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Application Note

High Sensitivity Analysis of Potential Mutagenic Boronic Acids Using Xevo™ TQ Absolute Tandem Quadrupole Mass Spectrometer with an ACQUITY™ Premier System

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Abstract

Boronic acids and their esters are common reagents used in the synthesis of organic compounds. Several boronic acids have been identified as potential mutagens and should be controlled at low levels using sensitive methods. This work describes an Ultra Performance Liquid Chromatography (UPLC) method with tandem quadrupole mass spectrometer for high sensitivity quantification of seven boronic acids with mutagenic potential. The chromatographic separation is achieved with an ACQUITY Premier BEH™ C_{18} Column. Quantification is performed using a Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer coupled to an ACQUITY Premier System. The quantification limits achievable for boronic acids with negative electrospray ionization (ESI) and multiple reaction monitoring (MRM) mode ranged from 0.005 to 0.05 ng/mL.

Benefits

- Highly selective and sensitive UPLC method with Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer in MRM mode for accurate identification and quantification of potential mutagenic boronic acids
- Robust separation of boronic acids using the ACQUITY Premier BEH C_{18} Column
- Low-level limits of quantification (LOQ) achievable with Xevo TQ Absolute Mass Spectrometer, ranging from 0.005 to 0.05 ng/mL for potential mutagenic boronic acids

Introduction

Boronic acids and their esters are important building blocks and key intermediates in the synthesis of pharmaceutical drug substances.[1] A published study reported that some boronic acids showed mutagenic activity in microbial assay.[1] Mutagenic compounds, often referred as genotoxic, are DNA-reactive substances that may damage DNA, leading to mutagenic mutations which could potentially cause cancer.[2,3] Therefore, highly sensitive methods are required for accurate identification and quantification according to the acceptable safety levels for mutagenic impurities.[3] In the case of boronic acids, they are used as intermediates during synthesis and may potentially be present as impurities in the drug substances.

Ultraviolet (UV)-based analytical methods are typically employed in pharmaceutical analysis for non-volatile compounds or gas chromatography (GC) with flame ionization detection (FID) for volatile compounds. However, these methods may not provide the desired low levels of detection needed for control of mutagenic compounds in drug substances. In these situations, mass detection is required to achieve the desired sensitivity. Tandem quadrupole mass spectrometry is a highly sensitive and selective technique often employed for low-level quantification of pharmaceutical compounds.[4]

In this work, a highly sensitive and selective UPLC-MS/MS method was developed for the analysis of potentially mutagenic boronic acids (Table 1). The method employed a Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer coupled with an ACQUITY Premier System.

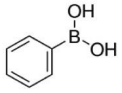
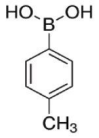
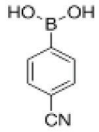
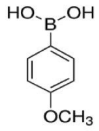
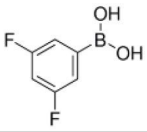
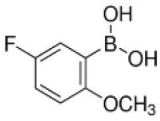
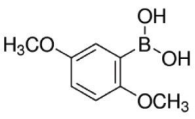
Compound	Monoisotopic mass (Da)	Formula	Structure
Phenyl boronic acid	122.05	$C_6H_7BO_2$	
4-Methylphenyl boronic acid	136.07	$C_7H_9BO_2$	
4-Cyanophenyl boronic acid	147.05	$C_7H_6BNO_2$	
4-Methoxyphenyl boronic acid	152.06	$C_7H_9BO_3$	
3,5-Difluorophenyl boronic acid	158.03	$C_6H_5BF_2O_2$	
5-Fluoro-2-methoxyphenyl boronic acid	170.06	$C_7H_8BFO_3$	
2,5-Dimethoxyphenyl boronic acid	182.08	$C_8H_{11}BO_4$	

Table 1. List of boronic acids and chemical information.

Experimental

Boronic acids were purchased from Fisher Scientific and mass spectrometry grade solvents were obtained from Sigma.

Sample Description

Individual stock standard solutions with boronic acids were prepared in methanol at 5.0 mg/mL. An equal volume of each stock solution was transferred to one vial and diluted with methanol to make a mixture standard solution at 10 µg/mL of each analyte. The mixture standard solution was then serially diluted with water to make LOD, LOQ, and linearity standard solutions.

Method Conditions

LC system:	ACQUITY Premier Binary System
Vials:	LCMS Maximum Recovery 2 mL volume, p/n: 600000670CV
Column(s):	ACQUITY Premier BEH C_{18} (2.1 x 100, 1.7 µm), p/n: 186009453
Column temp.:	40 °C
Sample temp.:	15 °C
Injection volume:	7.0 µL
Flow rate:	0.3 mL/min
Mobile phase A:	0.05% ammonium hydroxide in water
Mobile phase B:	Acetonitrile
Gradient:	Described in gradient table

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.300	98.0	2.0	6
1.0	0.300	98.0	2.0	6
5.0	0.300	5.0	95.0	6
5.6	0.300	5.0	95.0	6
5.7	0.300	98.0	2.0	6
8.0	0.300	98.0	2.0	6

MS Conditions

MS system:	Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer
Ionization mode:	ESI-
Acquisition:	MRM mode, described in Table 2
Capillary voltage:	1.0 V
Desolvation temp.:	550 °C
Desolvation gas flow:	950 L/Hr
Cone flow:	150 L/Hr

Nebulizer:	7.0 bar
Source temp.:	150 °C

Boronic acid	MRM transition (*m/z*)	Cone voltage (V)	Collison energy (eV)	Soft ionization
Phenyl	121.03>42.89	10	8	Yes
4-Methylphenyl	135.07>42.89	10	8	Yes
4-Cyanophenyl	146.04>42.87	10	8	Yes
4-Methoxyphenyl	151.03>42.89	10	10	No
3,5-Difluorophenyl	157.07>42.87	5	9	No
5-Fluoro-2-methoxyphenyl	169.05>42.88	10	8	Yes
2,5-Dimethoxyphenyl	181.08>42.89	10	8	Yes

Table 2. The MRM transitions for boronic acids.

Data Management

Instrument control:	MassLynx™ v4.2
Data processing:	TargetLynx™

Results and Discussion

The ACQUITY Premier BEH C_{18} Column successfully separated the boronic acids (Figure 1). The detection and quantification were performed using electrospray ionization (ESI) in negative ion mode using the Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer. The multiple reaction monitoring (MRM) experiment was performed by defining the precursor mass of the targeted compound for MS/MS fragmentation and then monitoring a product ion. The MRM transitions and ionization parameters for boronic acids were identified using IntelliStart™ within the MassLynx Software and confirmed via manual infusion.

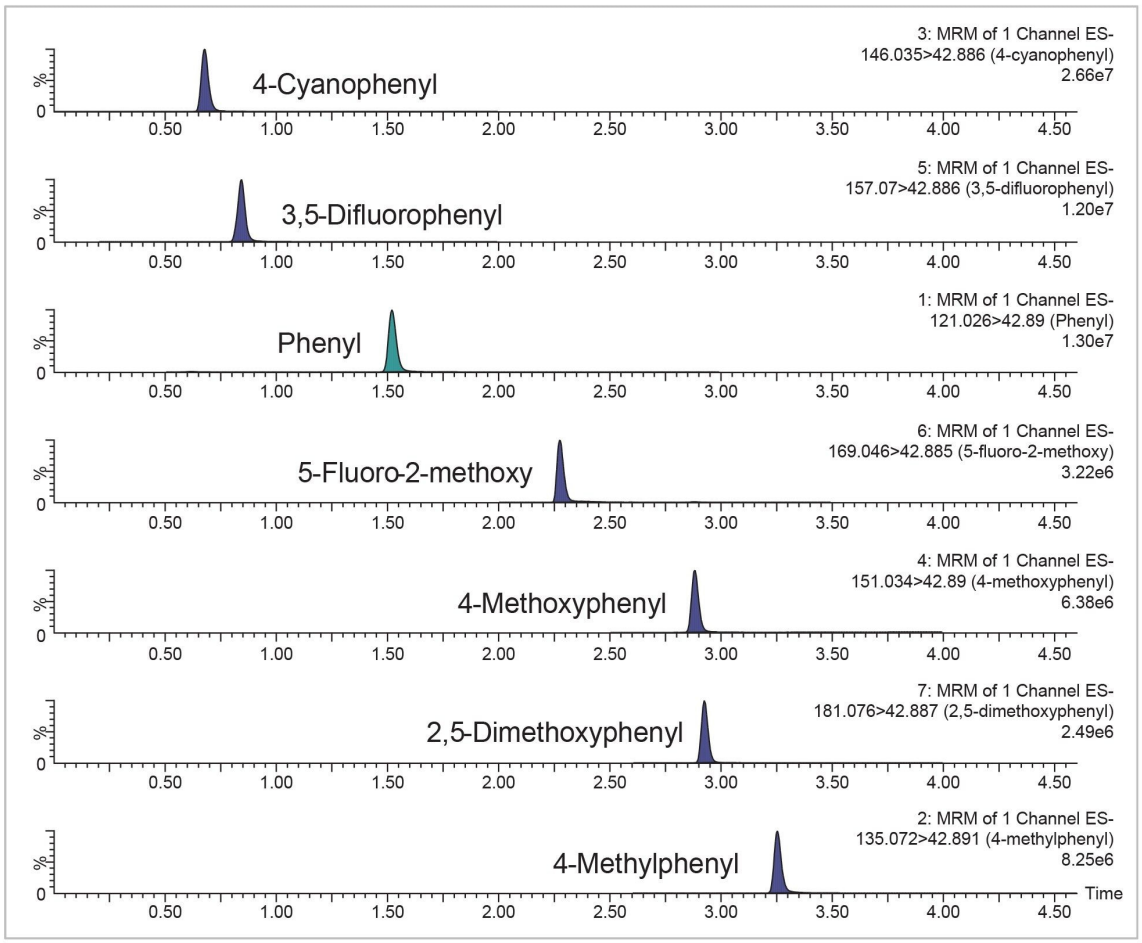


Figure 1. Chromatographic separation of boronic acids using a Xevo TQ Absolute in MRM acquisition mode. Standard solution at 10 ng/mL.

The limits of detection and quantitation (LOD and LOQ) for boronic acids were determined following the signal-to-noise (S/N) criteria of 3:1 and 10:1, respectively. Chromatograms representing LOD and LOQ concentration levels for boronic acids are shown in Figure 2. Based on the S/N criteria, LOD and LOQ ranged from 0.0025 to 0.025 ng/mL and 0.005 to 0.05 ng/mL, respectively. Additionally, the method exhibited a linear relationship between the MS responses and concentrations for boronic acids with the correlation coefficients of ≥0.996 (Figure 3).

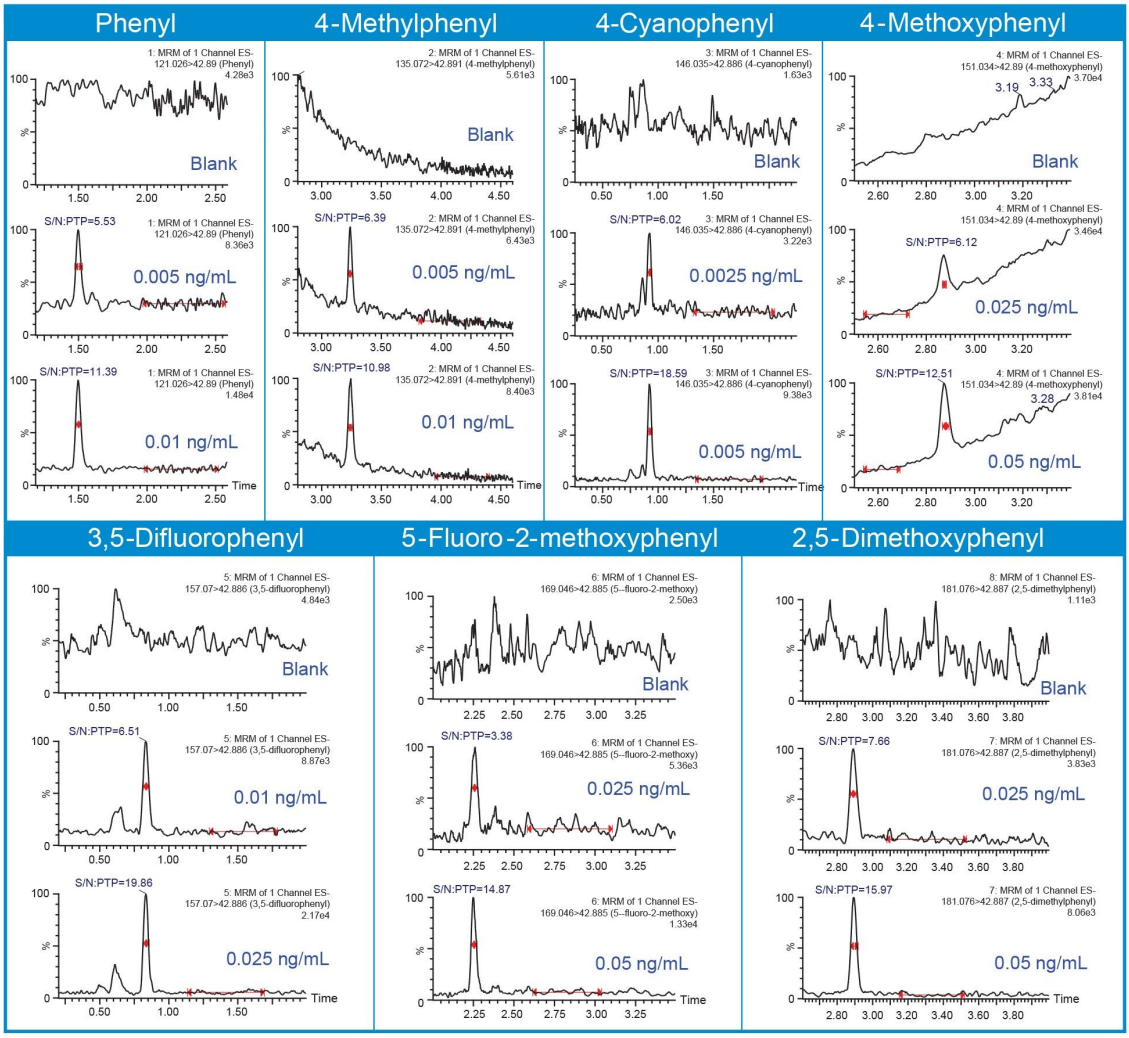


Figure 2. Representative chromatograms at the LOD (S/N≥3) and LOQ (S/N ≥10) concentrations for boronic acids with a Xevo TQ Absolute Mass Spectrometer.

Boronic acid	MRM transitions	Linearity range (ng/mL)	Linear fit R^2
Phenyl	121.03>42.89	0.01–50	0.9972
4-Methylphenyl	135.07>42.89	0.01–50	0.9982
4-Cyanophenyl	146.04>42.87	0.01–100	0.9974
4-Methoxyphenyl	151.03>42.89	0.005–50	0.9976
3,5-Difluorophenyl	157.07>42.87	0.05–100	0.9956
5-Fluoro-2-methoxyphenyl	169.05>42.88	0.05–50	0.9973
2,5-Dimethoxyphenyl	181.08>42.89	0.05–50	0.9971

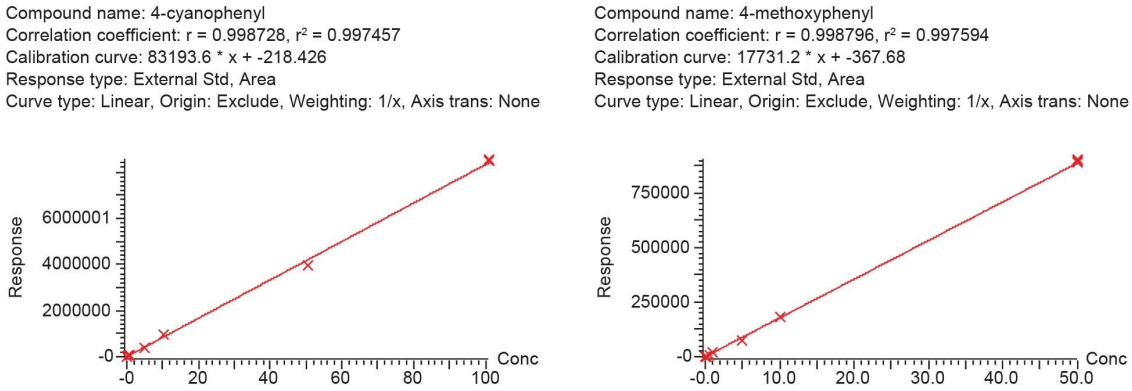


Figure 3. Method linearity with 1/x weighting acquired using the Xevo TQ Absolute Mass Spectrometer.

Demonstrating inter-day performance of the method for the same analysis ensures that the method generates consistent results, which is critical to the quality control and safety of the pharmaceutical products. For the inter-day study of the boronic acids method, the LOD and LOQ standard solutions were injected over several days. Performance on each day was evaluated by calculating S/N for the boronic acids and %RSD of the peaks areas. Results are summarized in Table 3. Across the days studied, all boronic acids exhibited a robust signal at the LOQ concentration with the S/N ratio well above 10:1. Additionally, the repeatability at the LOQ level was excellent with %RSD of the peak areas ≤6.86%. No internal standard was used in this work to correct for data variability.

Boronic acid	LOQ (ng/mL)	Day 1	Day 2	Day 6	Day 1	Day 2	Day 6
Phenyl	0.01	11.4	10.0	13.90	2.87	1.88	8.33
4-Methylphenyl	0.01	11.0	11.9	15.86	6.86	4.94	3.10
4-Cyanophenyl	0.005	18.6	16.5	12.45	5.10	5.26	5.64
4-Methoxyphenyl	0.05	12.5	10.42	13.65	6.34	4.98	6.31
3,5-Difluorophenyl	0.025	19.9	13.83	22.08	3.04	4.74	3.35
5-Fluoro-2-methoxyphenyl	0.05	14.9	13.59	12.28	3.34	5.26	3.23
2,5-Dimethoxyphenyl	0.05	16.0	18.84	20.42	4.84	6.69	3.70

Table 3. Inter-day method performance at the LOQ levels.

Conclusion

A highly sensitive UPLC-MS/MS method using the ACQUITY Premier I-Class System coupled with the Xevo TQ Absolute Mass Spectrometer was successfully developed for the ultra-low detection of potential mutagenic boronic acids, achieving LOQs of 0.005 to 0.05 ng/mL. The method demonstrated excellent inter-day performance at the LOQ level. A highly sensitive method is critical for the accurate identification and control of mutagenic compounds at the residual levels to help assure product quality and safety.

References

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4. Loos G, Van Schepdael A, Cabooter D. Quantitative Mass Spectrometry Methods for Pharmaceutical Analysis. *Philosophical Transactions*, 2016, 374:20150366.

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