

Development and Validation for Quantification of 7-Nitroso Impurity in Sitagliptin by Ultraperformance Liquid Chromatography with Triple Quadrupole Mass Spectrometry

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Validation parameters:

1. System suitability and Specificity chromatograms:

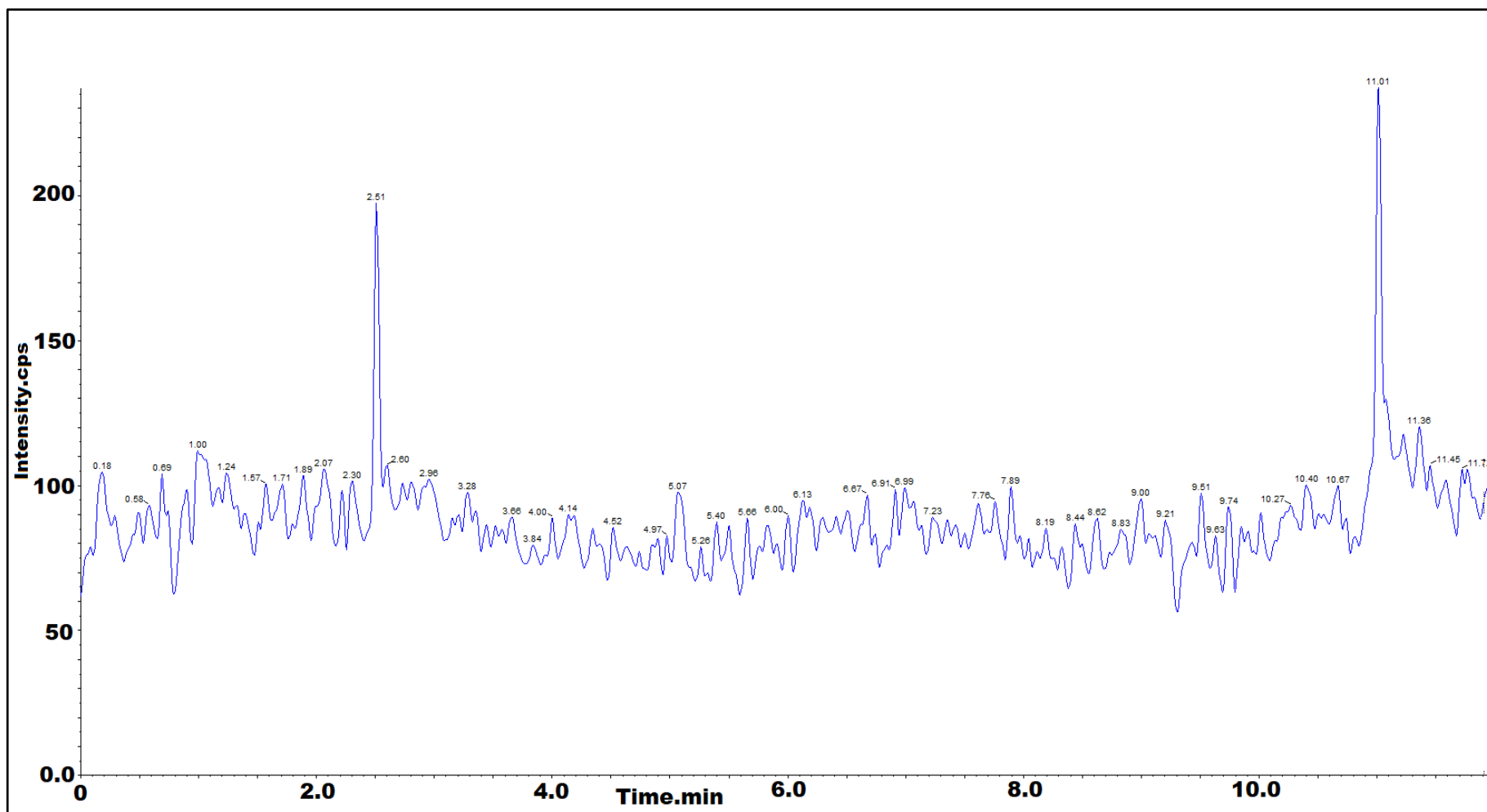


Figure S1: MS/MS chromatogram of Blank solution.

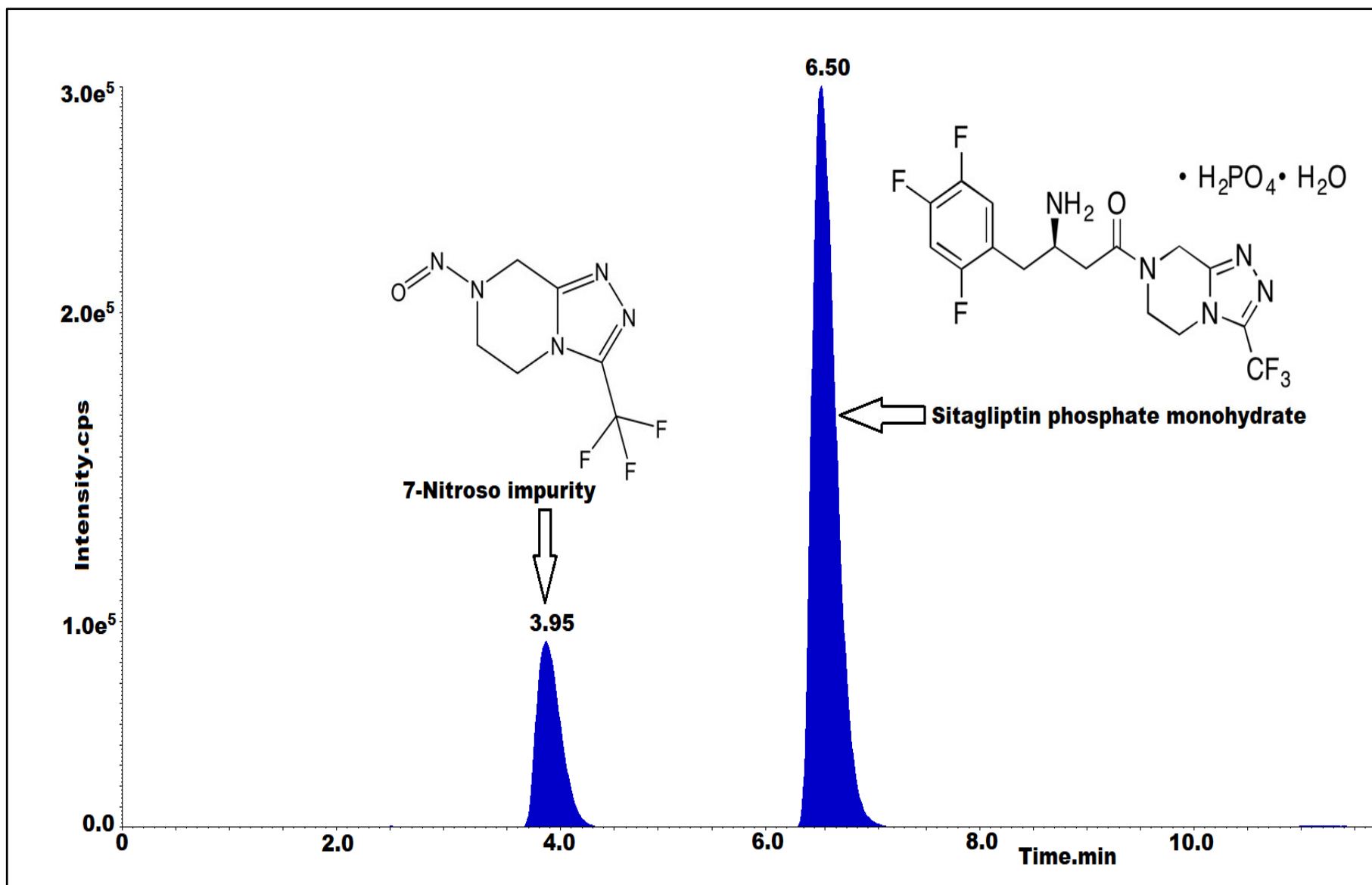


Figure S2: MS/MS chromatogram of 7-nitroso impurity spiked sample.

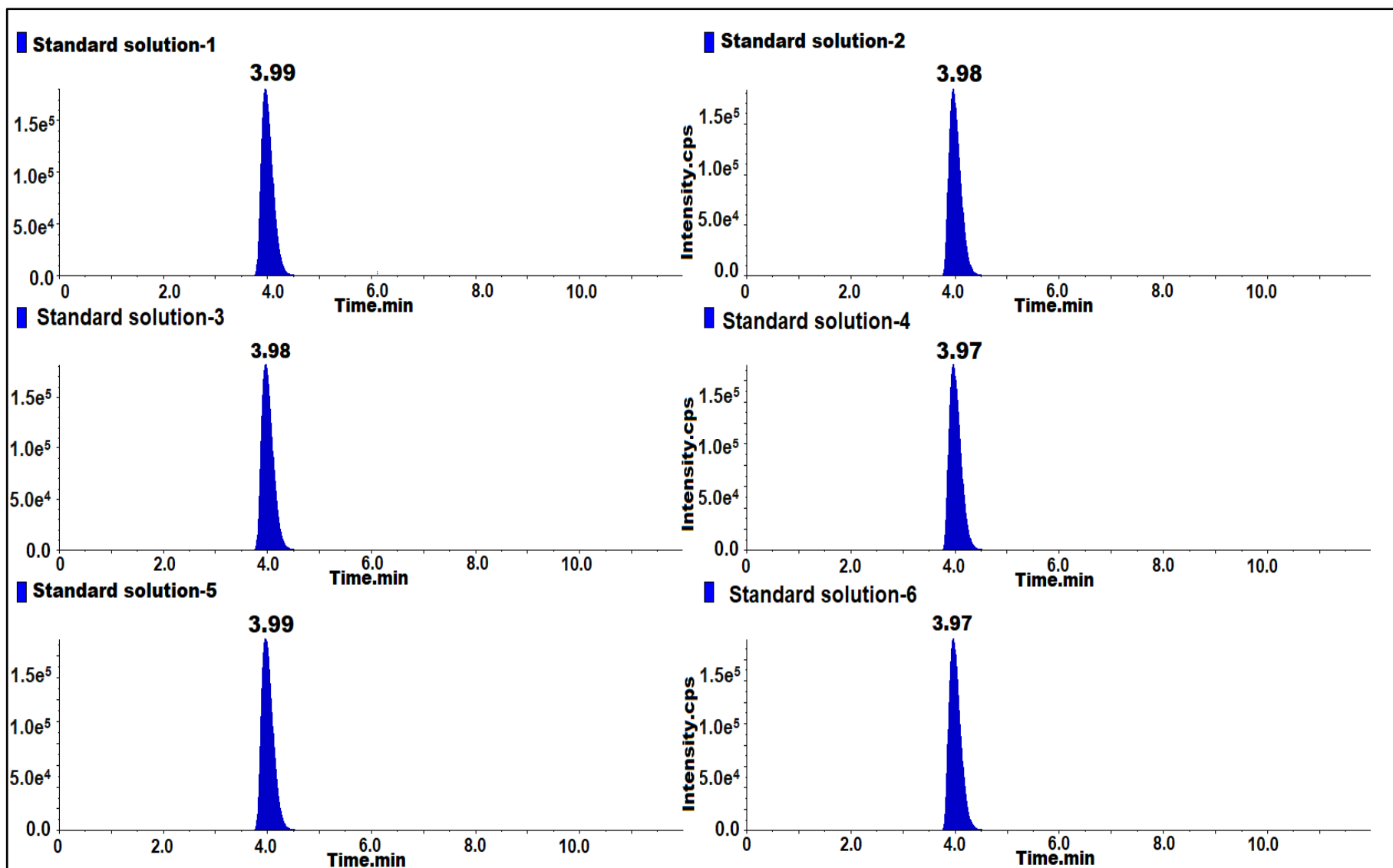


Figure S3: MS/MS chromatogram of System suitability standard solution.

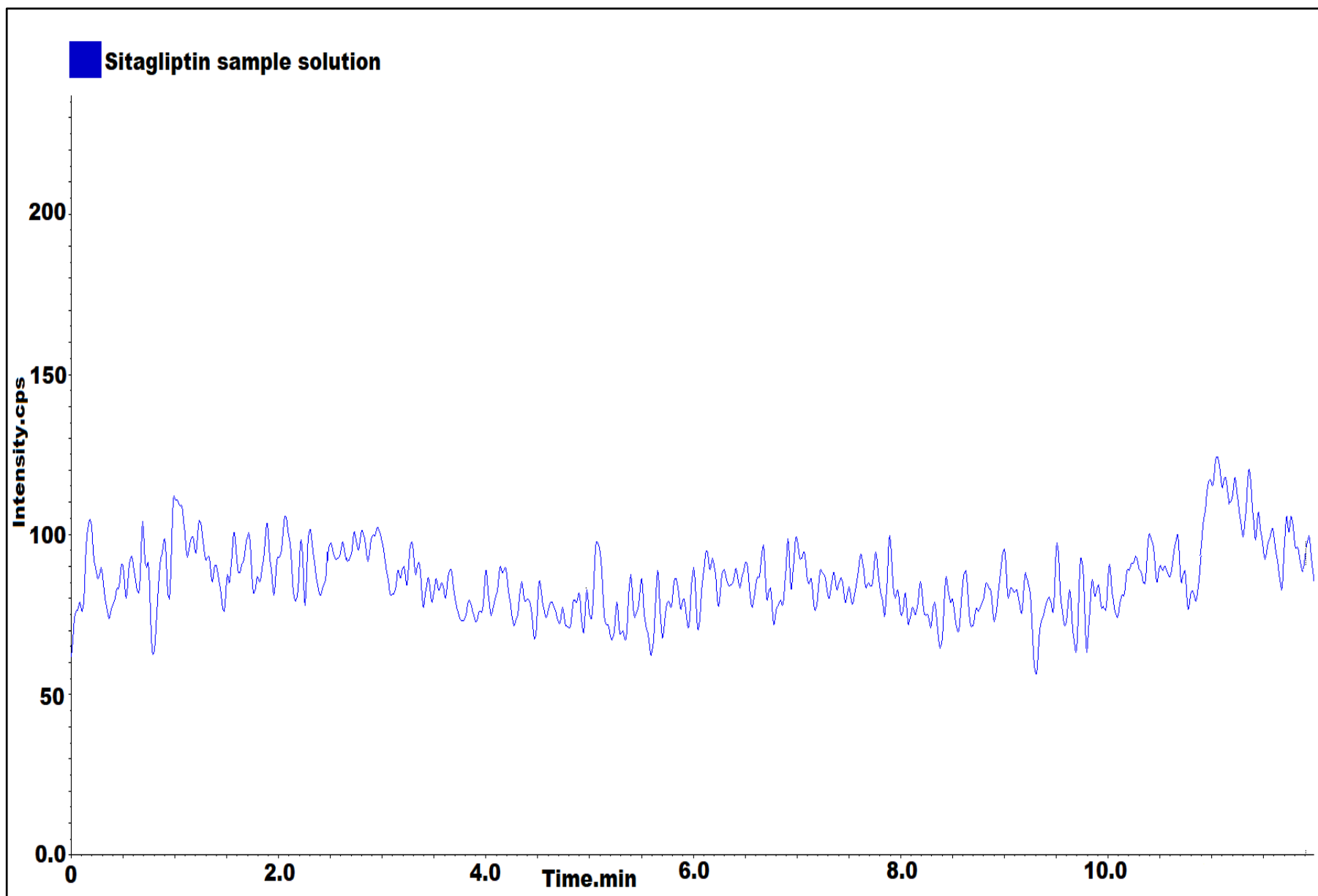


Figure S4: MS/MS chromatogram of Sitagliptin sample solution.

2. LOD, LOQ and LOQ precision

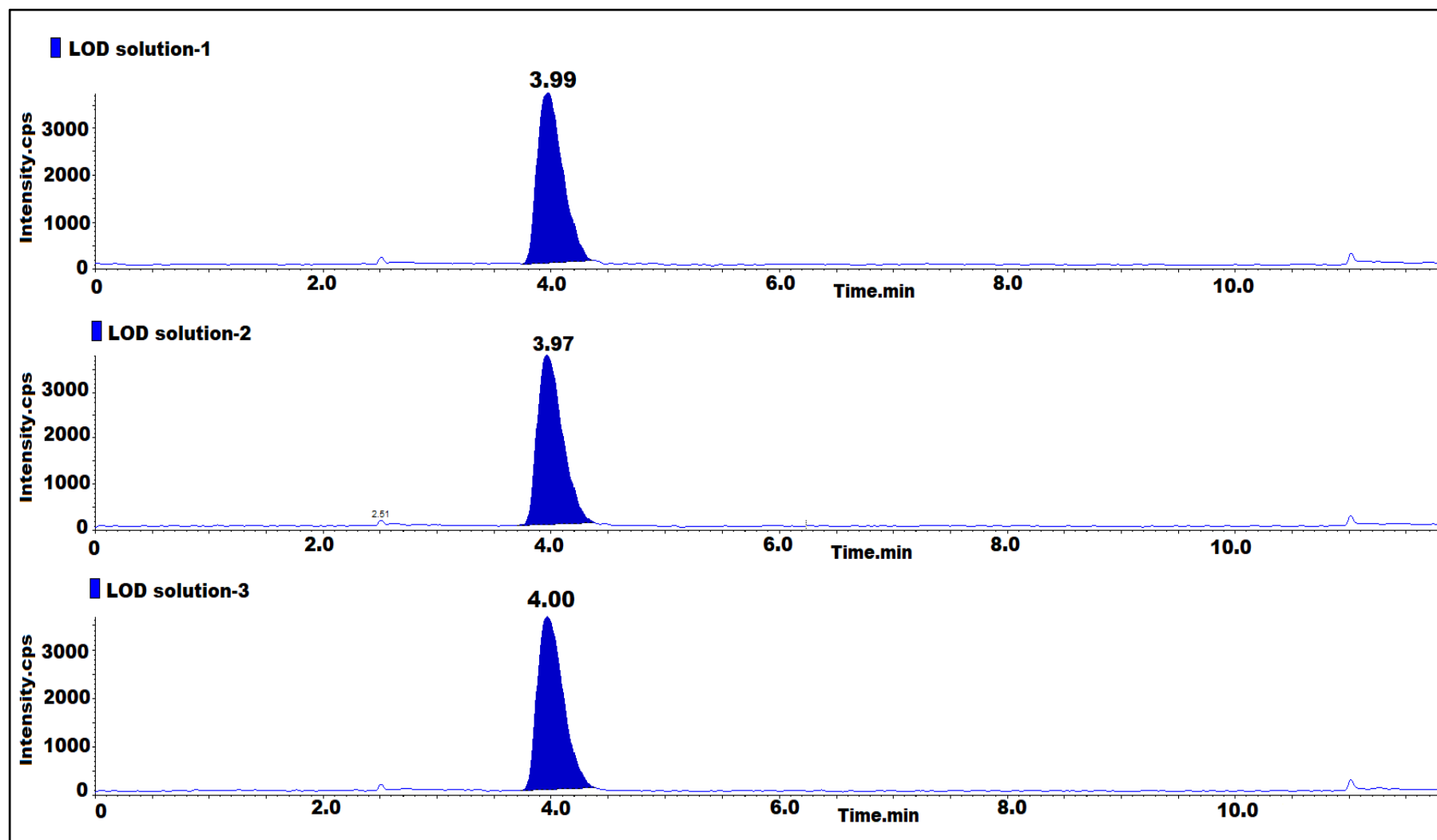


Figure S5: MS/MS chromatogram of LOD solution.

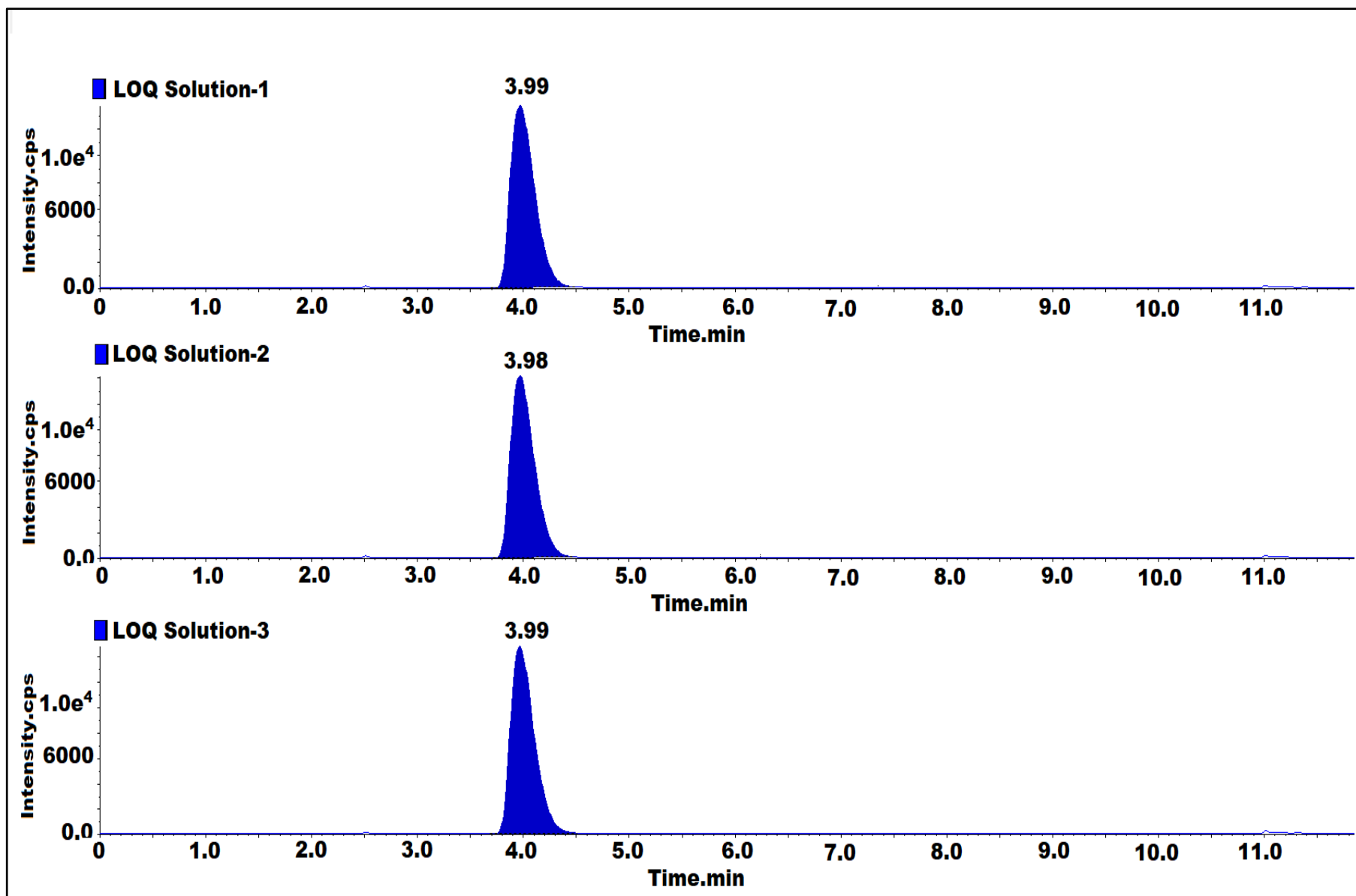


Figure S6: MS/MS chromatogram of LOQ solution.

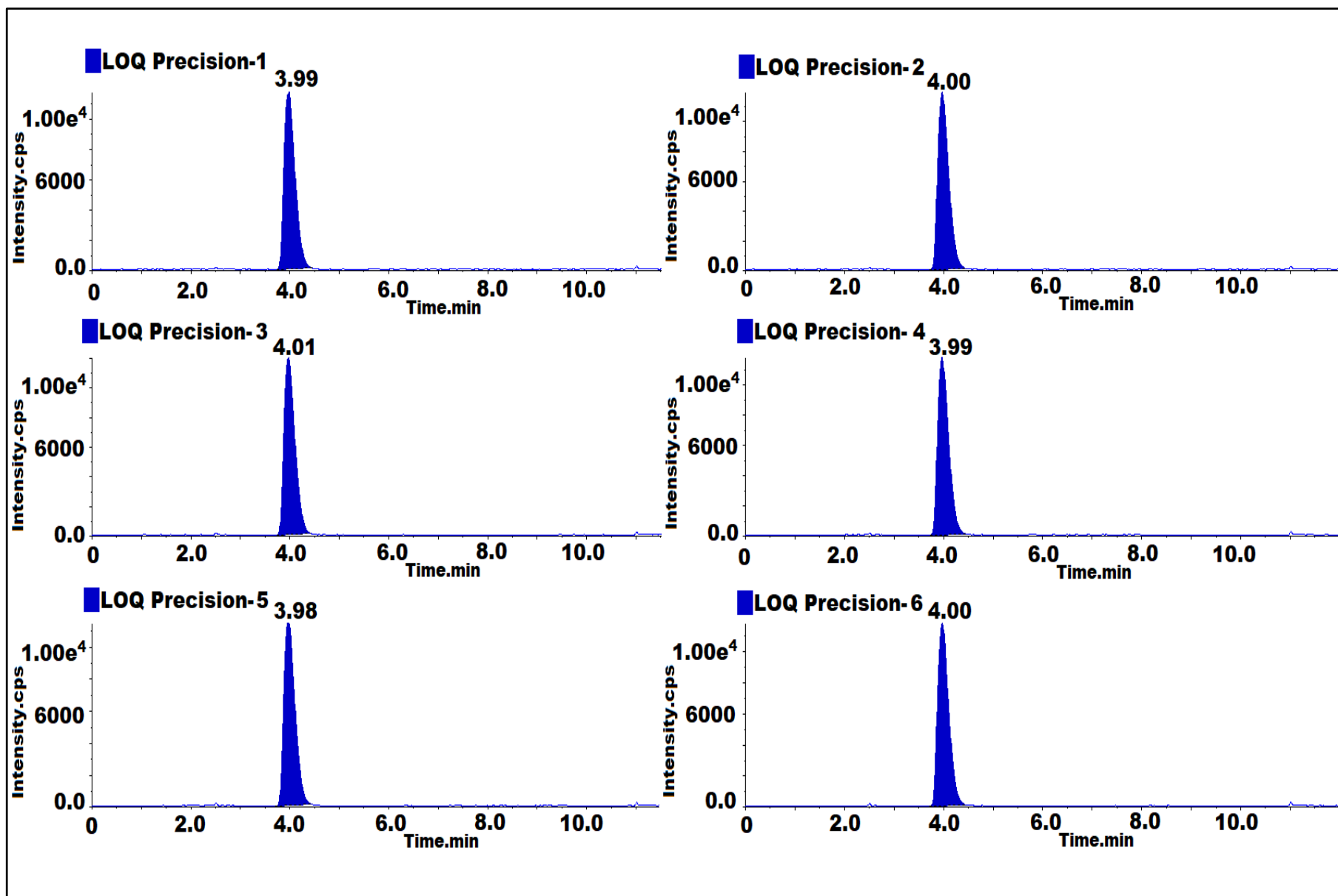


Figure S7: MS/MS chromatograms of LOQ Precision.

3. Linearity

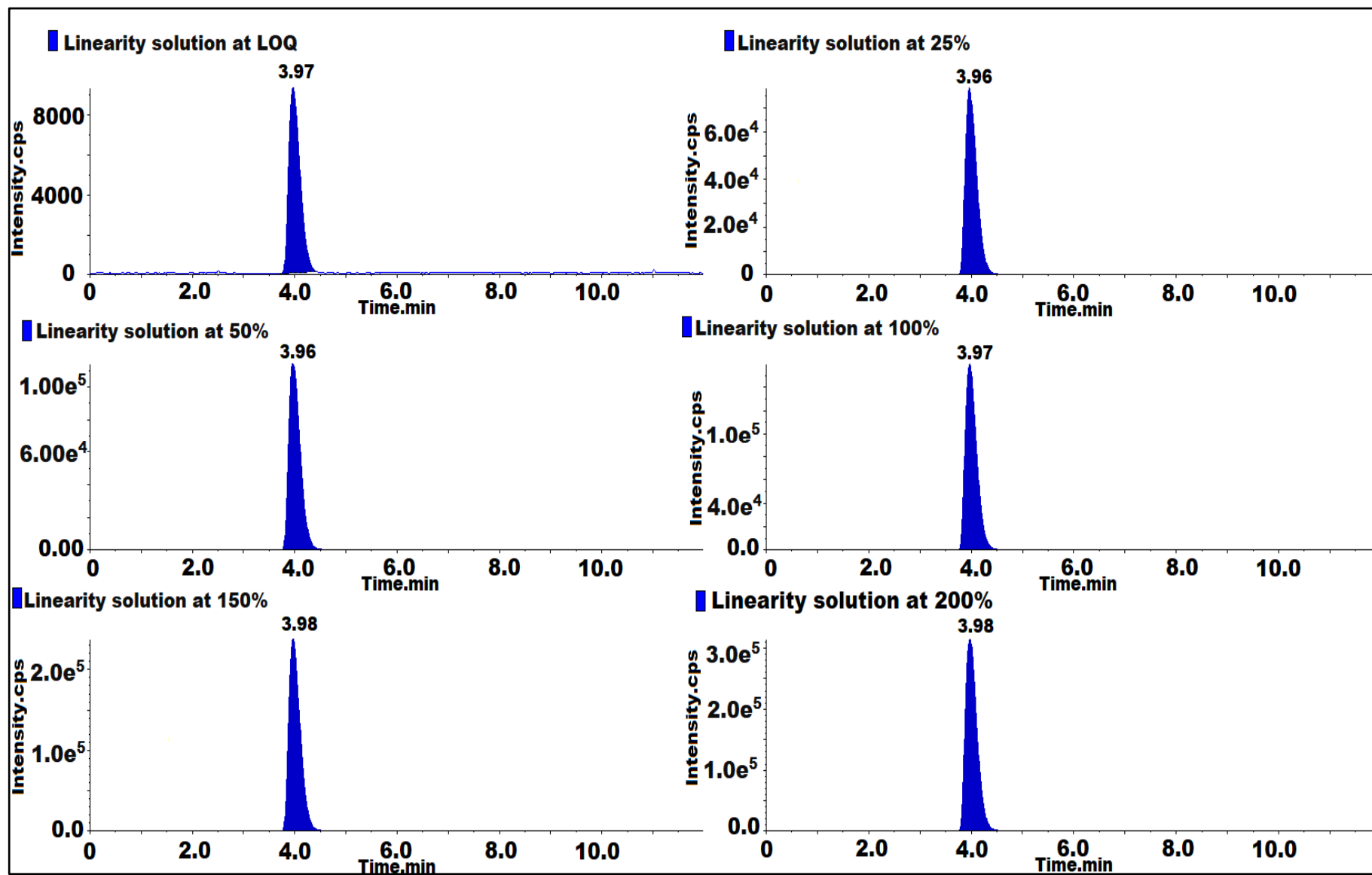


Figure S8: MS/MS chromatograms of Linearity solution.

4. Method precision

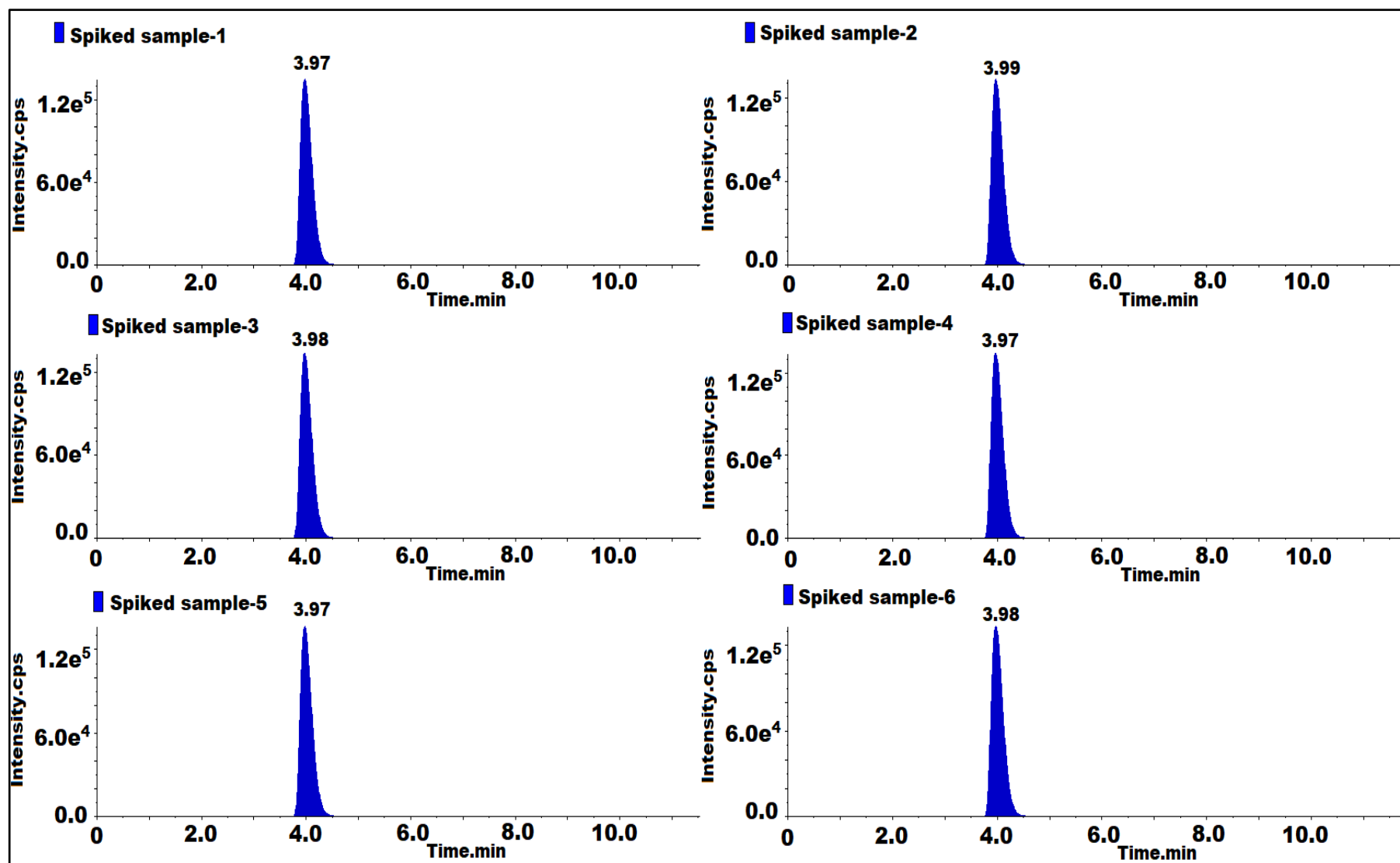


Figure S9: MS/MS chromatograms of Method precision.

5. Intermediate precision

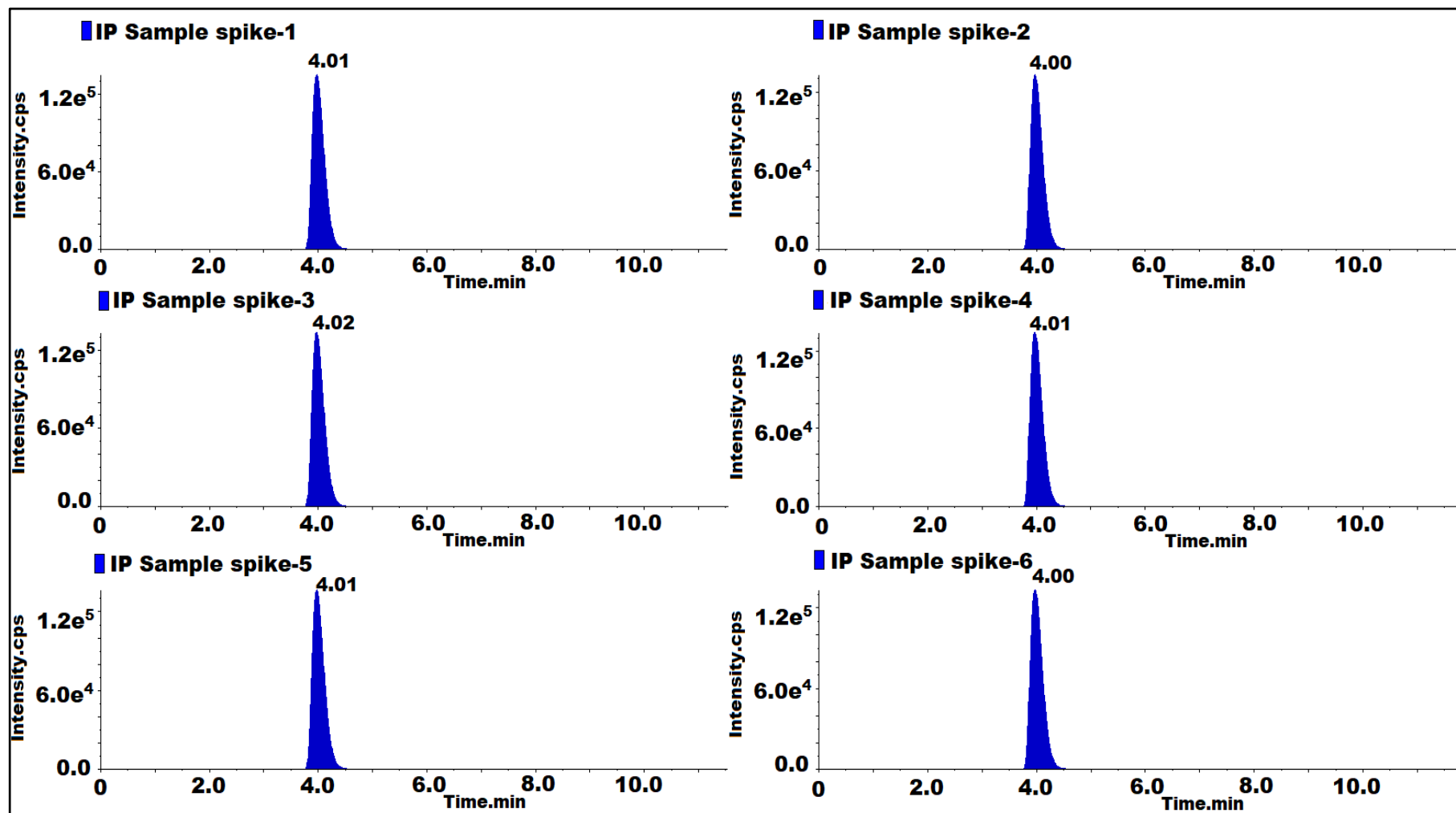


Figure S10: MS/MS chromatograms of Intermediate precision.

6. Accuracy

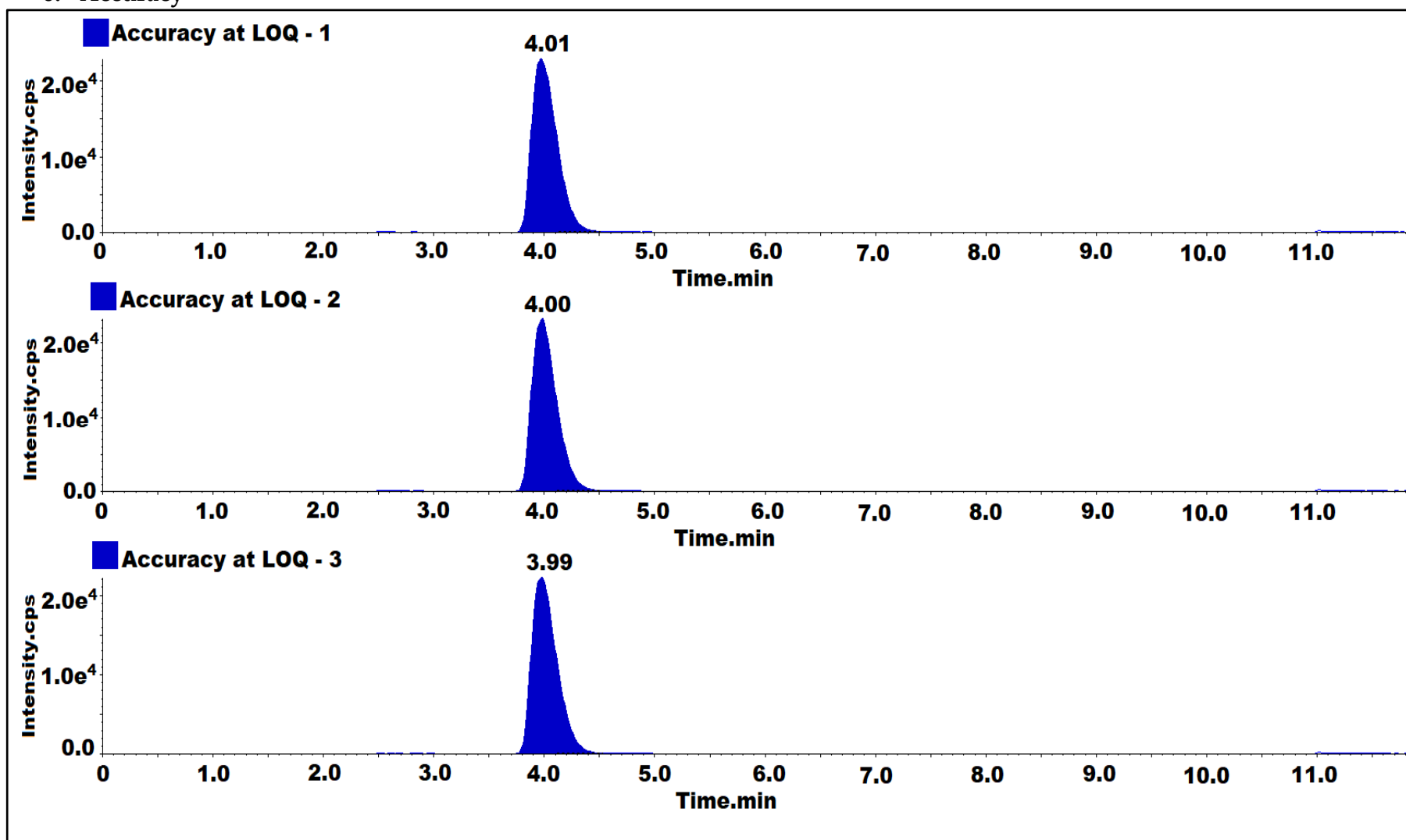


Figure S11: MS/MS chromatograms of LOQ Accuracy.

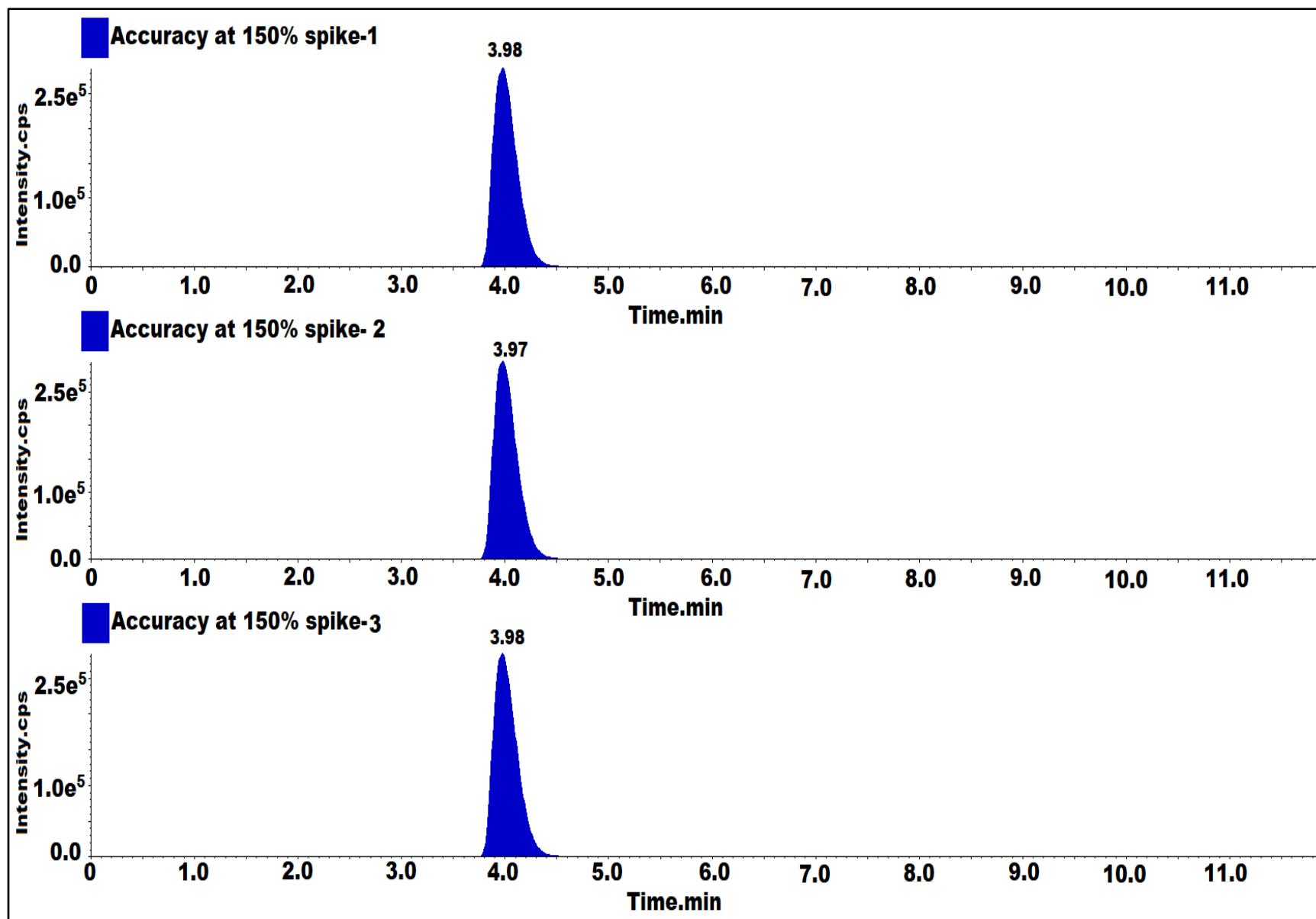


Figure S12: MS/MS chromatograms of LOQ Accuracy.

7. Solution stability

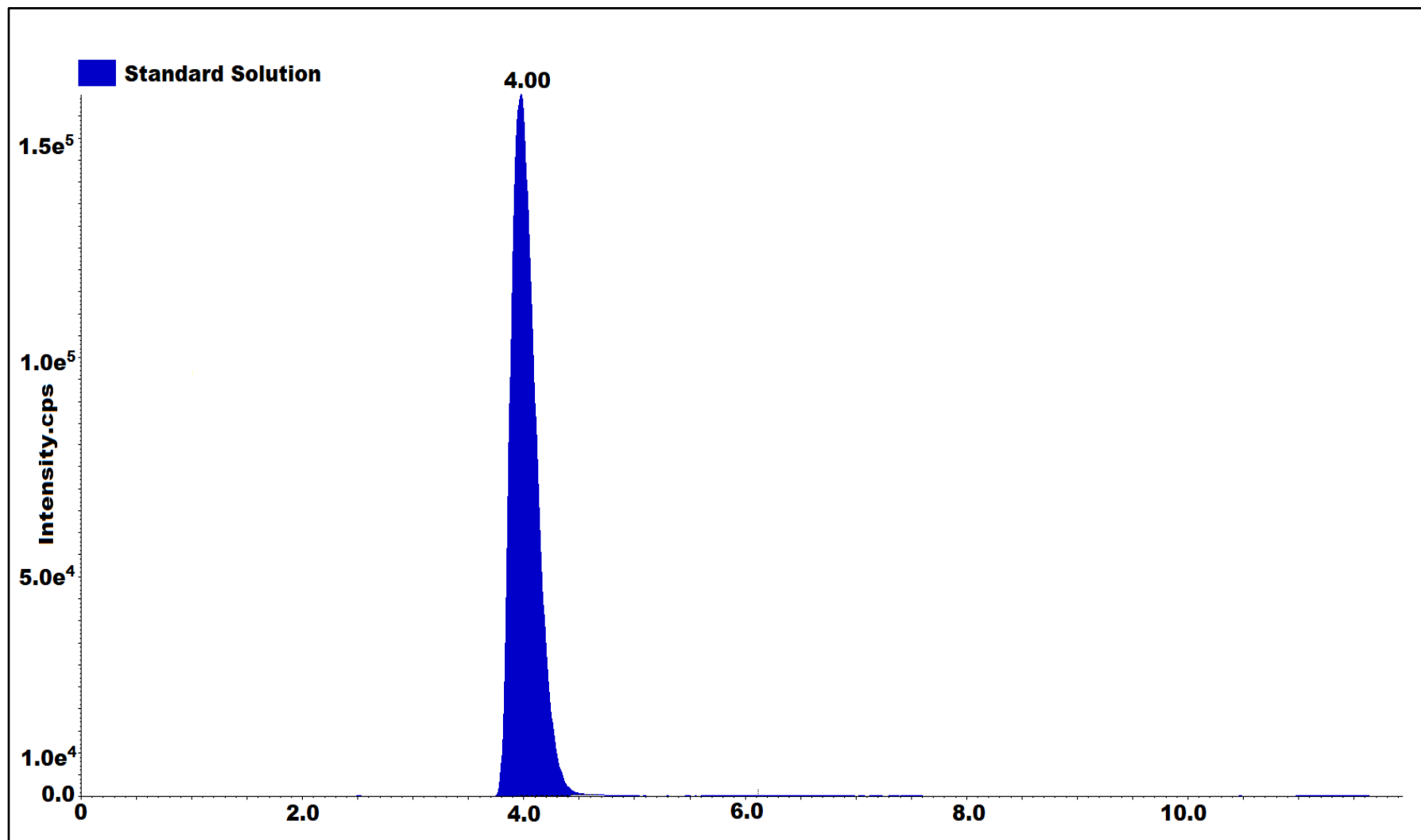


Figure S13: MS/MS chromatograms of Standard solution stability.

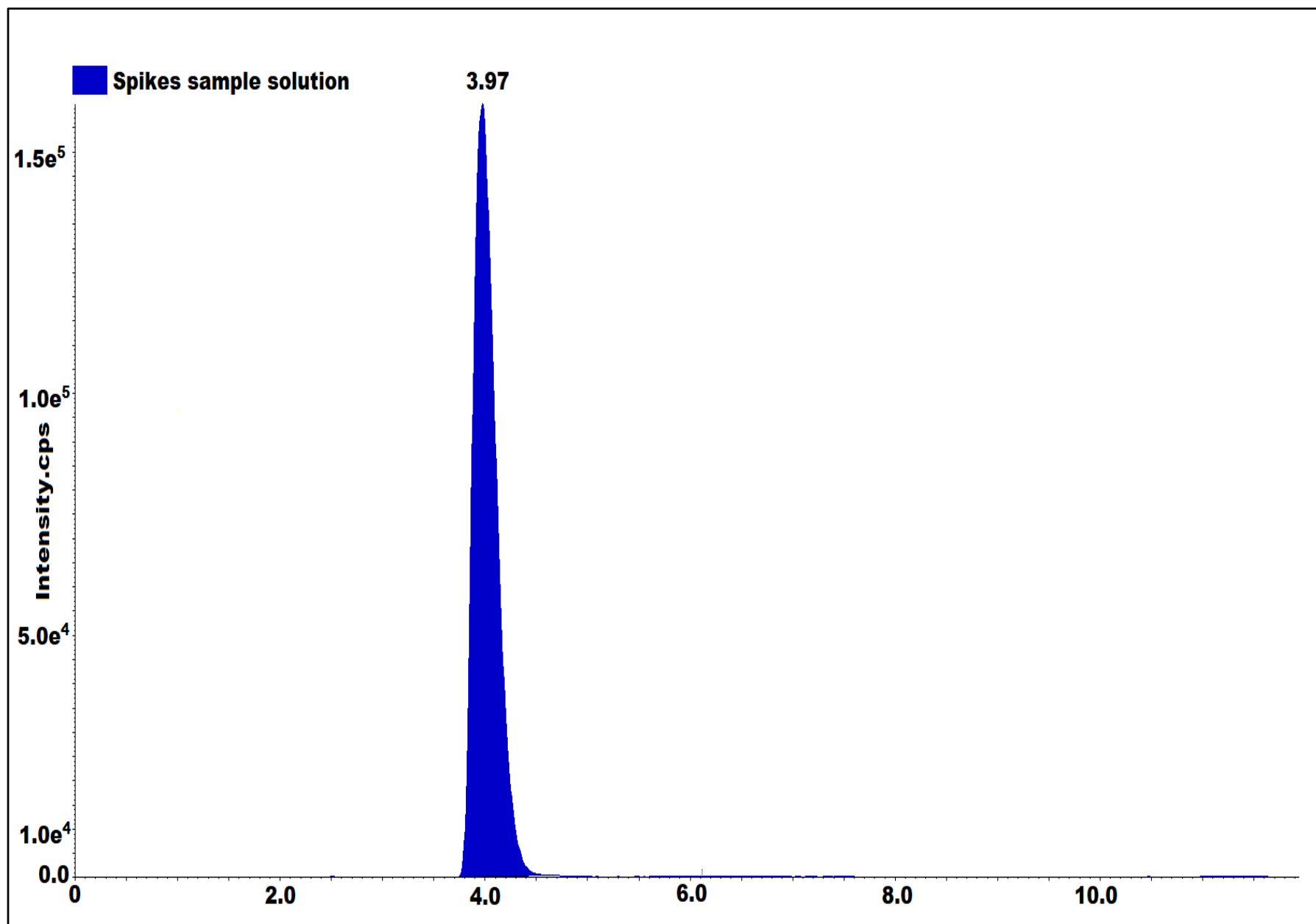


Figure S14: MS/MS chromatograms of Spiked sample solution stability.

8. Sitagliptin 7-nitroso impurity standard Qualification data

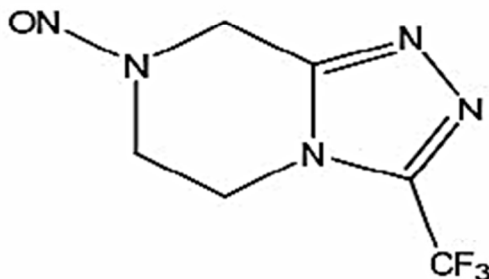
| CERTIFICATE OF ANALYSIS | | | |
|-----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|------------------|-------------------|
| Product Name | 7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrazine | | |
| IUPAC Name | 7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrazine | | |
| Synonyms | Not available | | |
| CAS # | Not available | | |
| Molecular Formula | C ₆ H ₆ F ₃ N ₅ O | Molecular weight | 221.14 |
| Longterm Storage Conditions | Store at refrigerator (2-8°C) for long term storage | | |
| Shipping Conditions | Product is stable to be shipped at room temperature | | |
| <div></div> | | | |
| Test | Specification | | Result |
| Appearance | Light Green Solid | | Light Green Solid |
| Solubility | Soluble in DMSO | | In DMSO |
| ¹ H NMR spectrum | Conform structure | | Confirms |
| Mass Spectrum | Conform molecular weight | | Confirms |
| Purity by HPLC - c | Not less than 90% | | 99.51% |
| Volatiles content % (by TGA) - b | Not specified | | 0.50% (w/w) |
| Ash content % (by TGA) - a | Not specified | | 1.39% (w/w) |
| Potency based on Mass balance $\{[100 - (b+a)] \times (c/100)\}$ | Not specified | | 97.63% |

Figure S15: 7-nitroso impurity Certificate of analysis.

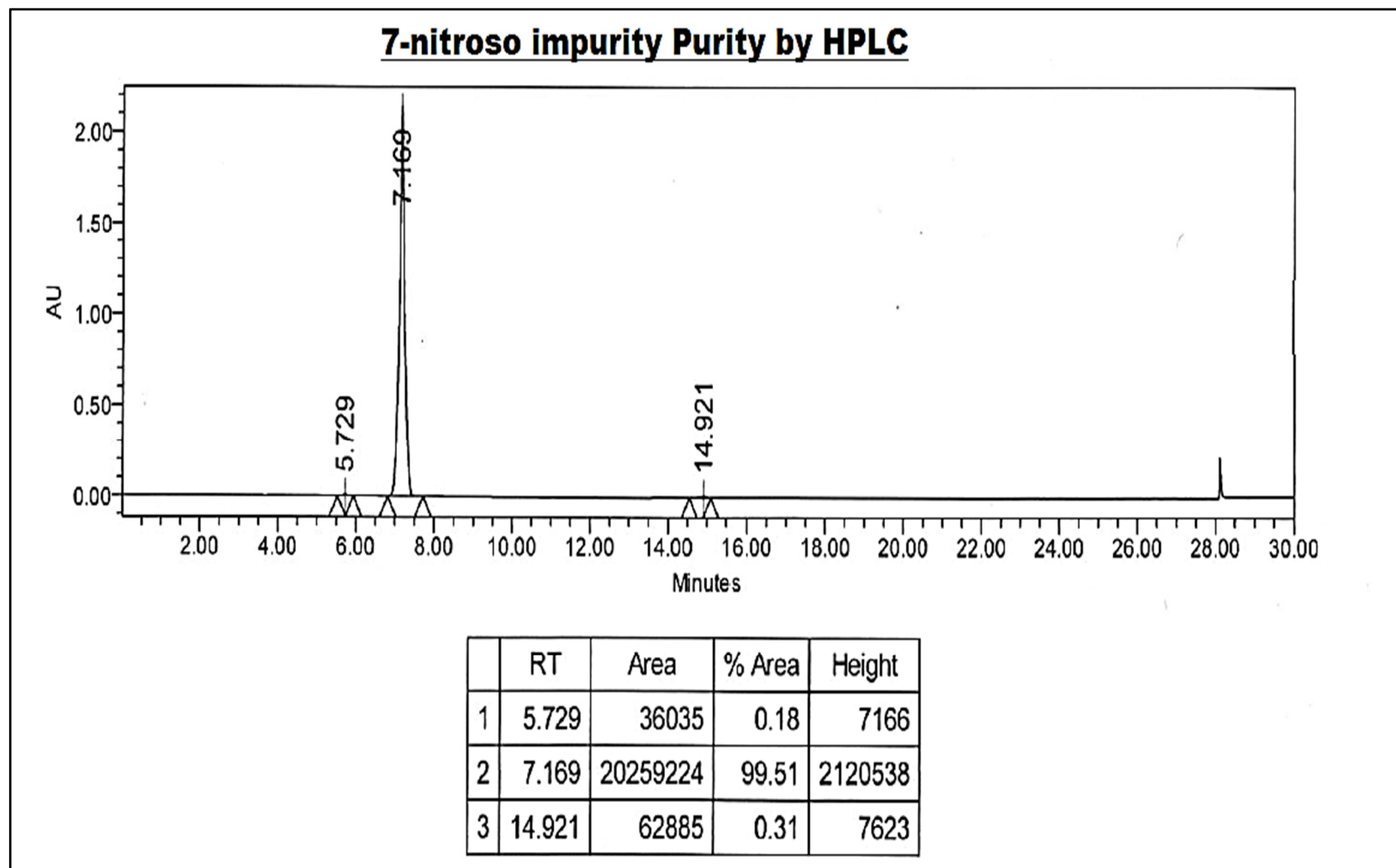


Figure S16: 7-nitroso impurity purity by HPLC chromatogram.

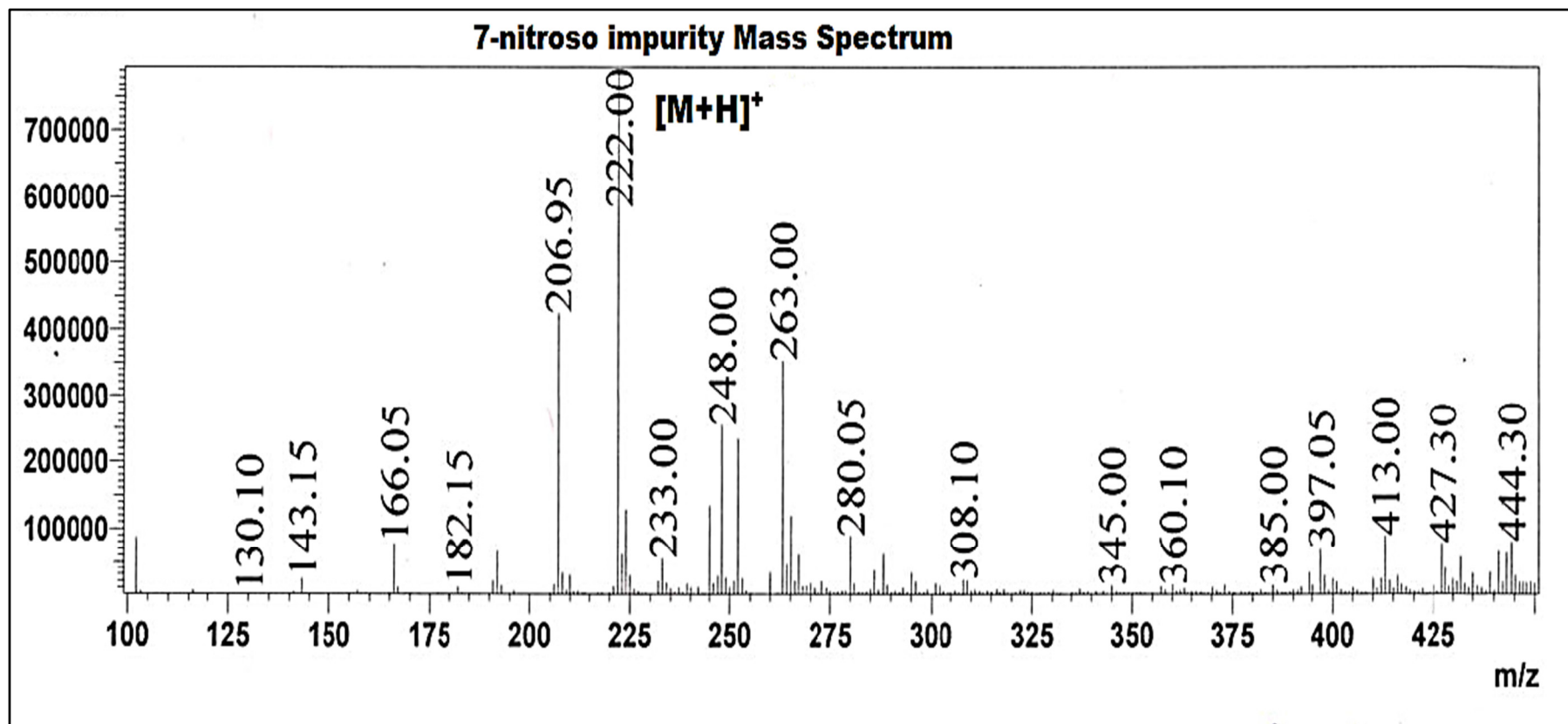


Figure S17: 7-nitroso impurity Mass spectrum.

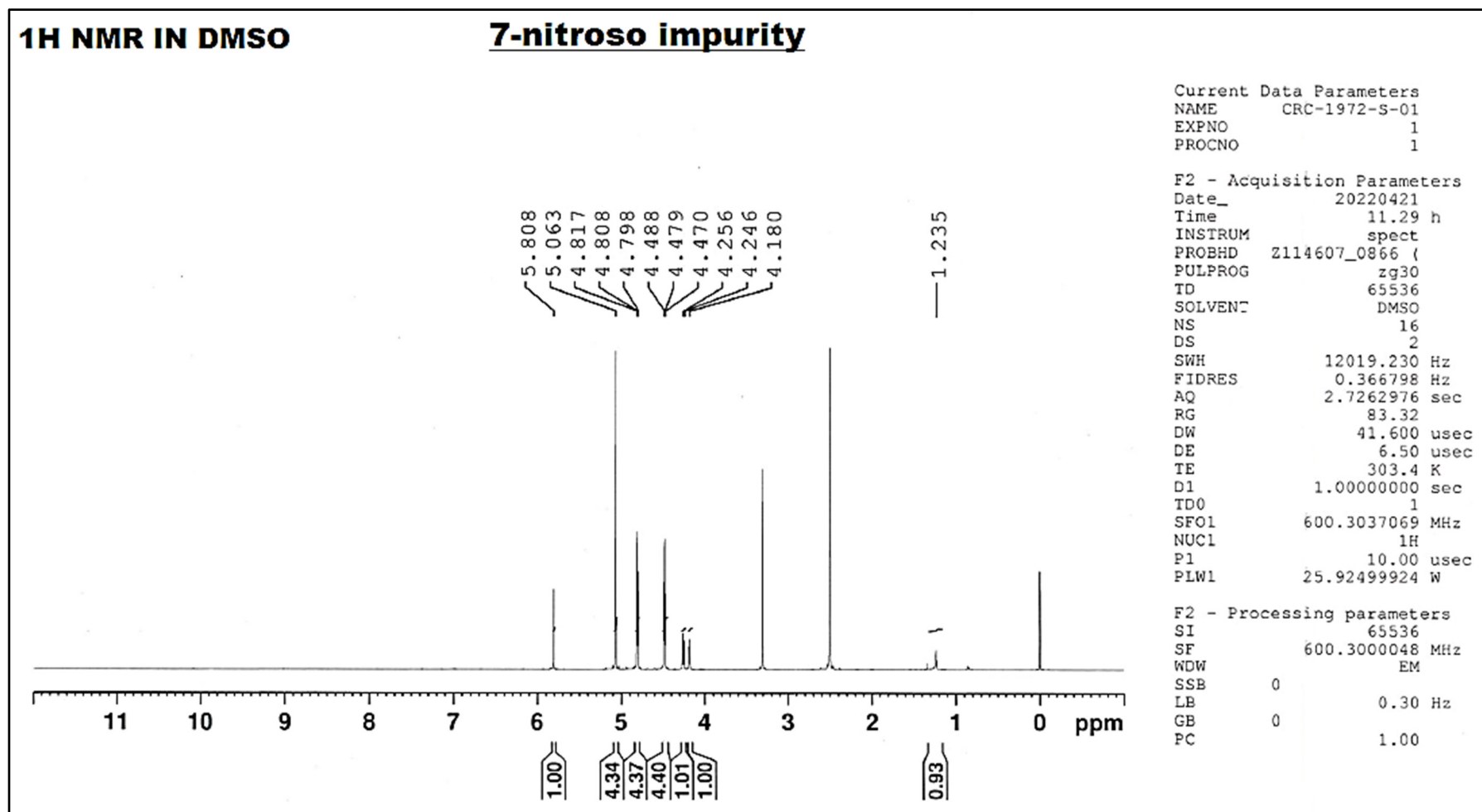


Figure S18: 7-nitroso impurity 1H NMR spectrum – 1.

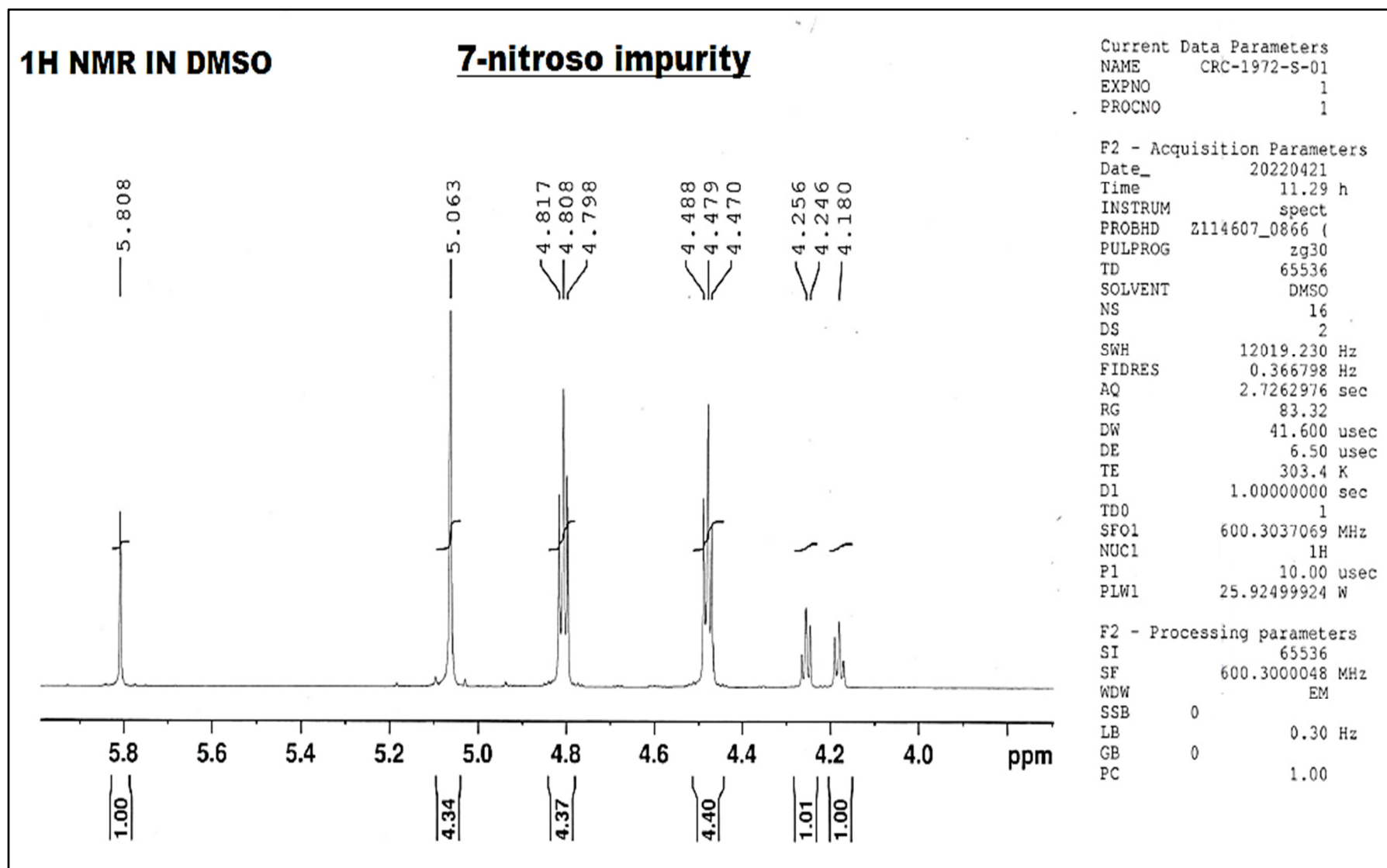


Figure S19: 7-nitroso impurity 1H NMR spectrum – 2.

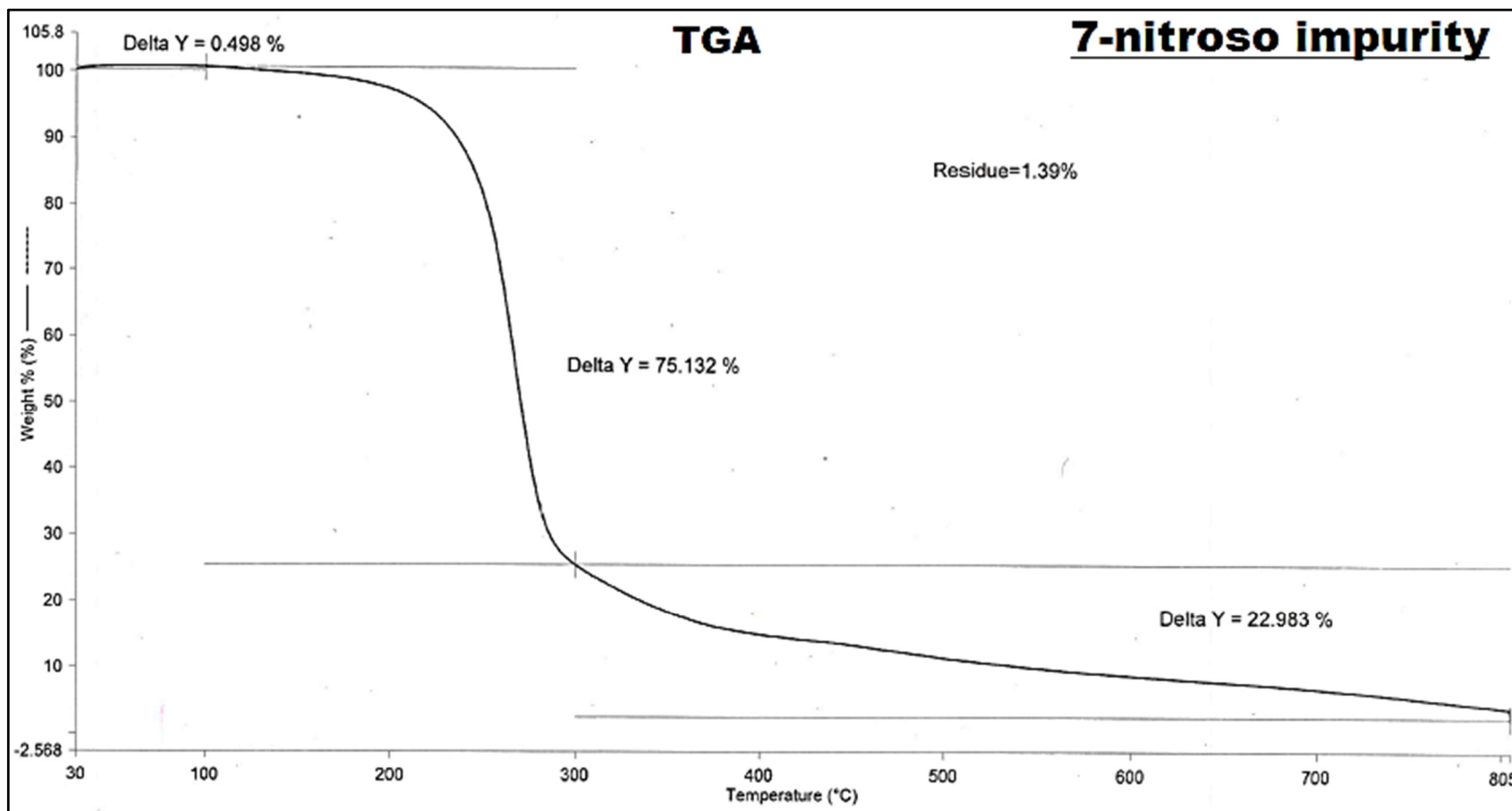


Figure S20: 7-nitroso impurity TGA graph.

7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4] triazolo[4,3-a] pyrazine probable parsing pyrolysis fragmentation.

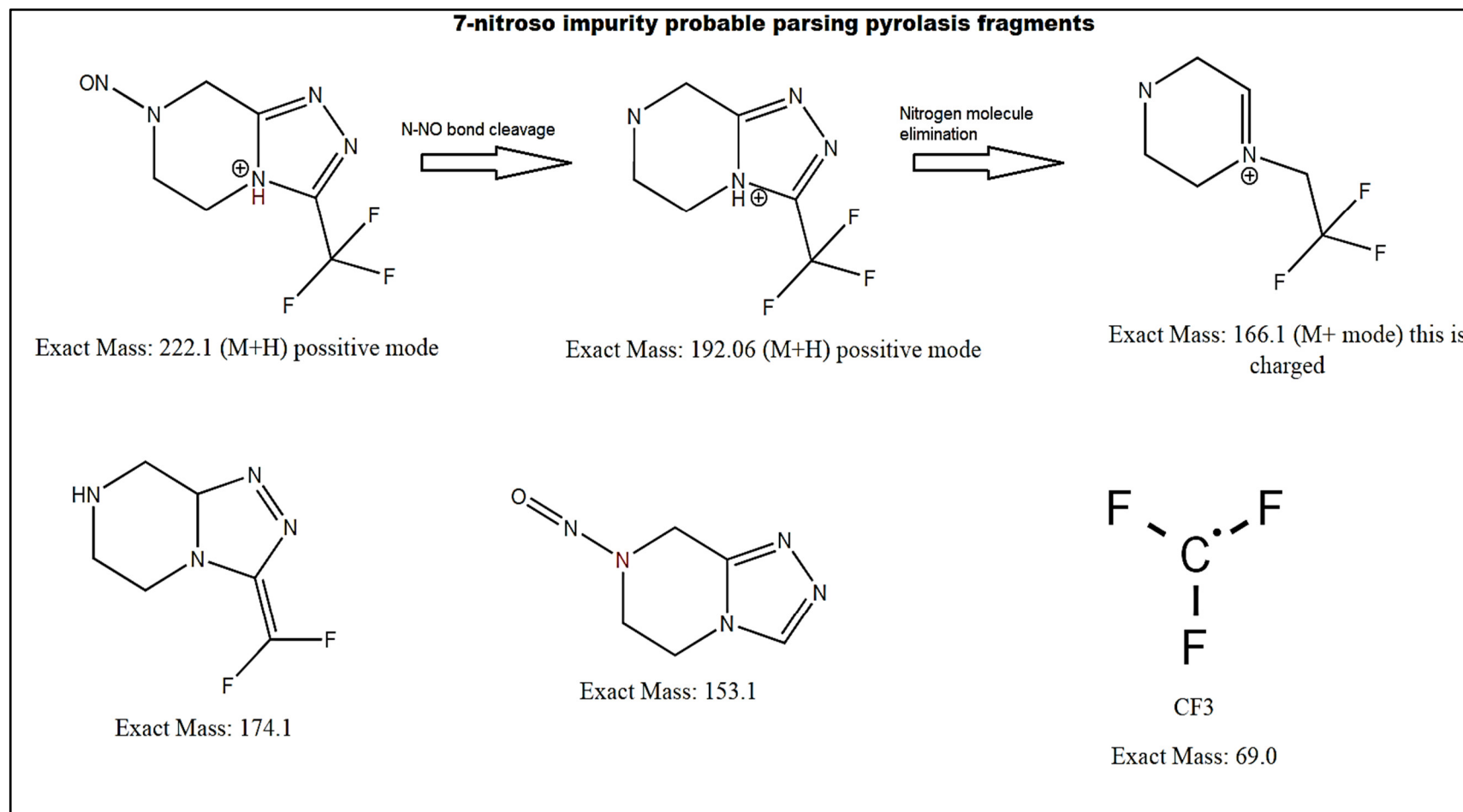


Figure S21: Probable Fragmentation pattern of 7-nitroso impurity in the mass ionization chamber due to parsing pyrolysis.