Quantitation of boronic acids at pg/mL levels of sensitivity

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Abstract

This poster details a highly sensitive analysis for the quantitation of boronic acids using LC-MS. The method utilizes multiple reaction monitoring (MRM) acquisition in negative ion mode, with a 7-minute runtime to allow for high throughput. The 4 compounds analyzed include phenylboronic acid, 4-methylphenylboronic acid, 5-fluoro-2-methoxyphenylboronic acid and 2,5-dimethoxyphenylboronic acid.

Introduction

Phenylboronic acid and its derivatives are capable of forming reversible complexes with polyhydroxy compounds, such as polysaccharides, glycolipids, glycoproteins and nucleotides. Phenylboronic acids can therefore be used to recognize polyhydroxy compounds in autonomic drug delivery systems or to regulate certain biological activities. Therefore, these compounds have received attention and interest from a large number of researchers.[1] In addition, phenylboronic acid and its derivatives are commonly used raw materials and intermediates for the synthesis of organic compounds. The 4 compounds analyzed in this study are phenyl boronic acid, 4-methyl phenyl boronic acid, 5-fluoro-2-methoxyphenyl boronic acid and 2,5-dimethoxyphenylboronic acid. The method developed allows for a high throughput of sample injections alongside ultra-high levels of sensitivity.

Materials and methods

Sample preparation

The 4 phenylboronic acid compounds were accurately weighed and then dissolved in methanol to create 1 mg/mL individual standards. These stock solutions were then diluted to prepare a 10 µg/mL mixed standard. The resulting mixed standard was diluted with 50:50 (v/v), methanol/water to multiple concentrations to prepare a calibration range.

HPLC conditions

HPLC system:	ExionLC AD system
Column:	Waters BEH C18 (100 x 3 mm, 1.7 µm)
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Injection volume (µL):	7
Flow rate (mL/min):	0.3
Column temperature (°C):	40

Table 1. HPLC gradient.

Time (min)	%A	%B
0	85	15
1	85	15
4	0	100
5	0	100
5.1	85	15
7	85	15

MS conditions

MS system:	SCIEX 7500 system
Acquisition mode:	Multiple reaction monitoring (MRM)
Ionization mode:	Negative electrospray ionization
Ion spray voltage (V):	1600
CAD gas:	9
GS1 (psi):	35
GS2 (psi):	70
Curtain gas (psi):	40
Temperature (°C):	500

Table 2. MRM transitions and optimized collision energy (CE).

Compound name	Q1 (m/z)	Q3 (m/z)	CE (V)
Phenylboronic acid	121.1	43	-22
4-Methylphenylboronic acid	135.1	43	-25
5-Fluoro-2-methoxyphenylboronic acid	169.1	43	-21
2,5-Dimethoxyphenylboronic acid	181.1	43	-40

Results

In this experiment, water and pure acetonitrile were used as the mobile phases because they produce more sensitivity for the compounds of interest than when modifiers are added to the eluent system. Direct quantitation of the 4 phenylboronic acid compounds was performed using the SCIEX 7500 system and a lower limit of quantitation (LLOQ) as low as 2 pg/mL was observed. The LLOQ ranged between 2 and 10 pg/mL for the 4 compounds analyzed (Table 3). The extracted ion chromatogram (XIC) of each compound at the LLOQ concentration is shown in Figure 1. The method demonstrated good selectivity, high sensitivity and no interference in the blank solvent injection. The linearity for each of the 4 compounds analyzed provided $R^2 > 0.99$, showing a good linear relationship for each analyte.

To investigate the reproducibility of the method, LLOQ solutions of the 4 compounds were injected multiple times for analysis. These results demonstrated high precision, as the %RSD of all compounds was <3 %. See Table 3 for the full details of the precision values achieved.

Table 3. LLOQ and precision of the 4 phenylboronic acid compounds.

Compound name	LLOQ (pg/mL)	%RSD
Phenylboronic acid	2	1.50
4-Methylphenylboronic acid	5	2.00
5-Fluoro-2-methoxyphenylboronic acid	10	1.90
2,5-Dimethoxyphenylboronic acid	10	0.43

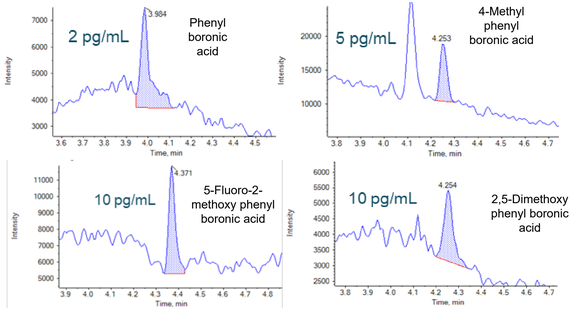

Figure 1. The extracted ion chromatograms (XICs) for the 4 phenylboronic acid compounds at the LLOQ.

CONCLUSIONS

In this method, water and pure acetonitrile were used as the mobile phase to directly quantify 4 phenylboronic acid compounds using the SCIEX 7500 system. The LC-MS/MS detection method has the advantages of a simple mobile phase, high sensitivity, good specificity and no interference in the blank. The LLOQ for the compounds analyzed was between 2 pg/mL and 10 pg/mL and the method demonstrated good reproducibility (RSD < 3%). The entire method is simple to operate, can be performed quickly and efficiently and can provide a reference for the quantitative determination of phenylboronic acid compounds.

REFERENCES

1 Xu D, Chu L Y. Research progress on the application of phenylboronic acid and its derivatives in medicine and chemical industry[J]. Chemical Industry and Engineering Progress, 2006, 25(9).

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