Application Note

Pharmaceuticals

Agilent

Trusted Answers

Low-Level Quantitation of N-Nitroso Dabigatran Etexilate Impurity in Dabigatran Etexilate Mesylate API Using the Agilent 6495C LC/TQ

Authors

Preeti Bharatiya,
Prasanth Joseph,
Vivek Dhyani, Saikat Banerjee,
and Kannan Balakrishnan
Agilent Technologies, Inc.

Abstract

A highly specific, sensitive, and reproducible method was developed for the quantitation of N-nitroso dabigatran etexilate impurity in dabigatran etexilate mesylate drug substance using an Agilent 6495C triple quadrupole LC/MS (LC/TQ) coupled to an Agilent 1290 Infinity II LC. Sensitivity at the picogram (pg) level was achieved by optimizing multiple reaction monitoring (MRM) parameters and source parameters. The developed MRM method demonstrated the limits of detection (LOD) and quantitation (LOQ) as 6 and 10 pg/mL, respectively, which corresponds to 0.0012 and 0.002 ppm with respect to a test concentration of 5 mg/mL. The method was found to be linear within the range of 10 to 1,000 pg/mL, with a regression coefficient of 0.9993 with linear regression and $1/x^2$ as the weighting factor. The analyte peak was free from interference and was well separated from the active pharmaceutical ingredient (API), highlighting the specificity of the method. Impurity in samples was confirmed by comparing analyte quantifier to qualifier ion ratios with that of the standard.

Introduction

Nitrosamines are of concern as most of them have been reported to be potent mutagens in rodents, and they are potential carcinogens. Therefore, it is important to control these impurities at or below the stipulated specification limits for nitrosamine analysis. API-derived complex nitrosamines are known as nitrosamine drug substance-related impurities (NDSRIs). Multiple recalls of pharmaceutical drug products due to the presence of NDSRIs have drawn the attention of regulators and manufacturers. By considering the complexity of the global pharmaceutical supply chain, manufacturers must be more diligent to protect consumers by screening their APIs and drug products for the presence of NDSRIs. NDSRIs can be formed from both secondary and tertiary amines. Secondary amines can easily undergo nitrosation in the presence of trace amounts of acid. In the case of tertiary amines, this reaction is possible due to nitrosative cleavage or dealkylation.[1] Dabigatran etexilate ($C_{34}H_{41}N_7O_5$) is a prodrug of dabigatran ($C_{25}H_{25}N_7O_3$). The mesylate salt of dabigatran etexilate is known to be therapeutically active and is widely used for the prevention of stroke, atrial fibrillations, and systemic embolism.[2] Dabigatran etexilate mesylate (1:1) salt of the prodrug is commercially marketed as an oral, solid capsule in the U.S. and Europe under the name Pradaxa.

Dabigatran etexilate mesylate ($C_{35}H_{45}N_7O_8S$) is a yellow-white powder. It has a solubility of 1.8 mg/mL in water and is freely soluble in methanol. For most NDSRIs, analytical methods are not yet available, so manufacturers will need to develop, qualify, and validate a method that is suitable for the sample matrix and specific for the nitrosamines in question.[3] The analytical method developed must be sensitive enough to quantify the nitrosamines of interest down to a level that corresponds to 10% of the specification limit.[4] In this application note, a highly specific, sensitive, and reproducible method was developed for the quantitation of N-nitroso dabigatran etexilate impurity (pictured in Figure 1) in dabigatran etexilate mesylate drug substance using a 6495C triple quadrupole LC/MS (LC/TQ) coupled to a 1290 Infinity II LC.

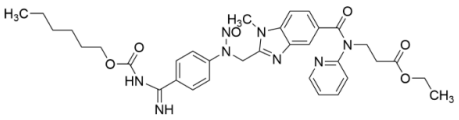

Figure 1. Structure of N-nitroso dabigatran etexilate impurity.

LC/MS/MS is an inherently selective and sensitive analytical technique that is well suited to identify and quantify mutagenic impurities at exceptionally low levels, and has been adopted widely in the pharmaceutical industry.[5] MRM mode was used for quantification as it selectively filters the precursor and product ions of the compound of interest, thus increasing the sensitivity and selectivity of the analysis. The Agilent Jet Stream (AJS) ionization source used in this application works by using thermal gradient focusing technology, which helps to increase the sensitivity of the instrument, reduce the contamination of the interface region, and reach lower detection limits with consistent results over multiple long batches.

Experimental

Chemicals and reagents

N-nitroso dabigatran etexilate impurity standard and dabigatran etexilate API samples were obtained from a pharmaceutical company. Other LC/MS-grade solvents—methanol (MeOH), water, and acetonitrile (ACN)—were purchased from Honeywell (Charlotte, NC, USA). Ammonium trifluoroacetate and formic acid were purchased from Sigma-Aldrich (Kansas, USA).

Instrument configuration

Configurations and parameters used for the HPLC are listed in Table 1. Parameters related to the compound and triple quadrupole MS are in Tables 2 and 3, respectively. Finally, preparation of the working standard solution for plotting the calibration curve is outlined in Table 4.

Table 1. LC configurations and parameters.

Parameter	Value
Instrument	Agilent 6495C LC/TQ coupled to an Agilent 1290 Infinity II LC
Needle Wash	A) MeOH:water (80:20) B) Water:MeOH (80:20)
Sample Diluent	MeOH:ACN (90:10, v/v) with 0.05% NH_4OH (of strength 25%)
Multisampler Temperature	10 °C
Injection Volume	7 µL
Analytical Column	Agilent Infinity Lab Poroshell HPH-C18, 150 × 3.0 mm, 2.7 µm (p/n 693975-502T)
Column Temperature	40 °C
Mobile Phase A	1 mM ammonium trifluoroacetate with formic acid in water
Mobile Phase B	MeOH: ACN
Flow Rate	0.5 mL/min
Run Time	20 min
Gradient	Time (min) %A %B 0 55 45 3 55 45 6 40 60 13 40 60 15 15 85 17 15 85 17.1 55 45 20 55 45

Table 2. Compound-related parameters in MRM mode.

Precursor Ion (*m/z*)	Product Ion (*m/z*)	Dwell (ms)	Fragmentor (V)	CAV	CE	Polarity
657.3	364.1	100	166	4	16	Positive
657.3	433.2	100	166	4	24	Positive
657.3	627.3	100	166	4	20	Positive

Table 3. Triple quadrupole MS source parameters.

Source/Gas Parameter	Value
Gas Flow	12 L/min
Gas Temperature	250 °C
Sheath Gas Flow	12 L/min
Sheath Gas Temperature	400 °C
iFunnel High Pressure RF	150 V
Polarity	Positive
Nozzle Voltage	2,000 V
Capillary Voltage	3,200 V
Nebulizer Pressure	35 psi
iFunnel Low Pressure RF	60 V

Table 4. Working standard solution preparation for plotting the calibration curve.

Stock ID	Stock Conc. (ng/mL)	Volume of Stock (mL)	Volume of Diluent (mL)	Final Volume (mL)	Final Conc. (ng/mL)	Working Standard (ID)
Stock	1,000,000.000	0.050	4.95	5.00	10,000.000	WS 1
WS 1	10,000.000	0.080	3.92	4.00	200.000	WS 2
WS 2	200.000	0.400	3.60	4.00	20.000	WS 3
WS 3	20.000	2.000	2.00	4.00	10.000	STD-I
STD-I	10.000	2.000	2.00	4.00	5.000	STD-H
STD-H	5.000	1.600	2.40	4.00	2.000	STD-G
STD-G	2.000	2.000	2.00	4.00	1.000	STD-F
STD-F	1.000	2.000	2.00	4.00	0.500	STD-E
STD-E	0.500	0.800	3.20	4.00	0.100	STD D (SPEC)
SPEC	0.100	2.000	2.00	4.00	0.050	STD-C
STD-C	0.050	1.600	2.40	4.00	0.020	STD-B
STD-B	0.020	2.000	2.00	4.00	0.010	STD A (LOQ)
LOQ	0.010	2.200	1.80	4.00	0.006	LOD

Sample preparation

Specification limit: The following calculation was used to determine the specification limit, where "MDD" represents maximum daily dose in mg, and "AI" represents acceptable intake:

Specification limit = AI ÷ MDD

The AI for N-nitroso dabigatran etexilate is 400 ng/day.[1] A dabigatran etexilate mesylate capsule at a dose of 150 mg, given orally, twice daily, gives an MDD of 300 mg. Therefore, the limit of N-nitroso dabigatran etexilate (ppm) was calculated as:

400 ng/day ÷ 300 mg/day = 1.33 ppm

This corresponds to 6.65 ng/mL absolute concentration with the API test concentration of 5 mg/mL.

The stock solution of dabigatran impurity was prepared by weighing 5 mg of the standard substance and dissolving it with 5 mL of MeOH to obtain a concentration of 1 mg/mL. The stock solution was stored between 2 and 8 °C and protected from light.

Preparation of working standard solution: Working standards were prepared with the diluent, and pH adjusted using NH_4OH to give a final concentration range of 0.01 to 1 ng/mL for the calibration plot. An optimized diluent was selected after studying the dabigatran etexilate mesylate API stability and degradation conditions.

Preparation of API sample solution: The dabigatran etexilate API drug sample was stored with desiccant at a temperature between 2 and 8 °C in the dark. API samples of 20 mg were dissolved in 4 mL of diluent in a volumetric flask, then vortexed at 2,000 rpm for 10 minutes.

Recovery sample preparation: Impurity standard solutions were spiked into samples to obtain a final concentration of 0.1 ng/mL in three different lots of APIs (sample reference, sample A, and sample B).

Data acquisition and data analysis

All samples were acquired using Agilent MassHunter acquisition software, version 12.0. MRM transitions were selected and compound parameters were optimized using the MassHunter Optimizer software within MassHunter acquisition software. A standard solution with a concentration of 100 ng/mL was introduced to the MS by Flow Injection Analysis with an injection volume of 5 µL. Method development was performed in positive ionization mode using the AJS ionization source for the optimization of compound parameters for N-nitroso dabigatran etexilate, including precursor ions, product ions, and collision energies. Through the automated workflow, three product ions for the impurity were selected for the creation of MRM based on their intensity. Gas temperatures, gas

flows, and dwell times were optimized to obtain the desired sensitivity and specificity. Instrument MRM parameters (Table 2) and source parameters (Table 3) were optimized to maximize sensitivity, while maintaining consistency in the method performance for large batches. Chromatograms were viewed using Agilent MassHunter Qualitative Analysis software. Quantitation of each batch was carried out using Agilent MassHunter Quantitative Analysis software, version 12.0.

Results and discussion

The stability of API solutions was studied by performing the analysis and observing any change in the chromatographic pattern compared with freshly prepared sample solutions. The mesylate salt of dabigatran etexilate provides an acidic microenvironment to the drug, which makes it susceptible to nitrosation in the presence of water or trace amount of moisture.[6] Therefore, an appropriate diluent was selected and optimized for accurate analysis.

To obtain the best chromatographic conditions, the mobile phase was optimized to provide sufficient selectivity and sensitivity and to achieve better resolution between the impurity and the API. Reversed-phase chromatography was performed using an Agilent Poroshell HPH-C18 column (3 × 150 mm, 2.7 µm), with a mobile phase consisting of ammonium trifluoroacetate and formic acid in water as aqueous phase, and a mixture of MeOH and ACN (80:20, v:v) as the organic phase. A typical chromatogram obtained by the proposed LC method, demonstrating the resolution between API and impurity with the symmetrical peak for N-nitroso dabigatran etexilate, is shown in Figure 2.

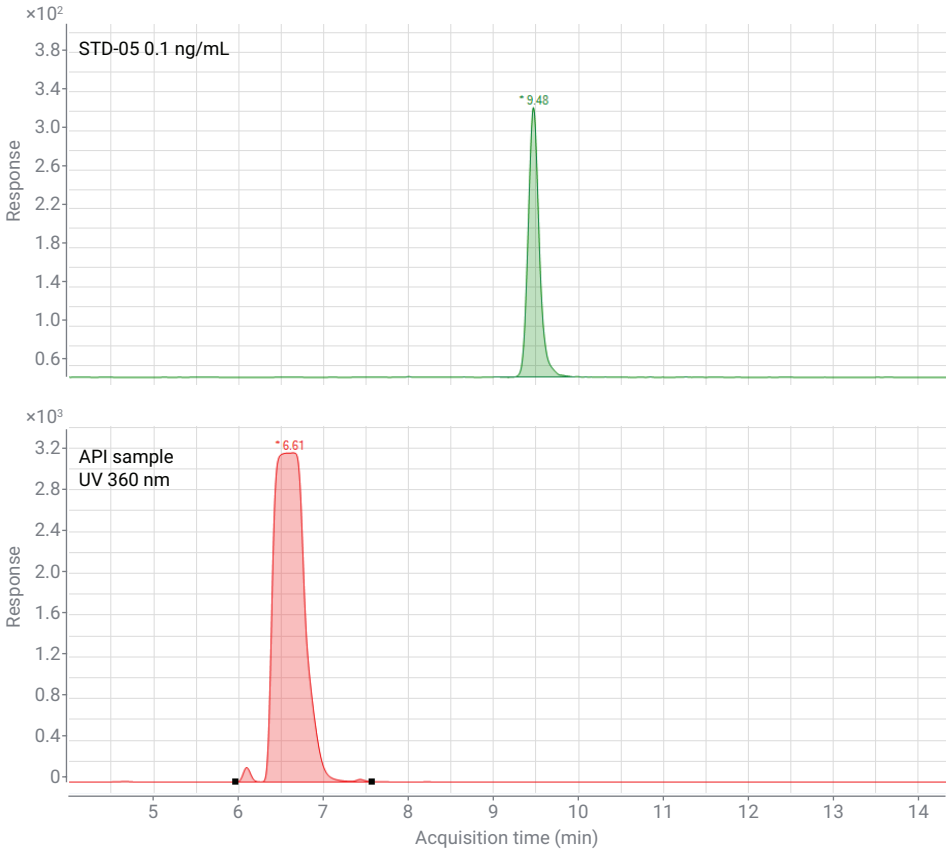

Figure 2. Representative MRM chromatogram of N-nitroso dabigatran etexilate impurity (0.1 ng/mL) compared to a diode array detector (DAD) chromatogram for dabigatran etexilate (API) sample.

A DAD was used to identify the API absorbance wavelength. UV chromatograms were acquired (as shown in Figure 2) to identify the retention times of higher concentrations of API, which were diverted to waste to avoid MS contamination. High concentrations of dabigatran etexilate API were diverted to waste using the built-in diverter valve and the diverter valve program shown in Table 5.

Table 5. Diverter valve program.

Start Time (min)	Scan Type	Diverter Valve
0	MRM	To waste
8.2	MRM	To MS
13	MRM	To waste
33	MRM	To MS

Critical method development parameters such as sensitivity, linearity, specificity, reproducibility, and recovery, were established. Sensitivity parameters, LOD and LOQ, were established, as defined by ICH guidelines, where the signal-to-noise (S/N) values were 3.3 and 10 for the LOD and LOQ, respectively. Calibration curves constructed for N-nitroso dabigatran were found to be linear within the 0.01 to 1.0 ng/mL (0.002 to 0.2 ppm with respect to API) concentration range. The value of R^2 was 0.9993 for the equation $y = mx + c$, where m is the slope and c is the intercept with $1/x^2$ as the weighting factor, as shown in Figure 4.

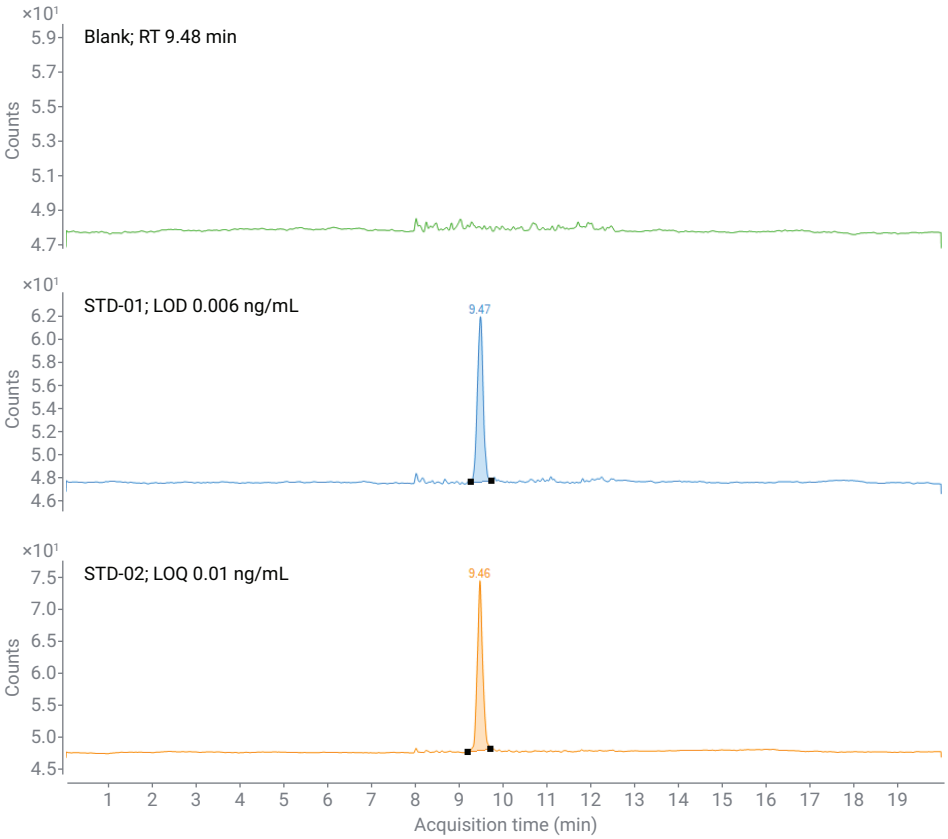

Figure 3. Representative MRM chromatogram of LOD 0.006 ng/mL (0.0012 ppm) and LOQ 0.01 ng/mL (0.002 ppm) with S/N > 70:1 and S/N > 190:1 respectively, calculated using root mean square (rms).

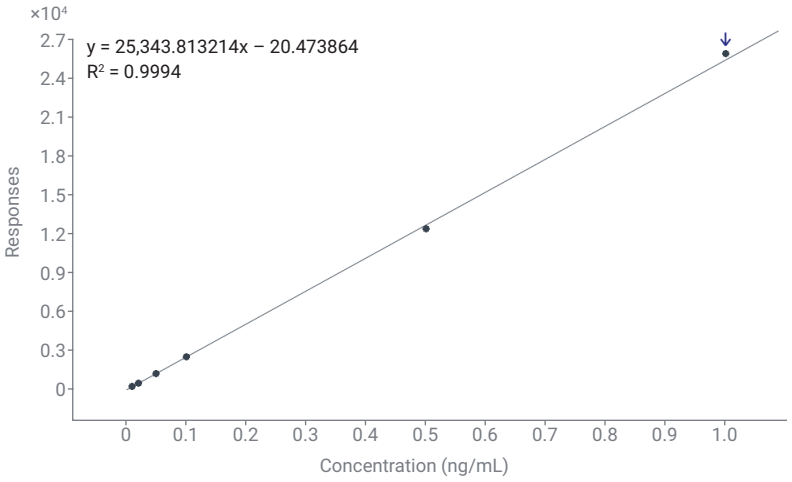

Figure 4. Calibration curve generated for N-nitroso dabigatran etexilate from 0.01 to 1.0 ng/mL.

The accuracy was evaluated by the determination of the analyte in solutions of different concentration levels, which were prepared and used for plotting the calibration curve. The accuracy in standard solutions was found to be 80 to 120%, which is within the acceptance criteria, as shown in Figure 5. A quantitative batch was created including the calibration curve standards, bracketing standard, as such sample and recovery samples where an integration parameter remained constant through standard, and sample as shown in Figure 6.

Batch Table

Sample: Blank-03 Sample Type: <All> Compound: N-NITROSODABIGATRAN

Sample				N-NITROS...	N-NITROSODABIGATRAN Results				
Name	Type	Level	Amt.	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy
Blank-01	Blank				9.27	1	0.00	0.001	
STD-01	CC		5	0.0060	9.47	132	0.01	0.001	
STD-02	Cal	1	5	0.0100	9.46	230	0.01	0.002	98.9
STD-03	Cal	2	5	0.0200	9.46	500	0.02	0.004	102.7
STD-04	Cal	3	5	0.0500	9.47	1227	0.05	0.010	98.5
STD-05	Cal	4	5	0.1000	9.47	2526	0.10	0.020	100.5
STD-06	Cal	5	5	0.5000	9.47	12327	0.49	0.097	97.4
STD-07	Cal	6	5	1.0000	9.46	25831	1.02	0.204	102.0
Blank-02	Blank				9.54	2	0.00	0.001	

Figure 5. Concentration of standard (0.01 to 1.0 ng/mL) plotted for the calibration curve.

Batch Table

Sample: Blank-01 Sample Type: <All> Compound: N-NITROSODABIGATR

Sample				N-NITROS...	N-NITROSODABIGATRAN Results			
Name	Type	Level	Amt.	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.
Blank-01	Blank				9.27	1	0.001	0.001
STD-01	CC		5	0.0060	9.47	132	0.006	0.001
STD-02	Cal	1	5	0.0100	9.46	230	0.010	0.002
STD-03	Cal	2	5	0.0200	9.46	500	0.021	0.004
STD-04	Cal	3	5	0.0500	9.47	1227	0.049	0.010
STD-05	Cal	4	5	0.1000	9.47	2526	0.100	0.020
STD-06	Cal	5	5	0.5000	9.47	12327	0.487	0.097
STD-07	Cal	6	5	1.0000	9.46	25831	1.020	0.204
Blank-02	Blank				9.54	2	0.001	0.001
CONTROL-SAMPLE-REF-R1	Sample		5		9.48	1789	0.071	0.014
CONTROL-SAMPLE-REF-R2	Sample		5		9.49	1742	0.070	0.014
CONTROL-SAMPLE-REF-R3	Sample		5		9.48	1709	0.068	0.014
CONTROL-SAMPLE-A-R1	Sample		5		9.49	1607	0.064	0.013
CONTROL-SAMPLE-A-R2	Sample		5		9.48	1608	0.064	0.013
CONTROL-SAMPLE-A-R3	Sample		5		9.48	1557	0.062	0.012
CONTROL-SAMPLE-B-R1	Sample		5		9.49	2189	0.087	0.017
CONTROL-SAMPLE-B-R2	Sample		5		9.48	2183	0.087	0.017
CONTROL-SAMPLE-B-R3	Sample		5		9.48	2169	0.086	0.017
STD-05	QC	5		0.5000	9.48	2466	0.098	0.098
REC-SAMPLE-REF-R1	Sample		5		9.49	4249	0.168	0.034
REC-SAMPLE-REF-R2	Sample		5		9.49	4248	0.168	0.034
REC-SAMPLE-REF-R3	Sample		5		9.49	4370	0.173	0.035
REC-SAMPLE-A-R1	Sample		5		9.49	4181	0.166	0.033
REC-SAMPLE-A-R2	Sample		5		9.49	4196	0.166	0.033
REC-SAMPLE-A-R3	Sample		5		9.49	4272	0.169	0.034
REC-SAMPLE-B-R1	Sample		5		9.49	4864	0.193	0.039
REC-SAMPLE-B-R2	Sample		5		9.49	4863	0.193	0.039
REC-SAMPLE-B-R3	Sample		5		9.49	4942	0.196	0.039
STD-05	QC	5		0.5000	9.48	2428	0.097	0.097
REC-SAMPLE-REF-P2-R1	Sample		5		9.49	4065	0.161	0.032
REC-SAMPLE-REF-P2-R2	Sample		5		9.49	4063	0.161	0.032
REC-SAMPLE-REF-P2-R3	Sample		5		9.48	4134	0.164	0.033
REC-SAMPLE-A-P2-R1	Sample		5		9.49	4344	0.172	0.034
REC-SAMPLE-A-P2-R2	Sample		5		9.49	4136	0.164	0.033
REC-SAMPLE-A-P2-R3	Sample		5		9.49	4178	0.166	0.033
REC-SAMPLE-B-P2-R1	Sample		5		9.49	4849	0.192	0.038
REC-SAMPLE-B-P2-R2	Sample		5		9.49	4845	0.192	0.038
REC-SAMPLE-B-P2-R3	Sample		5		9.49	4798	0.190	0.038
STD-05	QC	5		0.5000	9.48	2431	0.097	0.097
CONTROL-SAMPLE-P2-REF-R1	Sample		5		9.48	1806	0.072	0.014
CONTROL-SAMPLE-P2-REF-R2	Sample		5		9.49	1820	0.073	0.015
CONTROL-SAMPLE-P2-REF-R3	Sample		5		9.50	1821	0.073	0.015
CONTROL-SAMPLE-P2-A-R1	Sample		5		9.49	1584	0.063	0.013
CONTROL-SAMPLE-P2-A-R2	Sample		5		9.49	1595	0.064	0.013
CONTROL-SAMPLE-P2-A-R3	Sample		5		9.50	1534	0.061	0.012
CONTROL-SAMPLE-P2-B-R1	Sample		5		9.50	2189	0.087	0.017
CONTROL-SAMPLE-P2-B-R2	Sample		5		9.49	2177	0.087	0.017
CONTROL-SAMPLE-P2-B-R3	Sample		5		9.49	2160	0.086	0.017
Blank-03	Sample				9.79	1	0.001	0.001

API sample amount (5 mg/mL) | Concentration (ng/mL) | Concentration (ppm)

Figure 6. Quantitative result batch table for N-nitroso dabigatran etexilate impurity.

Specificity was established with sample preparation optimization in terms of extraction solvent (diluent) and chromatography conditions. Figures 7A to 7C show that the recoveries obtained for three different lots of APIs were between 90 and 110%.

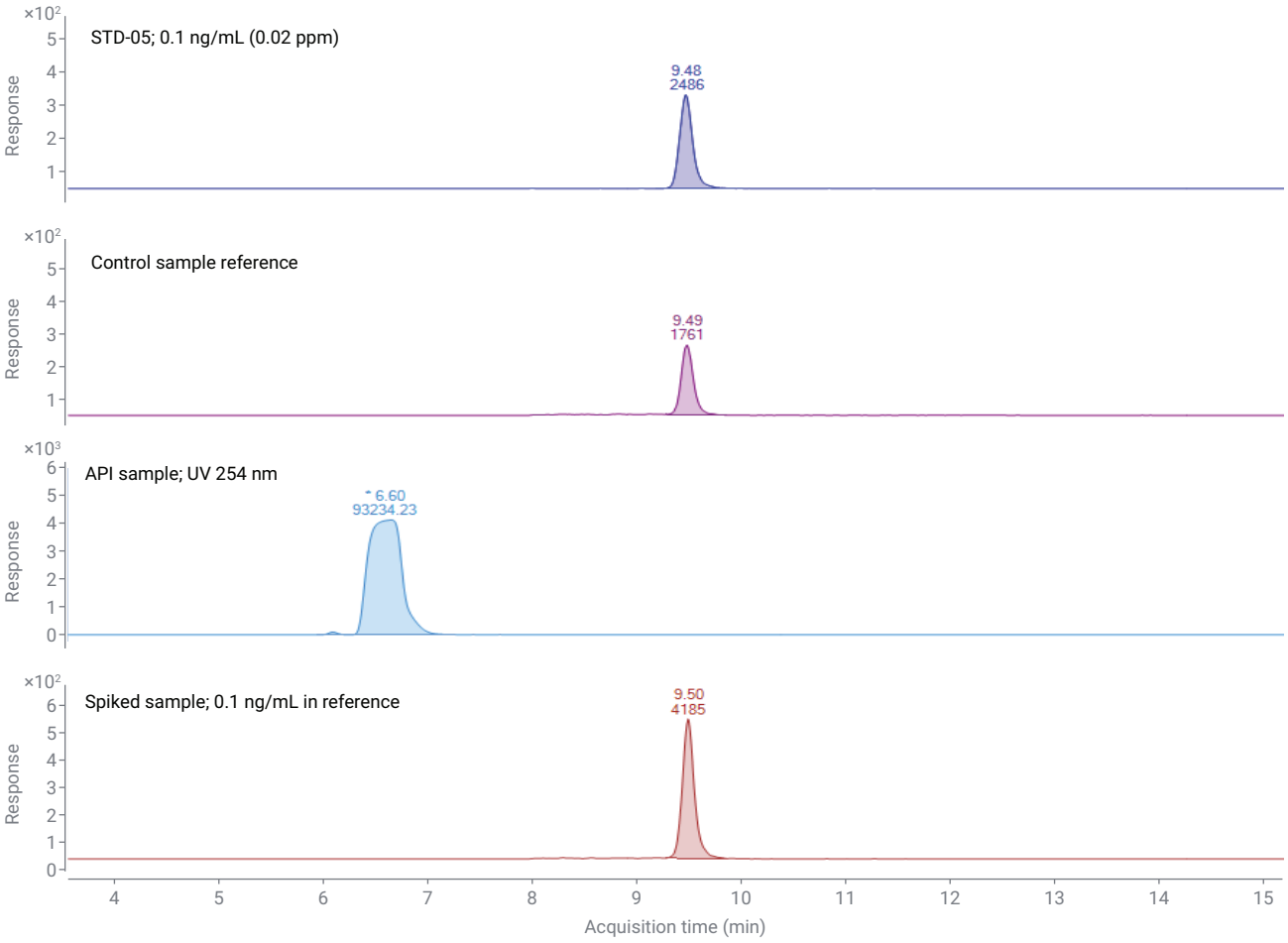

Figure 7A. Extracted ion chromatograms for 657/364 *m/z* of standard specification limit (0.1 ng/mL), control sample reference, and spiked sample reference (API load 5 mg/mL).

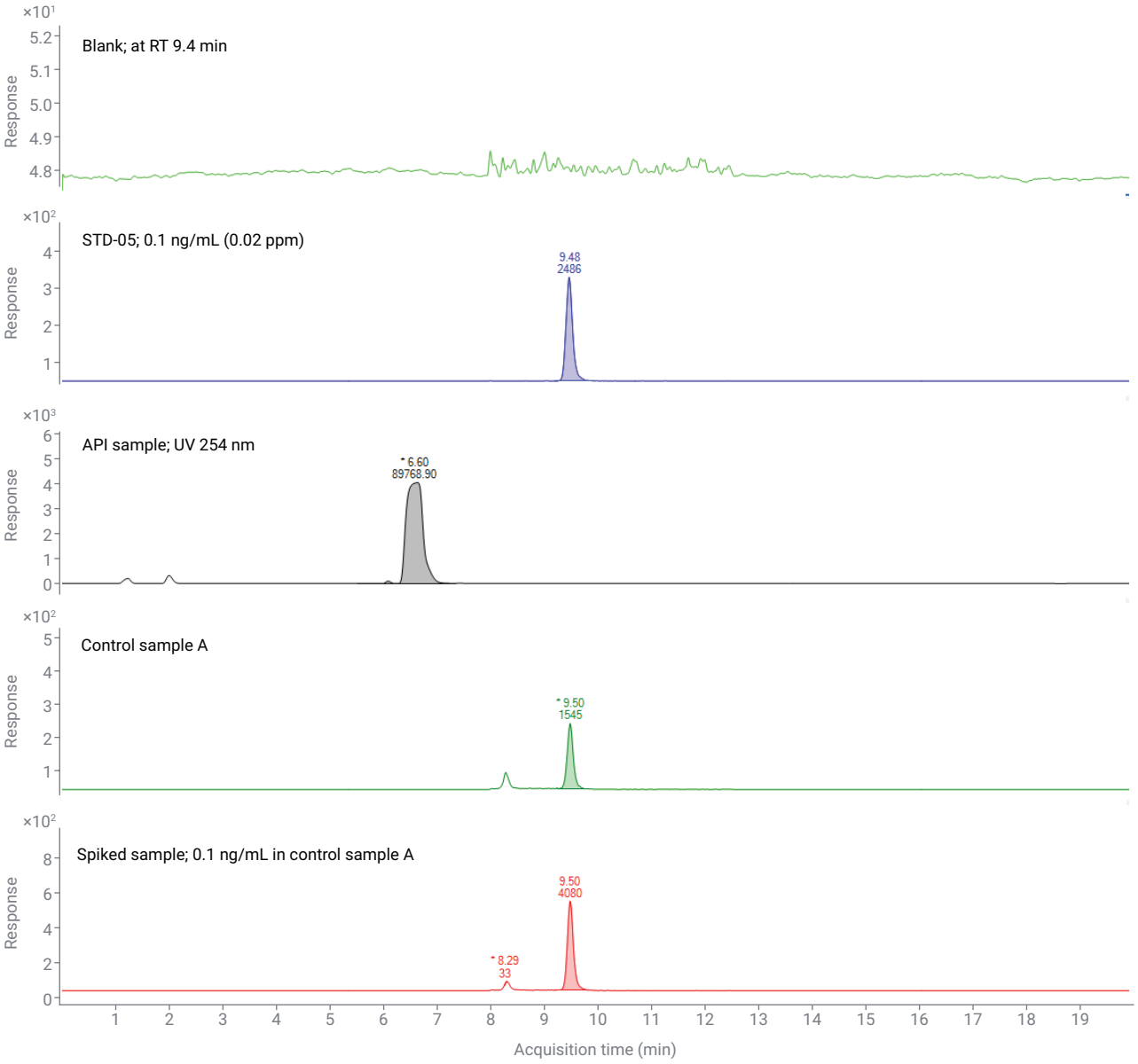

Figure 7B. Extracted ion chromatograms for 364/657 *m/z* of standard specification limit (0.1 ng/mL), control sample A, and spiked sample A (API load 5 mg/mL).

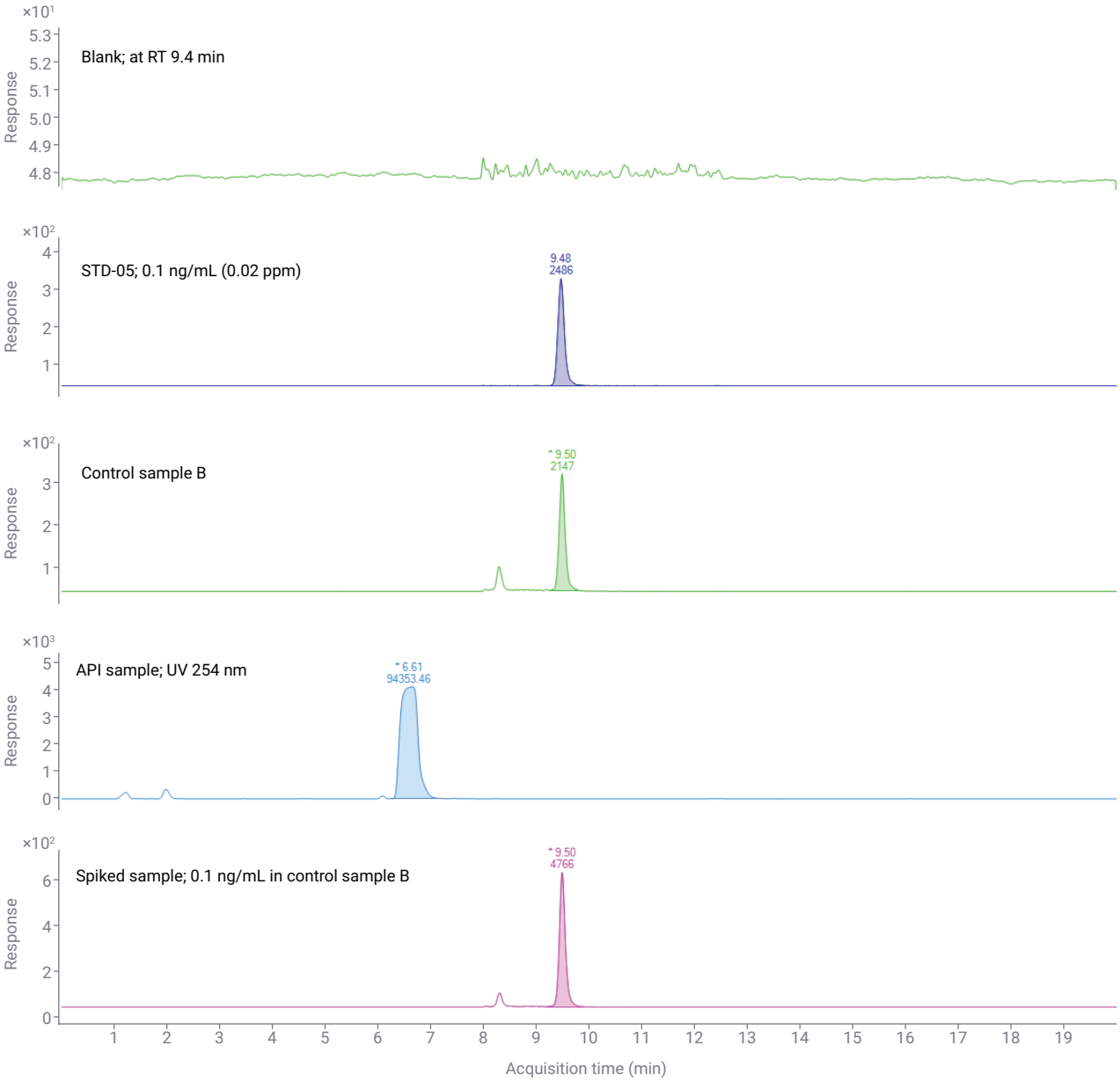

Figure 7C. Extracted ion chromatograms for 364/657 *m/z* of standard specification limit (0.1 ng/mL = 0.02 ppm), control sample B, and spiked sample B (API load 5 mg/mL).

Area response and retention time reproducibility for six consecutive injections of 0.1 ng/mL spiked sample are shown in Figure 8.

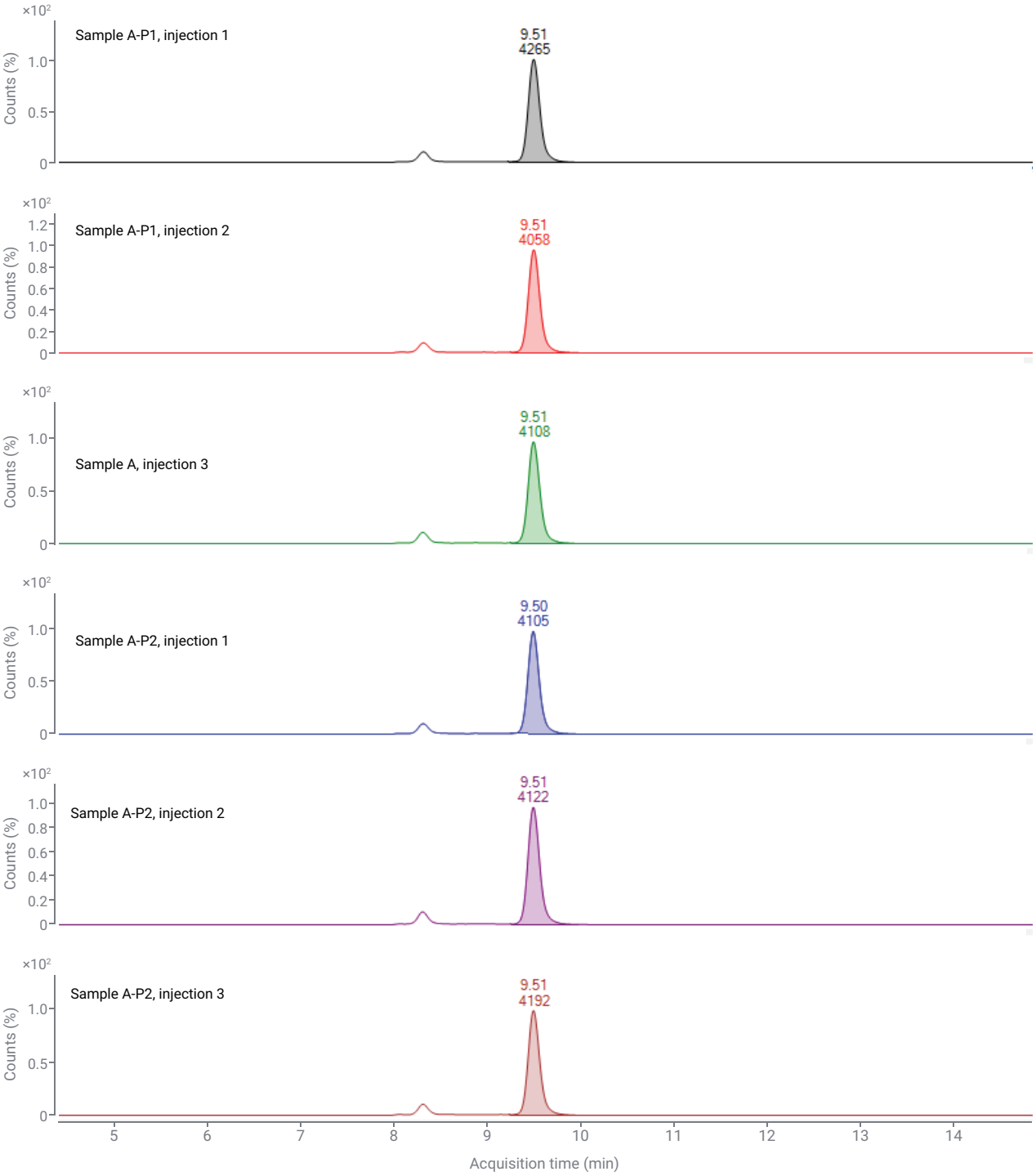

Figure 8. Area response and retention time reproducibility for six consecutive injections of 0.1 ng/mL spiked sample (including triplicate injections of two different preparations).

An MRM chromatogram representing method specificity is shown in Figure 9.

Table 6 shows the precision at 0.1 ng/mL when the method was performed with two different preparations and each triplicate injection.

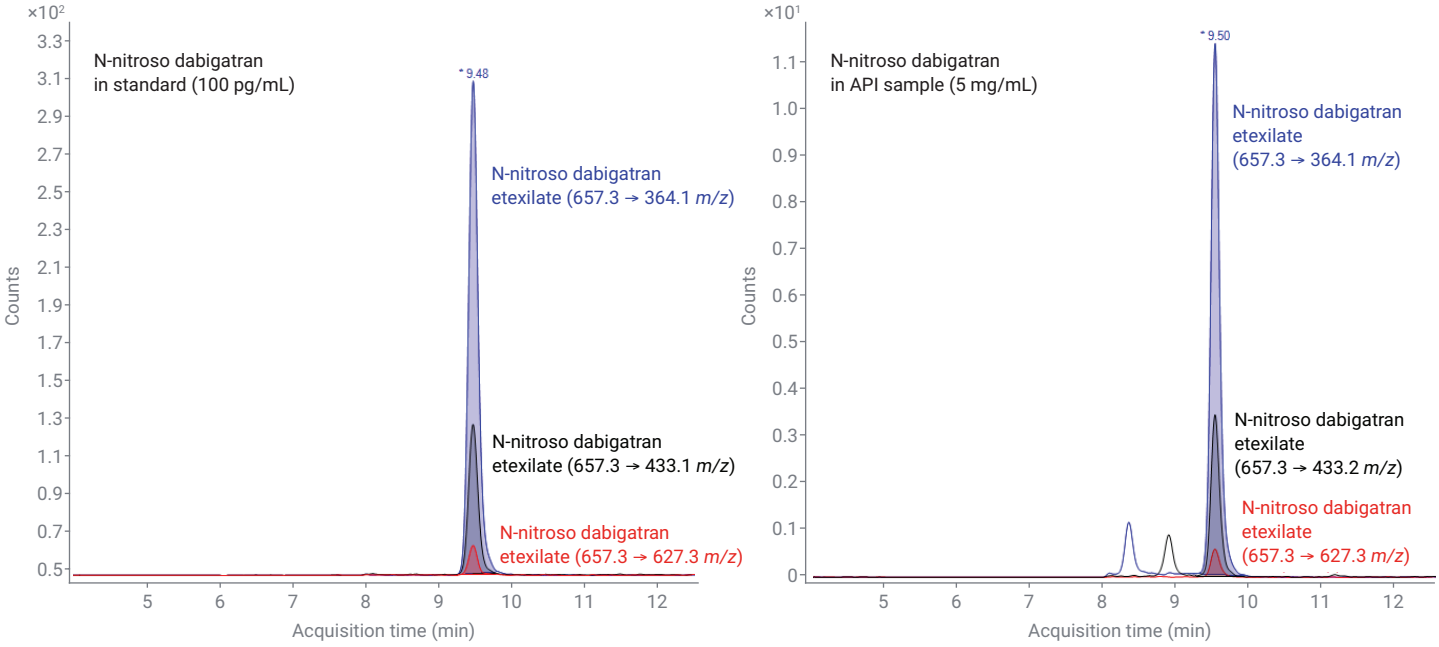

Figure 9. MRM chromatogram of standard at 0.1 ng/mL and control sample with overlaid quantifier and qualifier ions, representing method specificity.

Table 6. Precision at 0.1 ng/mL (0.02 ppm with respect to API load 5 mg/mL) performed with two different preparations and each triplicate injection.

Replicate		Standard	Control Sample			Recovery Sample		
No. of Replicates (n = 6)		Specification Limit	REF Sample	Sample A	Sample B	REF Sample	Sample A	Sample B
Preparation 1	R1	2,526	1,789	1,607	2,189	4,249	4,181	4,864
	R2	2,466	1,742	1,608	2,183	4,248	4,196	4,863
	R3	2,428	1,709	1,557	2,169	4,370	4,272	4,942
Preparation 2	R1	2,431	1,806	1,584	2,189	4,065	4,344	4,849
	R2	2,448	1,820	1,595	2,177	4,063	4,136	4,845
	R3	2,512	1,821	1,534	2,160	4,134	4,178	4,798
Average		**2,468.5**	**1,781.17**	**1,580.83**	**2,177.83**	**4,188.17**	**4,217.83**	**4,860.17**
Standard Deviation		38.02	41.86	27.06	10.59	111.14	69.48	42.70
%RSD		1.54	2.35	1.71	0.49	2.65	1.65	0.88
A = (Average response in spiked sample) – (Average response in control sample)		NA				2,407.00	2,637.00	2,682.33
(A ÷ Average response in standard) × 100						**97.51**	**106.83**	**108.66**

Responses of 0.1 ng/mL impurity standard in neat and spiked samples were plotted in a radar plot, displayed in Figure 10.

Recovery data at 0.1 ng/mL in three different lots of APIs are displayed in Table 7 and Figure 11.

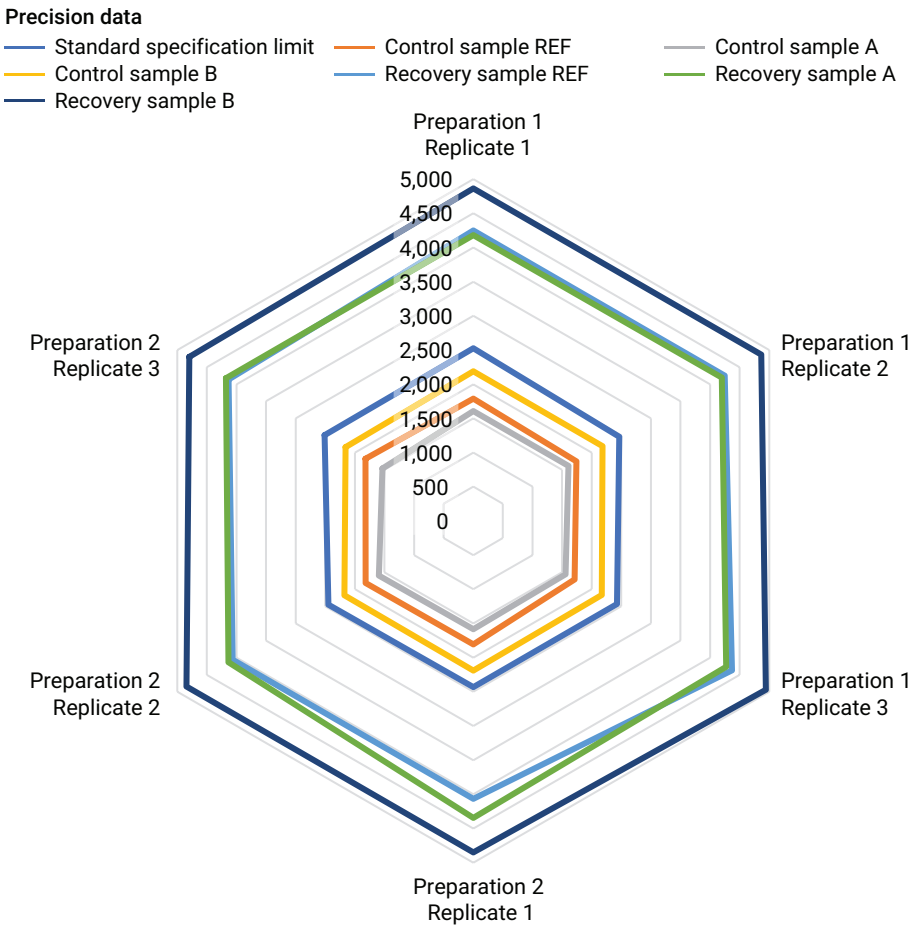

Figure 10. Radar plot of responses of 0.1 ng/mL impurity standard in neat and spiked samples (0.02 ppm with respect to API load 5 mg/mL). Performed with two different preparations and each injected in triplicate.

Table 7. Recovery data at 0.1 ng/mL (0.02 ppm with respect to API load 5 mg/mL) in three different lots of APIs (sample reference, sample A, and sample B).

Replicate (R)	Standard	Control Sample			Spiked Sample		
n = 3	Specification Limit	REF	A	B	REF	A	B
R1	2,526	1,789	1,607	2,189	4,249	4,181	4,864
R2	2,466	1,742	1,608	2,183	4,248	4,196	4,863
R3	2,428	1,709	1,557	2,169	4,370	4,272	4,942
Average	2,473.33	1,746.67	1,590.67	2,180.33	4,289.00	4,216.33	4,889.67
A = (Average response in sample) – (Average response in control sample)	NA				2,542.33	2,625.67	2,709.30
(A ÷ Average response in standard) × 100					102.78	106.15	109.54

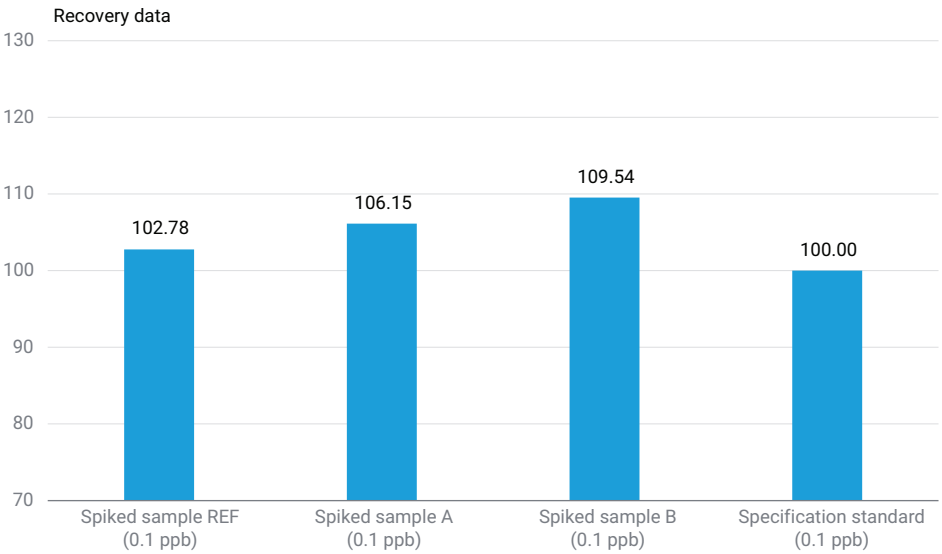

Figure 11. Recovery at 0.1 ng/mL (0.02 ppm with respect to API load 5 mg/mL), spiked in three different lots of APIs (reference, control A, and control B).

Conclusion

A sensitive and robust MRM method was developed to quantify N-nitroso dabigatran etexilate impurity in dabigatran etexilate API (drug substance) using the Agilent 6495C triple quadrupole LC/MS system. The ion funnel technology enabled high sensitivity and lower LOD and LOQ. The chromatographic method provided desirable separation between the impurity and the API to avoid interference. A diverter valve program was included to divert high-concentration API with the help of an integrated diverter valve to avoid contamination of the mass spectrometer. The method development produced many challenges considering the instability of API and its susceptibility to undergo hydrolytic, oxidative degradation. Careful selection of the diluent helped to maintain the stability of API in solution. A slow gradient method was adopted along with the selective MRM transition to establish the specificity. The developed method demonstrated excellent linearity over the range of 0.01 to 1.0 ng/mL (0.002 to 0.02 ppm) with an R^2 value greater than 0.9990. The LOD and LOQ values achieved were 6 and 10 pg/mL, respectively, for the targeted NDSRI. The S/N at LOQ was found to be more than 190:1, where the noise was calculated using an rms algorithm. The method provided recovery between 90 and 110%, which is within the acceptance criteria. The developed method was tested with multiple batches of API samples and proved to be useful for the routine quality control of dabigatran API.

References

1. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs) Guidance for Industry. August **2023**. https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs.
2. Bernardi, R.; Fröehlich, P.; Bergold, A. Development and Validation of a Stability-Indicating Liquid Chromatography Method for the Determination of Dabigatran Etexilate in Capsules. *J. AOAC Int.* January **2013**, *96(1)*, 37–41.
3. FDA guidance document: Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs.
4. FDA guidance document: Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Eight Nitrosamine Impurities in Metformin Drug Substance and Drug Product.
5. Schlingemann, J.; Burns, M. J.; Ponting, D. J.; Martins Avila, C.; Romero, N. E.; Jaywant, M. A.; Smith, G. F.; Ashworth, I. W.; Simon, S.; Saal, C.; *et al.* The Landscape of Potential Small and Drug Substance Related Nitrosamines in Pharmaceuticals, *Journal of Pharmaceutical Sciences* **2023**, *112(5)*, 1287–1304. https://doi.org/10.1016/j.xphs.2022.11.013.
6. V.V.S.R.N. Anji Karun Mutha; B.V. Narasimha Raju Katari. Hydrolytic Degradation Study of Dabigatran Etexilate Mesylate: Isolation and Structural Elucidation of New Degradants. *Analytical Chemistry Letters*, *8(3)*, 277–286, DOI: 10.1080/22297928.2018.1449664.

www.agilent.com

DE74422055

This information is subject to change without notice.

Printed in the USA, January 23, 2024
5994-7066EN

Agilent
Trusted Answers