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Trace-level Estimation of N-Nitroso Isavuconazonium Sulphate in Isavuconazonium Sulphate API Using Nominal Mass LC/MS

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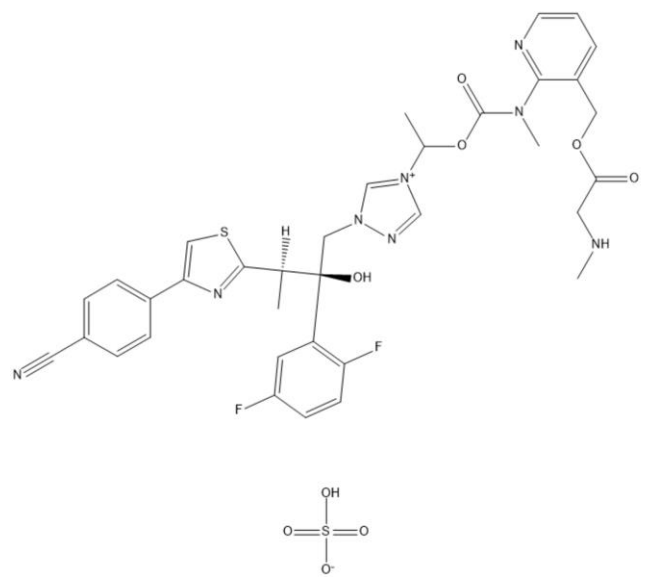
Introduction

The estimation of N-nitroso isavuconazonium sulphate (nitrosamine drug substance-related impurity or NDSRI) is a critical parameter for quality control of isavuconazonium sulphate API.

The estimation of NDSRIs is troublesome, as the APIs are sensitive to moisture and pH changes. APIs are generally unstable in solution and tend to convert into their respective NDSRIs. This affects the possibility of estimating the actual amount of NDSRI in the API or formulation. Analysts have also expressed concern regarding the ruggedness of sample preparation techniques. This study addresses these issues and represents a sensitive and stable method of analyzing N-nitroso isavuconazonium sulphate in isavuconazonium sulphate using an LC/TQ system.

Isavuconazonium Sulphate



N-Nitroso Isavuconazonium Sulphate

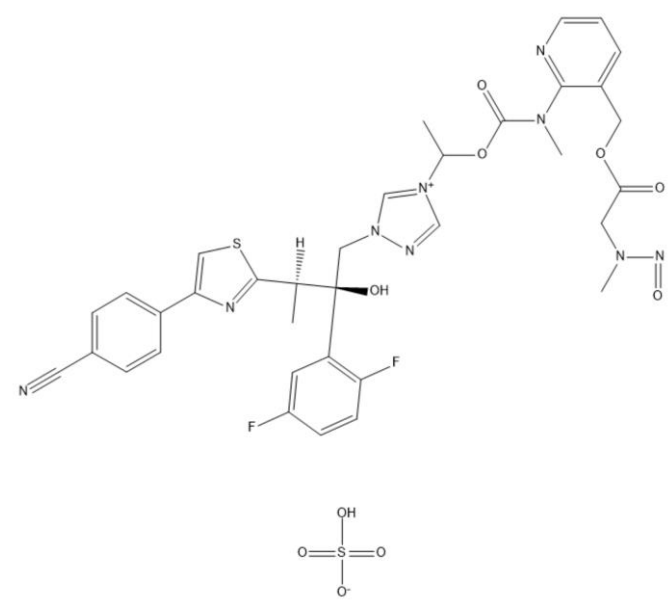


Figure 1. Structures of isavuconazonium and N-nitroso isavuconazonium sulphate.

Experimental

Method parameters

Mobile phase A: 1 mM ammonium trifluoro acetate with 0.004% formic acid in water

Mobile phase B: Acetonitrile

Flow rate: 0.8 mL/min

Injection volume: 20 µL

Diluent: Methanol:water: 1:1 with 0.1% formic acid

Column: Poroshell HPH-C18, 3.0 x 150 mm, 2.7 µm (p/n: 693975-502

The gradient program was as follows:

Time (min)	%A	%B
0.00	75	25
1.00	75	25
16.00	2	98
20.00	2	98
20.10	75	25
24.00	75	25

Source parameters used for AJS ESI ion source.

Parameter	Value
Gas flow	13
Nebulizer	35
Sheath gas	11
Capillary voltage	1,600
Nozzle voltage	1,400
Gas temperature °C	280
Sheath gas temperature °C	325
High-pressure funnel (V)	150
Low-pressure funnel (V)	60

MRM transitions used for N-nitroso isavuconazonium sulphate.

Parent m/z	Product m/z	ID	Collision Energy
747.2	164.8	Quantifier	24
	121.0	Qualifier	76

Results and Discussion

The specification limit to quantitate N-nitroso isavuconazonium sulphate is 0.03 µg/gm, which converts to a 0.03 ppb w.r.t test concentration of 1 mg/mL. An LOD of 1 ppt and LOQ of 5 ppt could be achieved using the above-mentioned method, with a projected test concentration of 1 mg/mL. An R2 value greater than 0.998 was achieved for a concentration linearity of a dynamic range of 1 to 1,000 ppt using the NDSRI impurity standard. The API and the impurity were both stable for multiple days in solution and no conversion of API was observed during the analysis period.

A three-day interday stability study was performed for the impurity standard solution. The experiment showed an area RSD of 2.2% for a total of 18 injections in a set of six injections, for three consecutive days at specification-level concentration.

Figure 2. Agilent 6495D LC/TQ.

Results and Discussion

Ruggedness of sample preparation was also tested at two QC levels (10 and 100 ppt) for three different preparations, prepared on three different days. The area RSD was found to be 2.9 and 3.5, respectively, for a total of 18 injections of each level.

There were around 2 minutes of separation between the API and the impurity, in terms of retention time with the given chromatographic conditions.

No blank interference was observed during batch analysis.

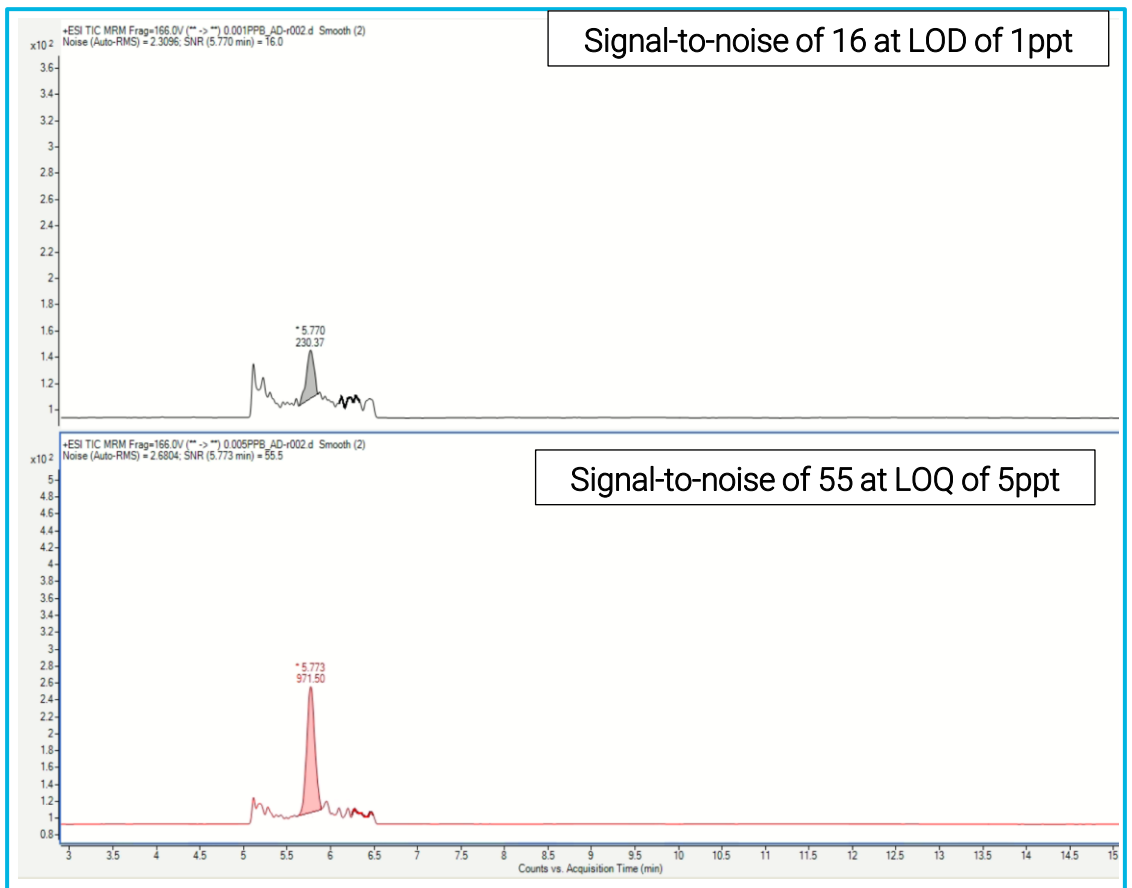


Figure 3. Responses at LOD and LOQ levels.

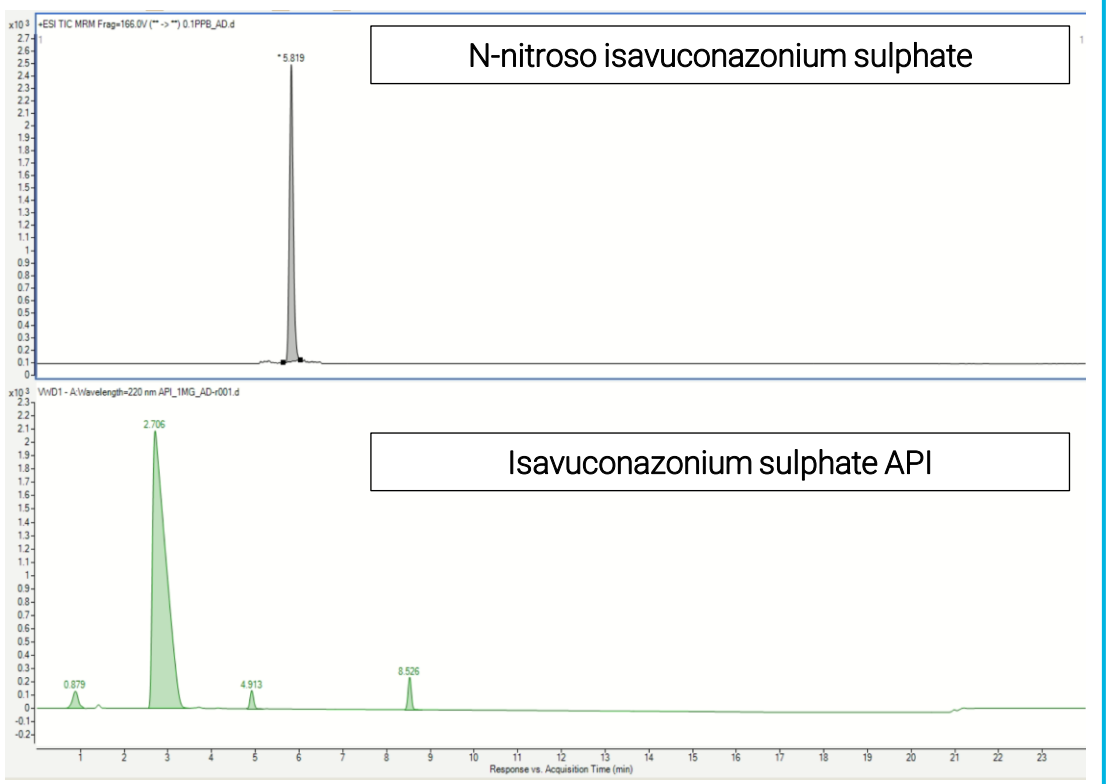


Figure 4. Separation between API and NDSRI.

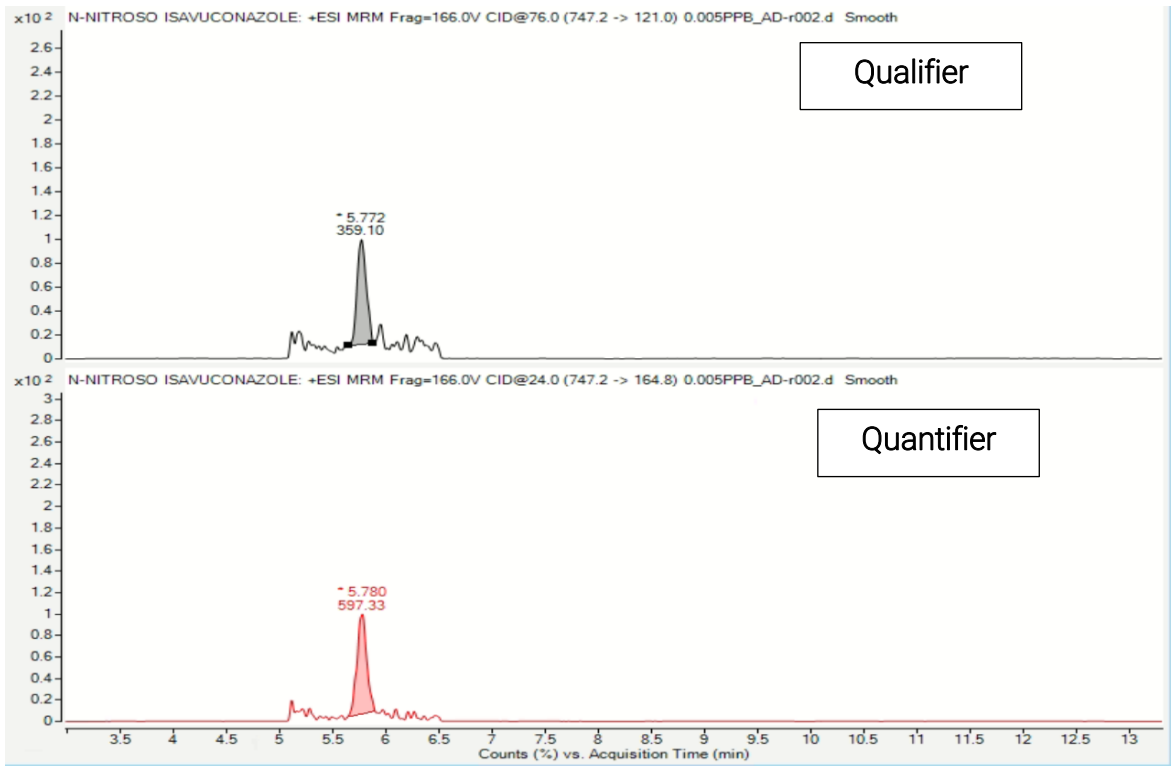


Figure 5. Qualifier and quantifier extracted ion chromatogram at LOQ.

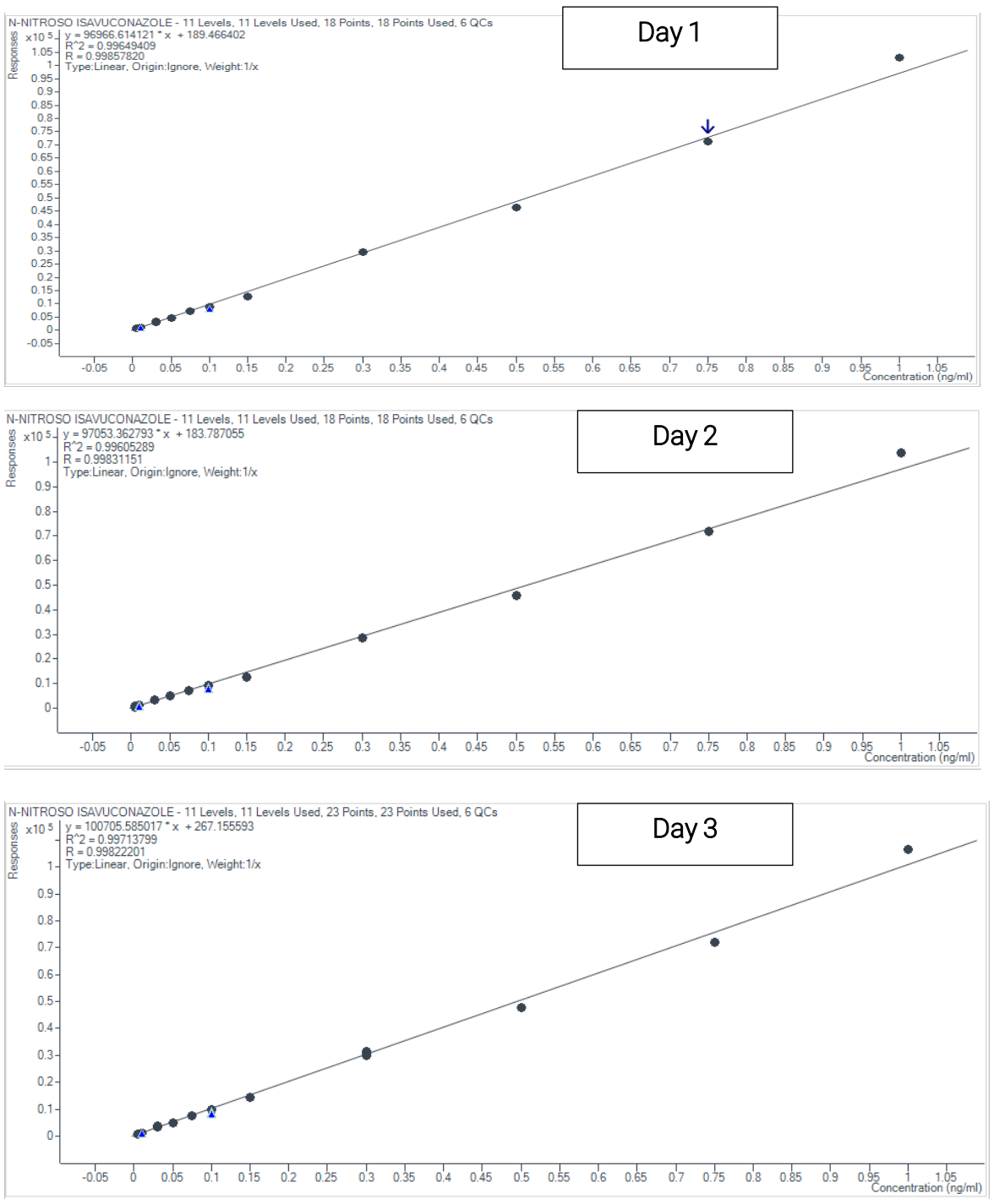


Figure 6. Concentration calibration curves acquired on 3 consecutive days.

Results and Discussion

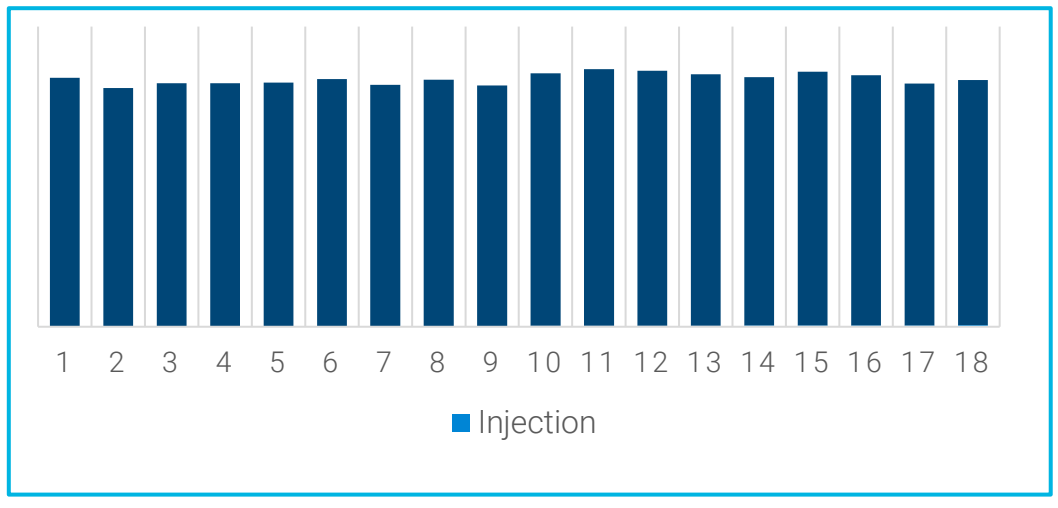


Figure 7. Bar plot to represent specification-level response variation.

Conclusions

- A sensitive, precise method has been developed for quantitating the NDSRI of isavuconazonium sulphate
- An LOD of 1 ppt and an LOQ of 5PPT have been achieved using this method
- The linear dynamic range of the impurity was tested for 200-fold concentration increments (5 to 1,000 ppt) and found linear responses with R^2 values of 0.99 and above
- There is a considerable separation between the impurity and the API
- The stability study, performed over three consecutive days, proves the method to be stable over time

References

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