

# Analysis of Boronic Acids Without Chemical Derivatisation

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## Introduction

Boronic acids play an important role in chemistry, so their analysis is frequently required in a service laboratory. Analysis by mass spectrometry has proved to be unpredictable with no single technique being reliable. There are often unexpected (and not easily identifiable) additional ions formed that can obscure the true molecular weight information.

Historically, the analysis of boronic acids has been achieved by derivatisation.<sup>[1,2]</sup> One such method required the boronic acid to be reacted with an excess of a diol, such as ethane-1,2-diol or propan-1,3-diol. The resultant product was then analysed by EI or CI.

The ongoing study presented here represents the initial steps in determining a reliable method for the analysis of boronic acids without derivatisation. The project is utilising a variety of ionisation techniques, in both positive and negative modes, and a variety of solvents to determine differences in results obtained from the analysis of six small aryl boronic acids. An inter-laboratory comparison of many instruments should also highlight differences between the ion sources.

## Experimental

The six boronic acids are shown in Figure 1. The solvents used are shown in Table 1. Data presented here were obtained at Durham University and Swansea University.

Durham University: Samples were diluted to 50 µg/mL in the desired solvent and infused into Xevo QToF MS (Waters UK, Ltd). The standard electrospray (ESI) ion source was fitted with an atmospheric pressure chemical ionisation (APCI) corona needle to perform ESCI (high-speed switching between ESI and APCI). Samples were analysed in positive and negative ionisation modes. The mass spectrometer was optimised for the mass range  $m/z$  50 to 650.

Swansea University: Samples were diluted to 10 µg/mL in the desired solvent. The samples were analysed by electrospray infusion using the low-flow Ion Max ESI source and, for nano-electrospray (nano-ESI), with a TriVersa Nanomate (Advion Biosciences Inc.) interfaced to an LTQ Orbitrap XL (Thermo Fisher Scientific Inc.). Samples were analysed in positive and negative ionisation modes. The mass spectrometer was optimised for the mass range  $m/z$  50 to 500.

## Results

Each boronic acid was successfully analysed. The choice of solvent has shown a significant effect on ionisation. Only 2,5-dimethoxyphenyl boronic acid ionised well in positive mode giving mainly  $[M+Na]^+$  in both ESI and nano-ESI, and  $[M+H]^+$  in positive mode APCI. This boronic acid failed to ionise well in negative mode on the Orbitrap. The remaining five boronic acids all ionised in negative mode, giving  $[M-H]^-$ , but failed to ionise well in positive mode. The bar chart plots of Solvent vs. Ion Intensity can be seen in Figure 2.

Interpretation of the spectra is not trivial. It can be seen from both Figures 3 and 4 that there are ions present which are not easily identified. Some of the ion species do contain the characteristic isotope pattern of boron. Ion identification has been assisted by high resolution accurate mass data: Figure 3 (<sup>10</sup>B isotope)  $m/z$  182 =  $[M+H]^+$  and  $m/z$  204 =  $[M+Na]^+$ ;  $m/z$  305 appears to be  $[C_{10}H_{22}O_3Na]^+$  (no boron isotope pattern observed). Ions identified in Figure 4 (<sup>10</sup>B isotope) appear to be  $m/z$  165  $[M-H]^-$ ,  $m/z$  225  $[M+C_3H_6O]^+$  and  $m/z$  313  $[2M-OH]^-$ .

## References

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- [2] J. Haas, K.F. Blom, and C.H. Schwarz III, Anal. Chem., 1999, Vol. 71, 1574-1578

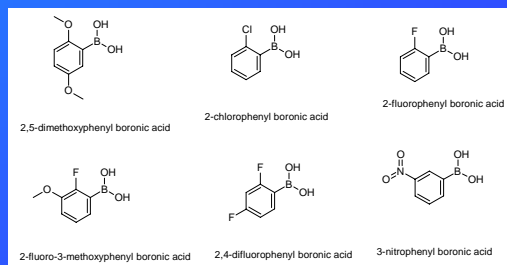


Figure 1: The six boronic acids used in the inter-laboratory comparison.

Table 1: Solvent matrices

A	CH <sub>3</sub> CN:CH <sub>2</sub> Cl <sub>2</sub>
B	CH <sub>3</sub> CN:CH <sub>3</sub> OH
C	CH <sub>3</sub> CN:H <sub>2</sub> O
D	CH <sub>3</sub> CN
E	CH <sub>2</sub> Cl <sub>2</sub>
F	CH <sub>2</sub> Cl <sub>2</sub> :CH <sub>3</sub> OH
G	CH <sub>3</sub> OH
H	CH <sub>3</sub> OH:H <sub>2</sub> O
I	H <sub>2</sub> O

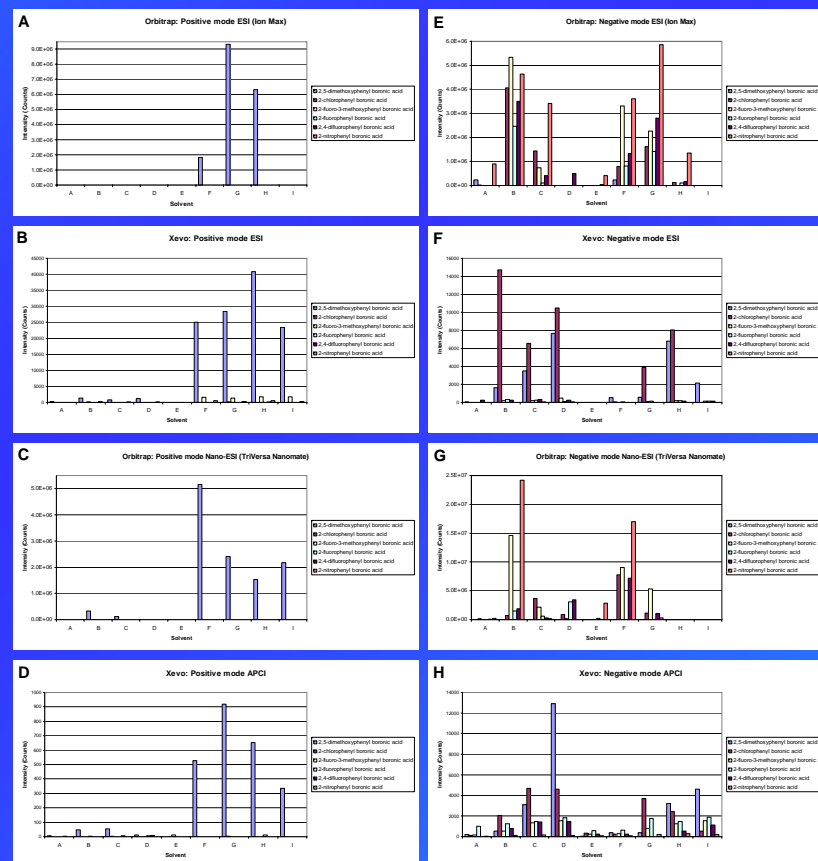


Figure 2: Bar chart plots of Solvent vs. Intensity (Ion Counts) for the boronic acids. Charts A to D represent data acquired in positive mode. Charts E to H represent data acquired in negative mode. See Table 1 for key to solvents. Refer to each chart for ionisation source and instrument.

## Summary

Boronic acids are used in a variety of applications, from synthetic chemistry<sup>[3]</sup> and drug delivery<sup>[4]</sup> to packing for chromatography columns.<sup>[5]</sup> The analysis of the boronic acids used in this study has been achieved without derivatisation.

In positive ion mode, the analysis of 2,5-dimethoxyphenyl boronic acid shows an interesting result that is independent of instrument or ionisation. It ionises well in positive mode in the presence of methanol but acetonitrile appears to be a hindrance. When dichloromethane is used ionisation fails; this is not surprising as this is a volatile aprotic solvent.

Dichloromethane failed to produce negative ions for any of the boronic acids.

The Xevo QToF generated an abundance of negative ions for 2,5-dimethoxyphenyl boronic acid and 2-chlorophenyl boronic acid relative to all other boronic acids tested, where as, bizarrely, the reverse trend was tentatively apparent for the Orbitrap. Water failed to provide any results in negative ion mode, with the exception of the Xevo APCI data which was mediocre. In summary, a mixture of methanol and acetonitrile would appear to be the best solvents to try first.

A common ion observed in negative ion mode is  $[2M-OH]^-$ , formed from dimerisation via dehydration. When methanol is used, solvent cluster ions can be observed. These higher  $m/z$  ions can complicate interpretation of an unknown boronic acid. In these circumstances, the use of a derivatisation protocol may be more beneficial in determining the molecular species of a truly unknown boronic acid.

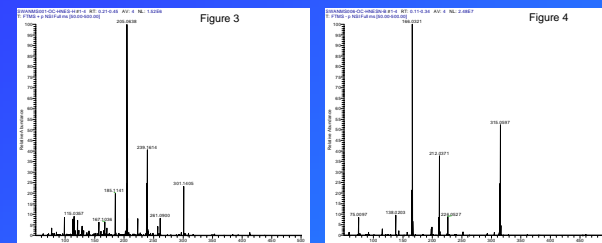


Figure 3 (above left). Mass spectrum of 2,5-dimethoxyphenyl boronic acid (MW=181 <sup>10</sup>B), in 1:1 methanol:water (Orbitrap positive mode, nano-ESI).

Figure 4 (above right). Mass spectrum of 2-nitrophenyl boronic acid (MW=166 <sup>10</sup>B) in 1:1 acetonitrile:methanol (Orbitrap negative mode, nano-ESI).

## Future Work

The collaboration will analyse these boronic acids on further ionisation sources, such as APPI and MALDI, and more instruments. Based on the data obtained so far, the number of solvents trialled can be reduced in future studies. This would allow more experimentation with a greater variety of boronic acids.

## Acknowledgments

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