Poster Reprint

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**TP 402**

# Quantitation of N-Nitroso Duloxetine N-Nitrosamine Drug Substance Related Impurities (NDSRI) in API and Formulation by 6475 LC/TQ

[1]Saravanan Thangavel, [2]Vivek Dhyani and [3]Saikat Banerjee

[1] Application Engineer, Agilent Technologies Inc.,

[2] Application Engineering Manager, Agilent Technologies Inc.,

[3] Product Sales Manager, Agilent Technologies Inc.,

## Introduction

Nitrosamine impurities are nitroso derivatives where the nitroso group is attached to an amine. The active pharmaceutical ingredient (API) derived from a class of nitrosamines are known as N-nitrosamine drug-substance-related impurities (NDSRI). Nitrosamine impurities in APIs and drug products pose safety concerns, even in small quantities, and thus are a major concern for drug makers [1].

Analytical methods must be sufficiently sensitive to quantify the nitrosamine of interest at levels as low as 10% of the specification limit and, where feasible, down to ≤5% of the specification limit, for the determination of the limit of detection. LC/MS/MS is an inherently selective and highly sensitive analytical technique that is well suited for the identification and quantification of trace-level impurities at extremely low concentrations. Consequently, LC/MS/MS has been widely adopted within the pharmaceutical industry, particularly for nitrosamine analysis [2-3].

Therefore, manufacturers must develop and qualify a dedicated analytical method that is appropriate for the specific sample matrix and targeted NDSRIs. An LC/MS/MS method based on multiple reaction monitoring (MRM) was developed for the detection and quantification of the N-nitroso duloxetine NDSRI impurity over a concentration range of 0.5 to 40 ppb (absolute standard concentration), using an atmospheric pressure chemical ionization (APCI) source on an Agilent 6475A LC/TQ instrument.

## Experimental

### Materials:

Calibration samples were prepared using a N-nitroso duloxetine analytical reference standard provided by a pharmaceutical company. Formulation pellets, placebo samples, and active pharmaceutical ingredient (API) materials were also supplied by the pharmaceutical company.

The following chemicals and reagents were used in the study: formic acid (MS grade, Fluka, Honeywell, Muskegon, Michigan, USA), methanol (MS grade, Biosolve, Dieuze, France), and water (MS grade, Honeywell, Muskegon, Michigan, USA).

## Experimental

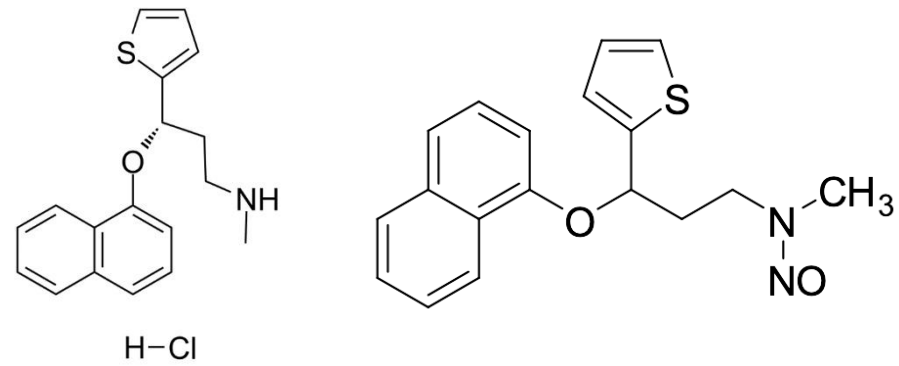


Figure 1. Duloxetine HCl and N-Nitroso Duloxetine

### Equipment:.

Figure 2. 6475 Triple Quadrupole LC/MS System

| LC and MS Conditions | | | |
|---|---|---|---|
| Column | Poroshell HPH C18 (4.6*150mm, 2.7µm) (Part number: 693975-702) | | |
| Mobile Phase A | 0.1% formic acid in water (1 ml of formic acid in 1000ml of Water) | | |
| Mobile Phase B | 100% Methanol | | |
| Flow Rate | 0.5 mL/min | | |
| Injection Volume | 8 µL | | |
| Sample Cooler Temperature | 5 °C | | |
| Column Temperature | 40 °C | | |
| Needle Wash | Methanol: water (90:10) | | |
| Elution | Gradient | | |
| Acquisition Time | 18 minutes | | |
| Gradient Program | Time (min) | %A | %B |
| | 0.00 | 90.00 | 10.00 |
| | 2.00 | 90.00 | 10.00 |
| | 8.00 | 10.00 | 90.00 |
| | 14.00 | 10.00 | 90.00 |
| | 14.50 | 90.00 | 10.00 |
| | 18.00 | 90.00 | 10.00 |
| Ionization Source | Atmospheric pressure chemical ionization source (p/n G1947B) | | |
| Gas Temperature | 300 ˚C | | |
| Gas Flow | 7 L/min | | |
| Nebulizer | 45 psi | | |
| Vaporizer Temperature | 350 ˚C | | |
| Capillary Voltage | 2000 V | | |
| Corona Current | 6 | | |

Table 1. 6475 LC/TQ Method Conditions

## Results and Discussion

### Chromatographic Separations of API and impurity by 6475 LC/TQ

Effective separation of duloxetine hydrochloride from the N-nitroso duloxetine impurity was critical to the success of this method. Extensive method development was undertaken, including systematic evaluation of multiple chromatographic columns and gradient conditions, to achieve optimal resolution between the API and the nitrosamine impurity. This separation was essential to minimize matrix effects arising from the high API concentration, which could otherwise compromise the accurate quantification of the target nitrosamine impurity.

The developed LC/MS/MS method achieved a chromatographic separation of approximately 2.5 minutes between duloxetine hydrochloride and N-nitroso duloxetine (Figure 3).

To further protect the detector, an inbuilt diverter valve program was implemented to prevent the large API load from entering the MS, thereby reducing the risk of contamination and signal suppression. In addition, MS/MS parameters were rigorously optimized to maximize sensitivity and ensure robust, reliable detection of N-nitroso duloxetine at trace levels.

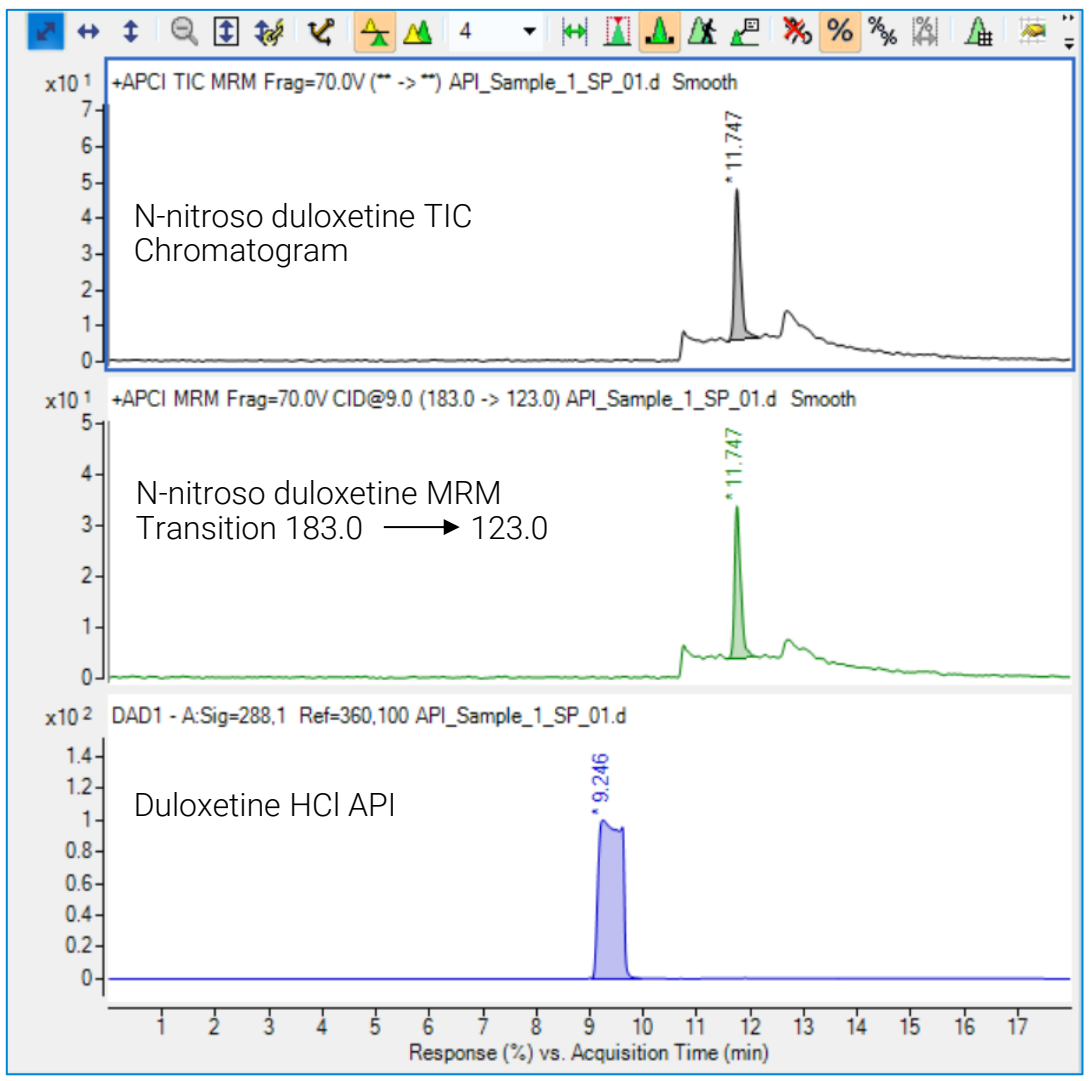


Figure 3. Duloxetine Hydrochloride and N-Nitroso Duloxetine Nitrosamine Impurity Separation by Agilent 6475 LC/TQ

### Signal to noise ratio at LOQ and LOD levels.

Signal to noise (S/N) ratios were determined at the LOD (0.5 ng/mL) and LOQ (2 ng/mL) (Figure 4).

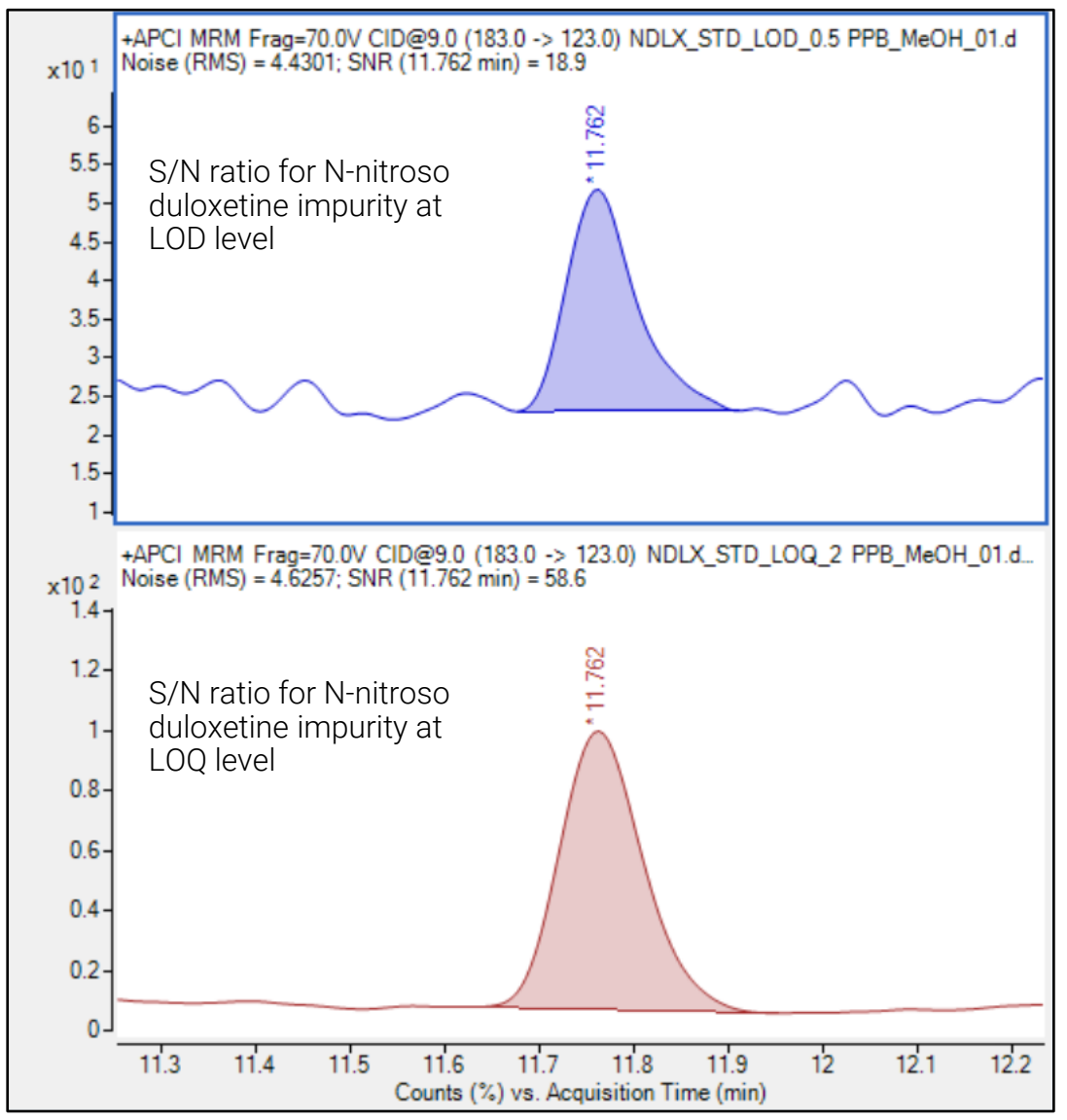


Figure 4. S/N Ratio at LOQ and LOD levels

### Linearity and Calibration curve plot:

A seven-point calibration curve was created over a concentration range of 0.5 ng/ml to 40 ng/ml (Figure 5). The calibration demonstrated excellent linearity, with a $R^2$ value greater than 0.99, meeting the established acceptance criteria.

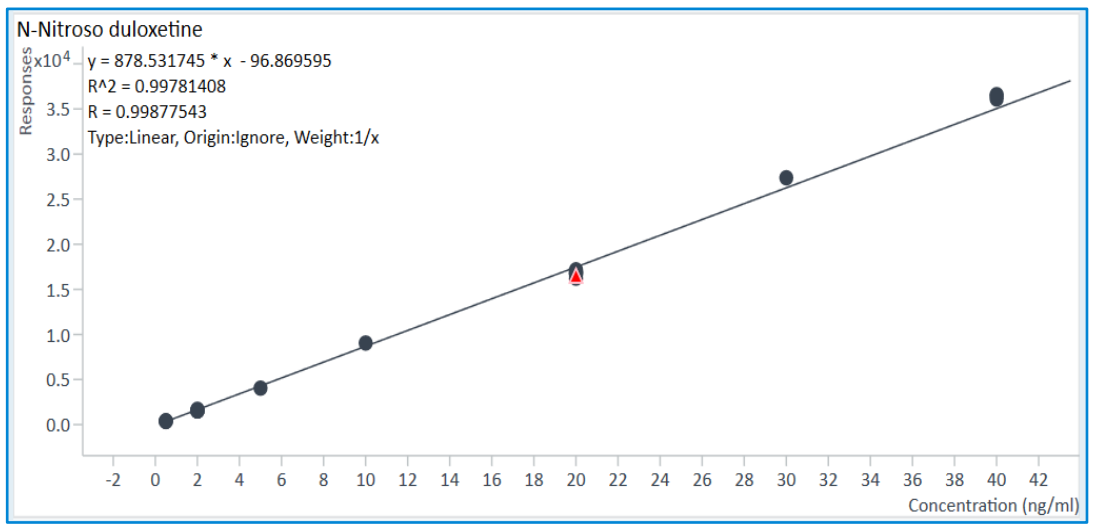


Figure 5. Calibration Curve

## Results and Discussion

### Duloxetine Hydrochloride API & Formulation: Linearity, Reproducibility at LOQ and Recovery Study Table:

Recovery of the N-nitroso duloxetine impurity, reproducibility at the LOQ, and linearity were evaluated in API samples.

The observed correlation co-efficient is greater than 0.99 and recovery was observed in the range of 80-120% (Table 2). The %RSD at LOD and specification levels was less than 5%, indicating good method precision.

| Sample | N-Nitroso duloxetine Results | | |
|---|---|---|---|
| | RT | Final Conc. | Accuracy |
| Blank_01.d | | | |
| Blank_01.d | | | |
| Blank_02.d | | | |
| NDLX_STD_0.5 PPB.d | 11.762 | 0.5128 | 102.6 |
| NDLX_STD_2 PPB.d | 11.757 | 1.9478 | 97.4 |
| NDLX_STD_5 PPB.d | 11.757 | 4.76 | 95.2 |
| NDLX_STD_10 PPB.d | 11.757 | 10.4136 | 104.1 |
| NDLX_STD_20 PPB.d | 11.762 | 19.3463 | 96.7 |
| NDLX_STD_30 PPB.d | 11.762 | 35.4329 | 118.1 |
| NDLX_STD_40 PPB.d | 11.757 | 41.3561 | 103.4 |
| Blank_03.d | | | |
| BKT_STD_01.d | 11.762 | 19.4181 | 97.1 |
| Blank_04.d | 11.798 | 0.1647 | |
| NDLX_STD_LOD_0.5 PPB_01.d | 11.762 | 0.5679 | 113.6 |
| NDLX_STD_LOQ_2 PPB_01.d | 11.762 | 1.9657 | 98.3 |
| Blank_05.d | 11.768 | 0.1258 | |
| BKT_STD_02.d | 11.762 | 19.5707 | 97.9 |
| Blank_06.d | 11.976 | 0.1377 | |
| Blank_07.d | 11.716 | 0.1201 | |
| BKT_STD_03.d | 11.762 | 18.9419 | 94.7 |
| Blank_08.d | | | |
| BKT_STD_04.d | 11.813 | 19.5535 | 97.8 |
| BKT_STD_05.d | 11.813 | 19.5939 | 98 |
| API_SP_01.d | 11.747 | 7.235 | |
| API_SP_02.d | 11.752 | 6.6194 | |
| APILOQ_Spike_SP_01.d | 11.798 | 8.6407 | |
| API_LOQ_Spike_SP_02.d | 11.788 | 8.6927 | |
| API_100% Spec_Spike_SP_01.d | 11.839 | 24.6857 | |
| API_100% Spec_Spike_SP_02.d | 11.844 | 24.1982 | |
| BKT_STD_06.d | 11.849 | 19.3045 | 96.5 |
| API_200% Spec_Spike_SP_01.d | 11.839 | 45.1074 | |
| API_200% Spec_Spike_SP_02.d | 11.844 | 45.5522 | |
| API_Sample_2.d | 11.773 | 4.828 | |
| API_Sample_3.d | 11.773 | 4.5051 | |
| BKT_STD_07.d | 11.849 | 19.1144 | 95.6 |
| Placebo_SP_01.d | 11.701 | 1.0492 | |
| Formulation_SP_01.d | 11.696 | 1.8962 | |
| Formulation_SP_02.d | 11.711 | 1.953 | |
| Formulation_LOQ Spike_SP_01.d | 11.772 | 3.9215 | |
| Formulation_LOQ Spike_SP_02.d | 11.829 | 3.8141 | |
| Formulation_100% Spec Spike_SP_01.d | 11.829 | 24.0634 | |
| Formulation_100% Spec Spike_SP_02.d | 11.803 | 23.1592 | |
| Formulation_200% Spec Spike_SP_01.d | 11.803 | 49.1228 | |
| Formulation_200% Spec Spike_SP_02.d | 11.803 | 49.7464 | |
| Formulation sample 2_SP_01.d | 11.737 | 89.5955 | |
| Formulation sample 3_SP_01.d | 11.737 | 93.5494 | |

Table 2. Duloxetine HCl API and Formulation Linearity and Recovery

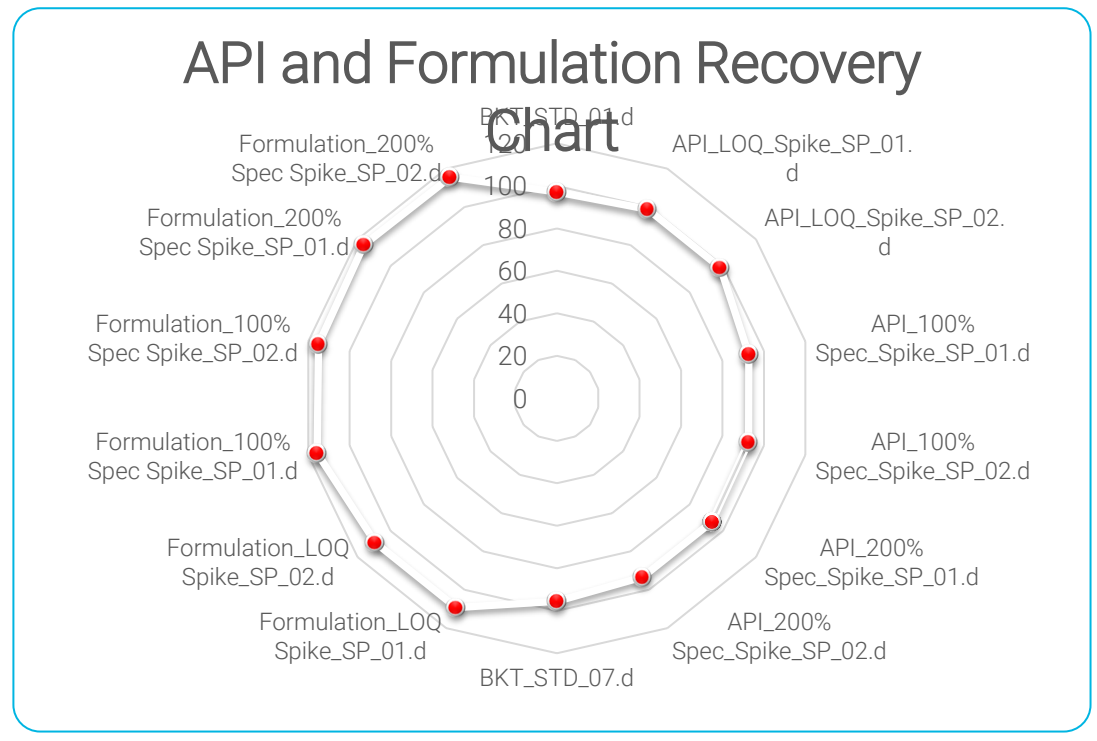


Figure 6. Duloxetine HCl API and Formulation Recovery

## Conclusions

- A highly sensitive and robust MRM LC/MS/MS method was developed for the quantitation of N-nitroso duloxetine in duloxetine hydrochloride API and finished formulation using the Agilent 6475A LC/TQ.
- The optimized chromatographic conditions provided effective separation of the analyte from the API, placebo, and formulation matrices, ensuring reliable quantitation.
- The calibration curve was linear over the concentration range of 0.5-40 ng/mL, with 1/*x* weighting. R and $R^2$ values were observed for both to be greater than 0.99.
- Recovery studies demonstrated efficient sample extraction, with recoveries ranging from 92-99% in API samples and 109-116% in pellet formulations at the LOQ, specification, and 200% specification levels.
- The method also showed excellent reproducibility across all evaluated concentration levels, confirming its suitability for routine analysis.

## References


1. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan.

2. EMA Guidelines: EMA/409815/2020 Rev.23; Effective from 10 October 2025.

3. FDA Guidance document on Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities, Docket Number: FDA-2020-D-1530, 04 Aug (2023).


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