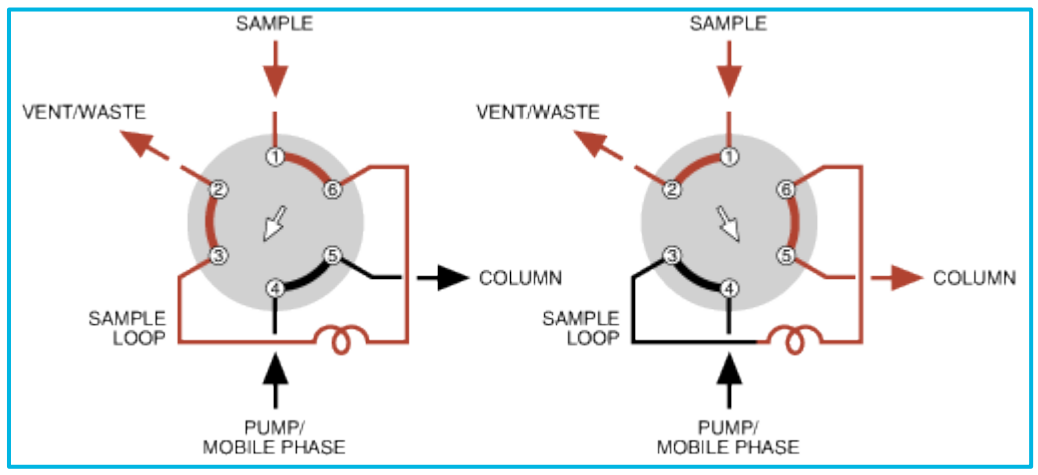

Figure 3: Built-in flow diverter valve program

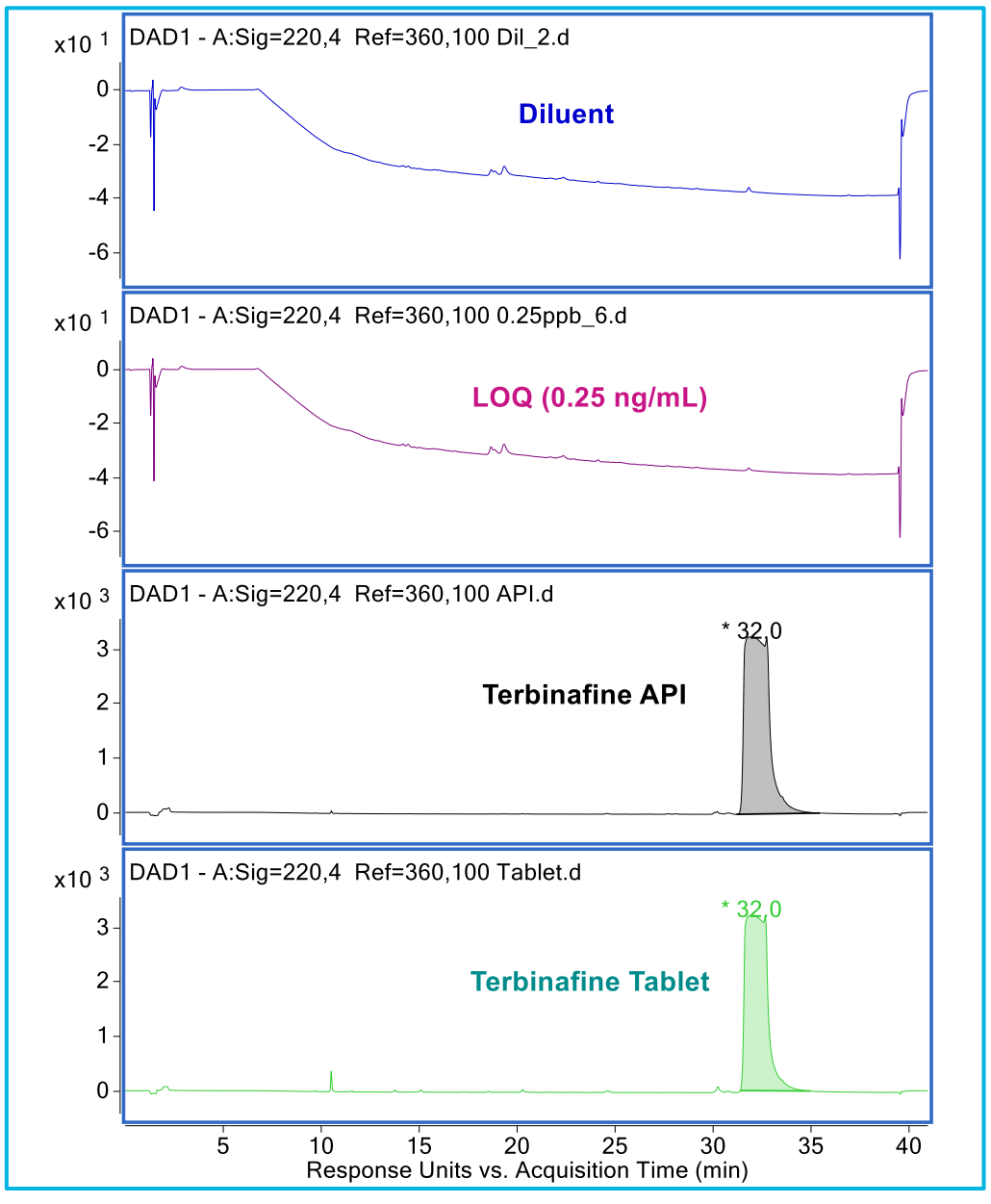

Figure 4: UV profile of diluent, LOQ, API, and terbinafine tablet

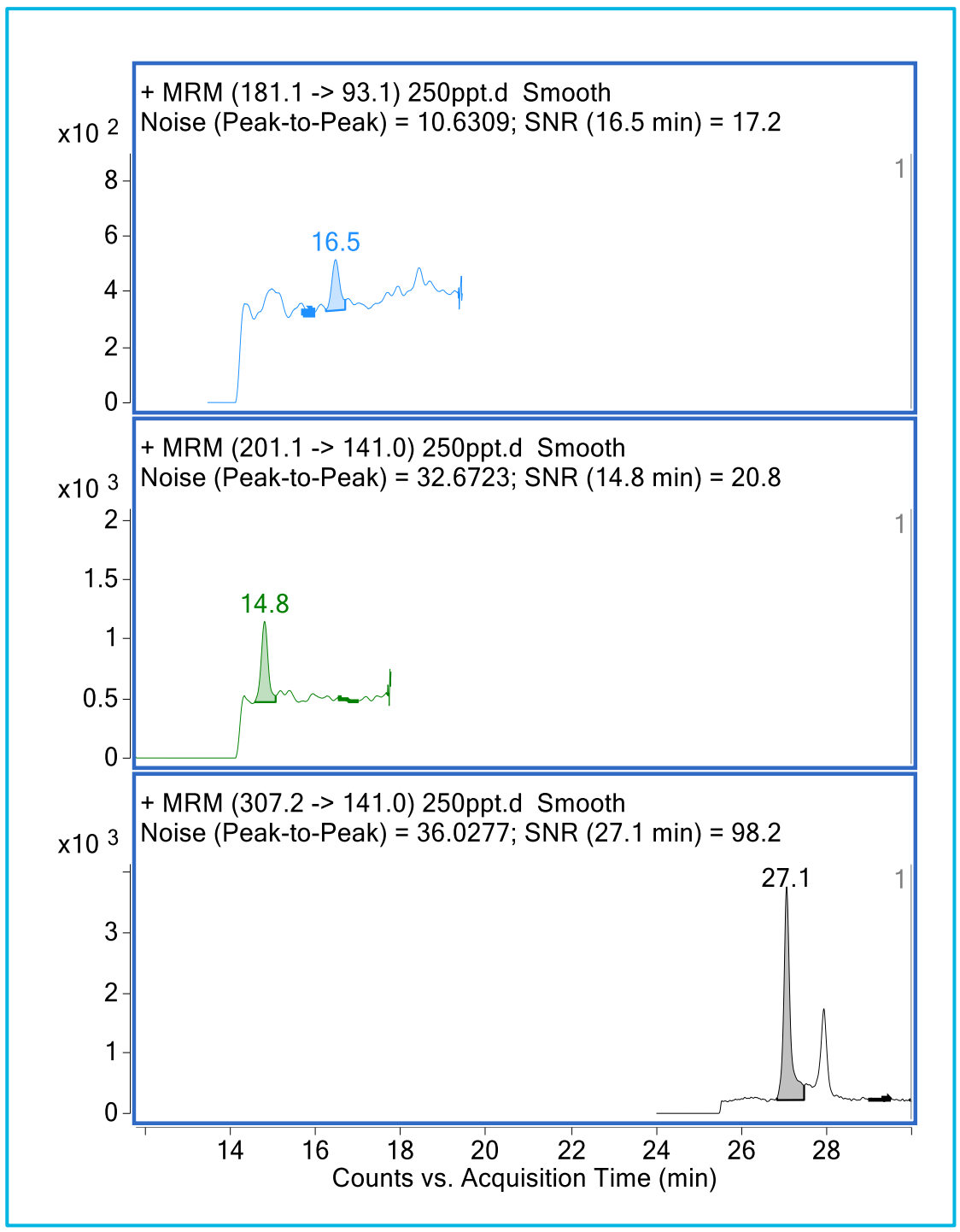

Figure 5: MRM profile of 3 terbinafine NDSRIs at LOQ; having signal to noise values above 15:1 using peak to peak algorithm.

Calibration, RSD and Recovery data:

A calibration plot was generated for all 3 NDSRIs between 0.125 to 2 ng/mL. The obtained plot having r2 values ≥0.997 confirmed a good linearity behavior for all NDSRI impurities (figure 6). The RSD values between 5% to 7%, calculated for six replicates at 0.25 ng/mL, reflected a good method reproducibility. The spike recovery performed at 0.25 ng in 37 mg drug resulted in a value between 96-108% reflecting that the method is working well at targeted <0.007 ppm level. Table 2 showcases, acceptable limits, targeted spike levels and obtained LOQ against drug.

Results and Discussion

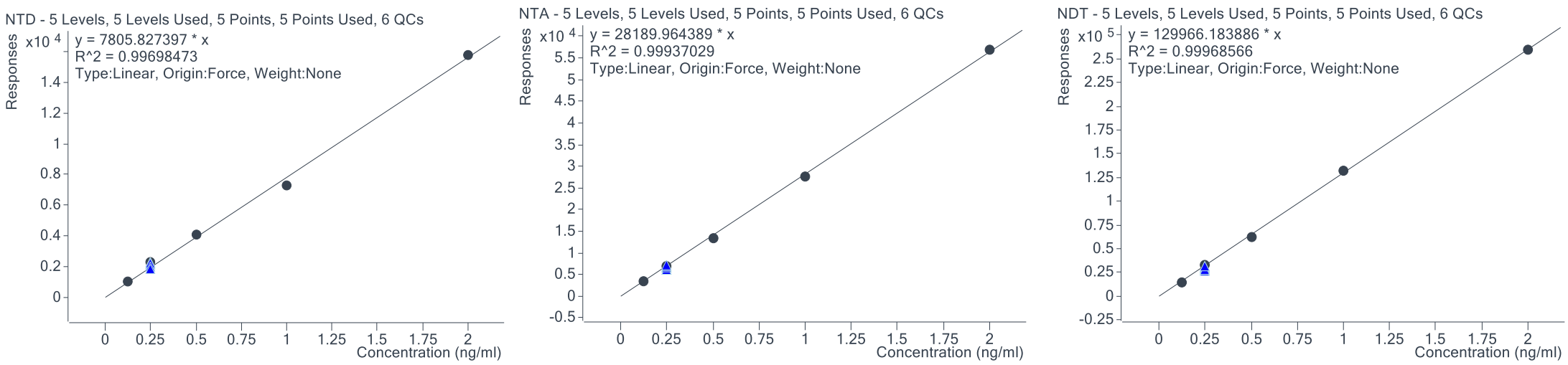

Figure 6: Calibration plot of three NDSRIs across 0.25 ng/mL to 2 ng/mL with r2 values ≥ 0.997

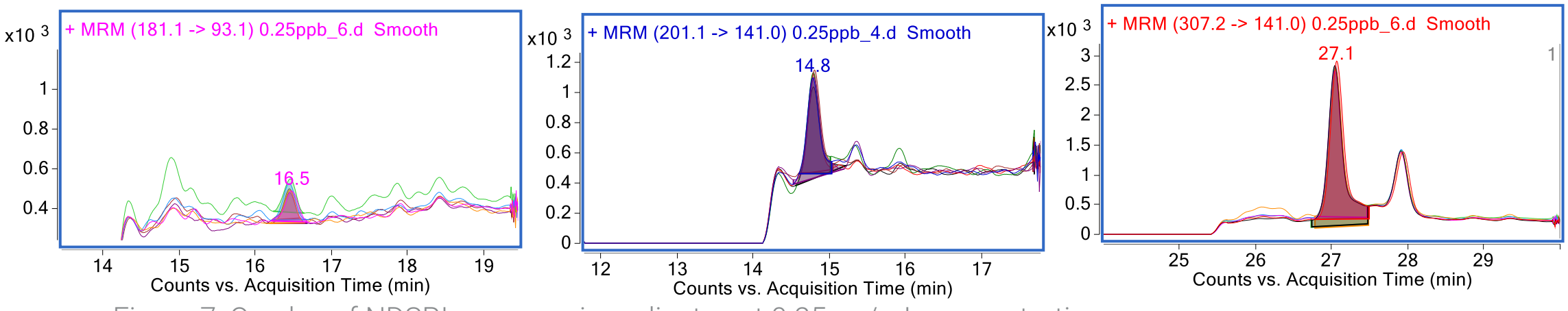

Figure 7: Overlay of NDSRIs across six replicates at 0.25 ng/mL concentration

Analyte	RSD at LOQ	S/N	Recovery in API	Recovery in Tablet
NTD	6.78 %	> 15:1	96.11 %	100.08 %
NTA	6.61 %	> 20:1	99.17 %	108.1 %
NDT	5.08 %	> 75:1	96.63 %	93.32 %

Table 2: Reproducibility, sensitivity and recovery data for 3 NDSRIs

Conclusions

1. A sensitive and robust method has been developed for NTA, NTD, and NDT in terbinafine samples.
2. The LOQ of 0.25 ng/mL with high reproducibility for all NDSRIs has been achieved.
3. Method linearity is obtained from 0.125 ng/mL to 2 ng/mL with r2 values above 0.997.
4. The recovery at LOQ values of 0.25 ng / 37 mg (0.007 ppm) is within 90-110% for all 3 NDSRIs.

References

1. Low-Level Quantitation of N-Nitroso Dabigatran Etexilate Impurity in Dabigatran Etexilate Mesylate API Using the Agilent 6495C. Agilent Application Note, 5994-7066EN.
2. Nitrosamine Impurities Application Guide. Agilent Application Guide, 5994-2393EN.

https://www.agilent.com/en/promotions/asms

DE-006650

Published in USA, May 15, 2025

Agilent
Trusted Answers