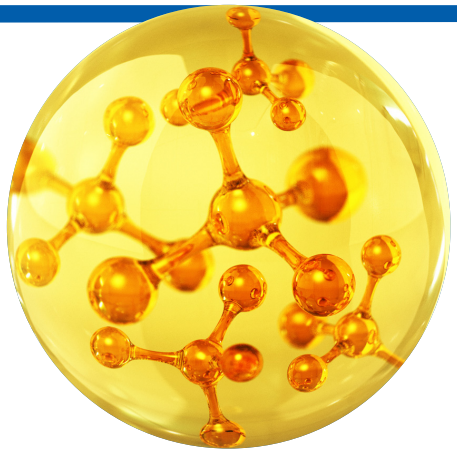

TN-1389

LC-MS/MS N-Nitroso Rasagiline Analysis using a SCIEX 5500+

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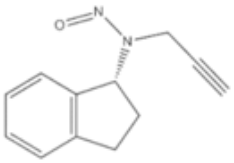
Introduction

Rasagiline is used to treat the symptoms of Parkinson's disease, either alone or in combination with other drugs. Parkinson's disease causes the death of cells which produce dopamine, a chemical which acts as a messenger promoting motivation and movement. An enzyme monoamine oxidase (MAO) breaks down neurotransmitters, but Rasagiline reduces the effectiveness of MAO so that dopamine is more readily available.

As Parkinson's disease is a long-term degenerative disease, any medication prescribed for its treatment is going to be taken for an extended period. As such, it is important to reduce the risk of contamination of any drugs where that contamination could have a deleterious effect on overall health. As Rasagiline is a secondary amine, it is possible for an N-nitroso impurity to be formed. Such impurities are termed as NDSRIs (nitroso drug substance related impurities). NDSRIs have the potential to be carcinogenic and it is therefore of paramount importance to have methods in place to test for low levels of NDSRI contamination.



N-Nitroso desmethyl doxepin

Molecular Formula: $C_{12}H_{12}N_2O$

Monoisotopic m/z: 200.09

Sample Preparation

Standard Preparation (Impurity)

1 mg of N-Nitroso Rasagiline impurity was dissolved in 1 mL diluent (80:20 v/v Methanol:Water) to give a final concentration of 1 mg/mL. The intermediate impurity standard was subsequently diluted to make neat standards using diluent (80:20 v/v Methanol:Water).

Sample Preparation

5 tablets were weighed to calculate the average mass of a tablet, which was found to be 200 mg.

20 mg of triturated tablet sample (corresponding to 0.1 mg of API) was dissolved in 1 mL diluent (80:20 v/v Methanol:Water) to give a final concentration of 0.1 mg/mL.

The sample was vortexed for 5 minutes, then centrifuged at 14000 rpm at 4 °C for 5 minutes. The samples were then filtered using 0.22 µm PVDF syringe filters.

LC Conditions

Column:	Kinetex™ Biphenyl, 2.6 µm
Dimensions:	150 x 3.0 mm
Part No.:	00F-4622-Y0
Mobile Phase:	A: 1 mM Ammonium Formate with 0.1% Formic acid in Water B: 0.1% Formic acid in Methanol
Gradient:	Time (min) % B 0 30 6 30 9 60 12 60 12.1 90 14 90 14.1 30 18 30
Flow Rate:	0.4 mL/min
Injection Volume:	10 µL
Temperature:	40 °C
LC System:	EXION LC 30 AD
Detection:	MS/MS
Detector:	SCIEX 5500+

MS/MS Conditions

Ion Source:	ESI
Polarity:	Positive
Source Temperature:	250 °C
GS1:	75
GS2:	50
CUR:	35
IS:	5500

Table 1. MS Transitions.

Analyte	Q1 Mass(Da)	Q3 Mass(Da)
N-Nitroso Rasagiline	218.1	117.2
N-Nitroso Rasagiline	218.1	115.1
N-Nitroso Rasagiline	218.1	91.1

Have questions or want more details on implementing this method? We would love to help!
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Results and Discussion

Figure 1. Representative Chromatogram: Rasagiline Tablet

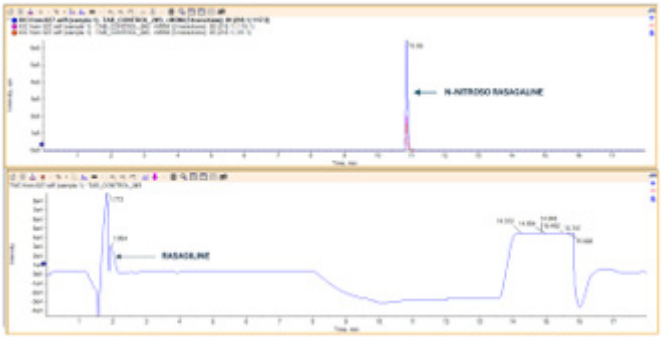

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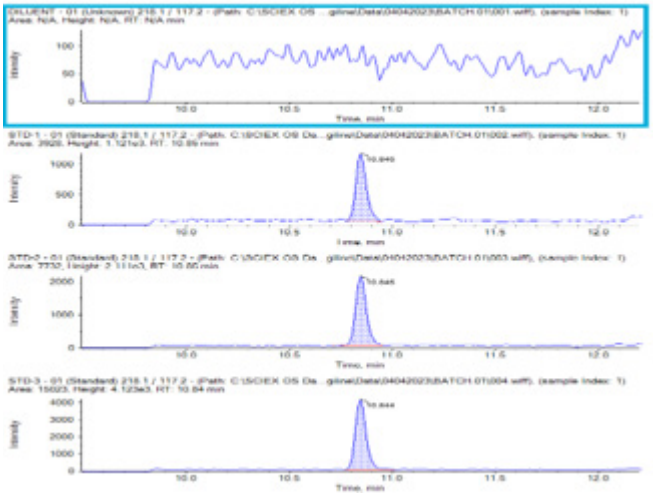
Figure 2. Representative Chromatograms: N-Nitroso Rasagiline



Diluent as blank

Limit of Detection (LOD)

Limit of Quantitation (LOQ)

Specification Limit Level

Table 2. Repeatability Data (% RSD) – N-Nitroso Rasagiline.

Injection No.	Area standard samples – Limit of quantitation (0.09 ng/mL)	Area standard samples – Specification level (1.8 ng/mL)
1	7360	145699
2	7569	142493
3	8048	144133
4	7732	144529
5	7541	143382
6	7454	146485
Average	**7617**	**144454**
SD (%)	**245**	**1468**
Precision (%)	**3.21**	**1.02**

Kinetex™ Biphenyl was selected for this application based on its Core-Shell particle architecture which minimizes analyte diffusion, thereby enhancing chromatographic efficiency. The 2.6 µm particle size offers a favorable balance between performance and system pressure, delivering high efficiency without the elevated backpressure typically associated with sub-2 µm materials.

The polar nature of the Biphenyl stationary phase promotes strong interactions with the N-nitroso drug impurity of interest, resulting in sharp, symmetrical peak shapes, optimal for integration and quantitation. The combination of high efficiency and selective retention enables resolution of N-Nitroso Rasagiline from the Rasagiline drug substance.

As shown in **Figure 1**, the upper chromatogram displays the extracted ion chromatograms (XICs) for the three monitored transitions of N-Nitroso Rasagiline (as listed in **Table 1**), while the lower chromatogram presents the total ion chromatogram (TIC) for the tablet matrix. The data confirm that N-Nitroso Rasagiline is well retained, eluting distinctly from both the solvent front and the Rasagiline peak. This retention window minimizes the risk of ion suppression and allows for the Rasagiline peak to be diverted to waste early in the run, thereby preserving the cleanliness of the MS interface.

Figure 2 presents EICs for N-Nitroso Rasagiline across various conditions. The impurity is not detected in the blank diluent, while both the limit of detection (LOD) and limit of quantitation (LOQ) chromatograms exhibit excellent signal-to-noise ratios. At the specification level—twice the LOQ—the signal remains strong and well-defined. In all cases, the peak shape is symmetrical and the impurity demonstrates high chromatographic efficiency, supporting reliable detection at trace levels.

Conclusions

This study demonstrates that the combination of Kinetex™ Biphenyl chromatography and a SCIEX 5500+ mass spectrometer enables reliable quantification of N-Nitroso Rasagiline at a specification level of 1.8 ng/mL. The method exhibited excellent precision, with a relative standard deviation (RSD) of 1.02% at the specification level and 3.21% at the limit of quantitation (0.09 ng/mL). These results confirm the method's suitability for trace-level analysis of N-nitroso impurities in drug products, offering both sensitivity and reproducibility.

Ordering Information

00F-4622-Y0 Kinetex 2.6 µm Biphenyl 150 x 3.0 mm

For ordering details of alternate particle sizes and dimensions please visit our Kinetex page.

When working with drug products it is advisable to protect columns with SecurityGuard Ultra, further details can be found here.

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

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