

EPSRC National Mass Spectrometry Centre, Swansea

Application Note No 6

Boronic Acid Analysis by Mass Spectrometry – I: Cis-diol Derivatisation for EI and CI Analysis

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Introduction

Mass spectrometry analysis of boronic acids by conventional methods, using a variety of ionisation modes, has often failed to show molecular species. In particular, EI and positive ion CI have generally been unsuccessful in our laboratory. We have therefore developed a derivatisation method to allow effective EI and CI analysis by characterisation of the less reactive ester form of the boronic acid.

The esterification is based on a well documented reaction [1], commonly used in recent decades in affinity chromatography methods for the analysis of a wide variety of cis-diols [2]. In our application, the reaction is effectively used for the reverse effect, using the diol to facilitate analysis of the boronic acid, rather than using the acid to enable analysis of the diol.

We recognise that it would be preferable to analyse the boronic acids without derivatisation, and research is currently in progress to establish more reliable methods for this. Electrospray, APCI and MALDI all show promise, and are the subject of initial studies. Any useful findings should be published on this website and more widely as they progress.

Experimental details

INSTRUMENTATION

- Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK) with robotic desorption probe.
- MAT95 double focussing magnetic sector mass spectrometer (ThermoFisher Scientific)
- LTQ-Orbitrap (ThermoFisher Scientific)

MATERIALS AND REAGENTS

- Methanol (HPLC grade, Fisher Scientific)
- Dichloromethane (DCM) (HPLC grade, Fisher Scientific)
- 1,2-ethanediol (ethyleneglycol) (BDH AnalaR) 10% (v/v) solution in HPLC grade methanol;
- Perfluorotributylamine (PFTBA) accurate mass reference compound (Aldrich).

METHODS

Sample Preparation For MS Analysis

Samples were dissolved in the customer specified solvent (DCM or methanol), and diluted into methanol, to a suitable concentration and volume for the robotic probe (ca. 0.1mg in 100µL; inject 1µL). **Derivatisation** was achieved by adding 10µL of the ethyleneglycol solution to a duplicate analysis sample.

MS ACQUISITION PARAMETERS

Positive ion analysis EI and CI:

Quattro II: (spectra of Figures 2,3,5,6)

Electron ionisation (EI): source temp. 200°C; electron energy 70eV; electron trap current ca. 150µA.

Chemical ionisation (CI): ammonia reagent gas; source temp. 170°C; electron energy 50eV; electron emission ca. 1mA.

Desorption probe current: 0mA for 1 minute, then 500mA for 30 secs, then 1500mA for 2 minutes.

MAT95: (EI accurate mass measurement): source temp. 120°C; electron energy 70eV; electron trap current 100µA. Accurate mass measurement by computer-controlled peak matching using the m/z 264 ion of PFTBA as reference.

Positive ion analysis ESI:

LTQ-Orbitrap: (spectra of Figure 1)

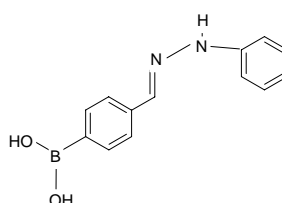
The sample was dissolved in DCM and diluted in 1:1 methanol/water (conc. ca. 10⁻⁷ mmol.), then 2µL loop injected into a stream of 1:1 methanol/water for transport to the mass spectrometer.

ESI spray conditions: Source Temperature 275°C; Sheath Gas flow ca 5 units; Capillary (ionising) voltage ca 3.4kV.

ANALYTES

Customer samples were used by kind permission as listed in the acknowledgements. This Note focusses on the structure below. See Footnote for additional structures for which the described method has also been successful.

A



C₁₄H₁₃O₄N₂B
Mass ¹⁰B = 283
Mass ¹¹B = 284

Results and Discussion

(Please see Appendix for enlarged mass spectra)

Sample **A** was characterised to our satisfaction by ESI MS, giving the spectrum shown in Figure 1, which shows (a) the predicted isotope pattern with exact masses of the $[M+H]^+$ species of **A**; (b) the observed corresponding data (e.g. ^{10}B isotope: measured mass = 284.1080; calculated = 284.1077; $\Delta = 0.9$ ppm; ^{11}B isotope – see figure).

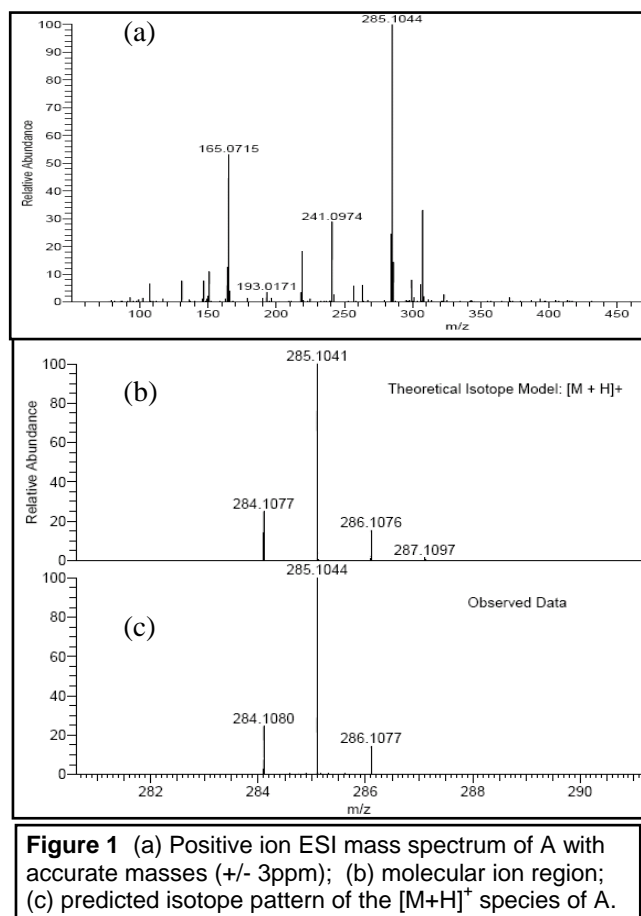


Figure 1 (a) Positive ion ESI mass spectrum of **A** with accurate masses (± 3 ppm); (b) molecular ion region; (c) predicted isotope pattern of the $[M+H]^+$ species of **A**.

Figures 2 and 3 show the mass spectrum of **A** using EI and CI, where there is clearly no evidence of the proposed species. Figure 4 shows the expected derivatisation reaction and product species with the salient compound information. Figures 5 and 6 show the EI and CI spectra after addition of ethylene glycol by the method described.

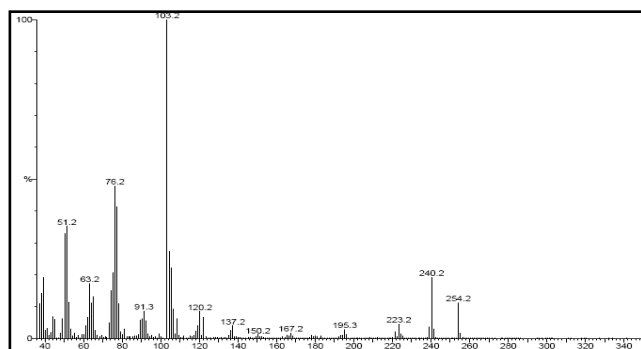


Figure 2 EI mass spectrum of **A**

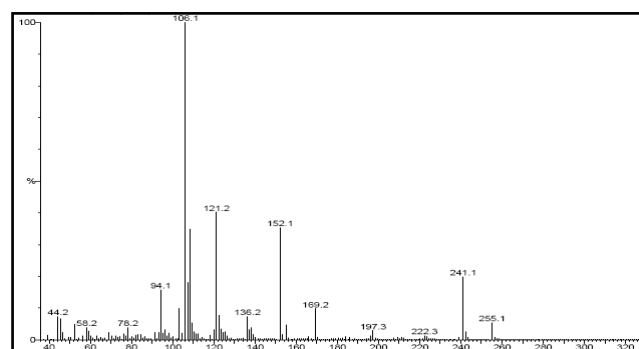


Figure 3 – $\text{Cl}(\text{NH}_3)$ mass spectrum of **A**

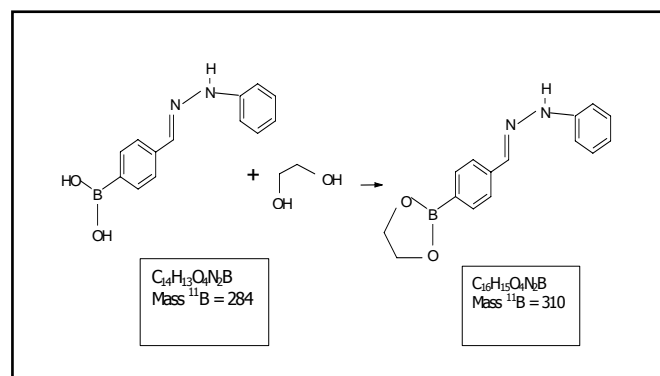


Figure 4 Reaction of **A** with 1,2-ethanediol (ethyleneglycol)

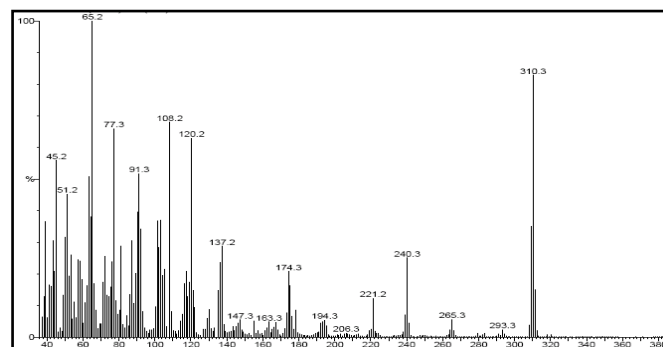


Figure 5 EI spectrum of **A** after reaction with ethyleneglycol

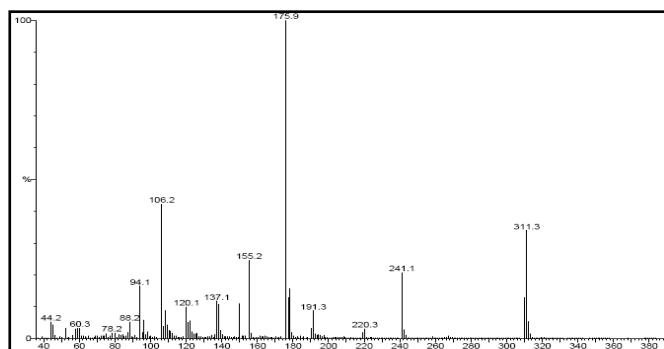


Figure 6 CI spectrum of A after reaction with ethyleneglycol

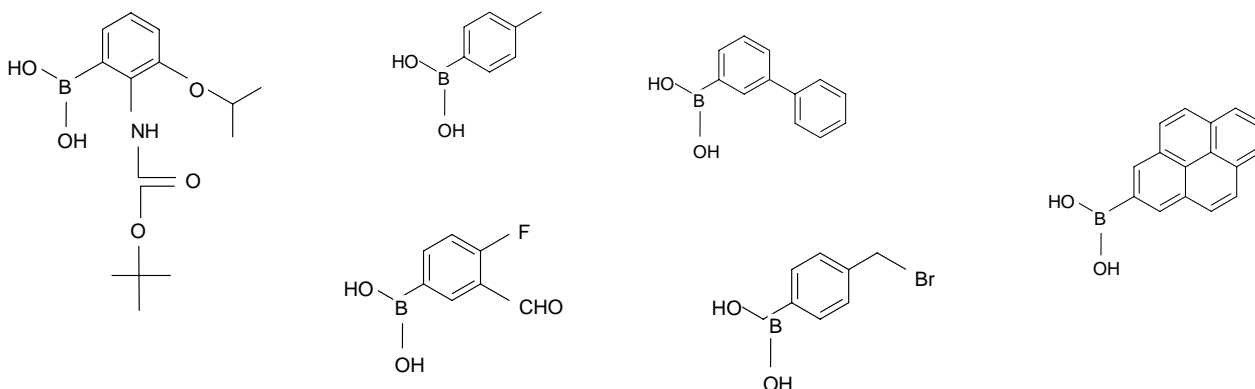
It can thus be seen that performance of the derivatisation has allowed successful analysis by EI and CI: The expected isotope pattern is observed, and the accurate mass of the EI molecular species gave additional evidence (calculated mass ^{11}B isotope $\text{M}^+ = 310.1119$; measured mass = 310.1119).

Conclusion

Our initial findings by this small study indicate that the method can be easily and effectively applied to a variety of analytes, and can be useful to confirm the identity of boronic acids when analysis of the unaltered species fails by alternative methods, or alternative ionisation modes are not available.

Footnote

Additional structures for which this method has been successful:



References

1. *Polyol Complexes and Structure of the Benzeneboronate Ion*, John P Lorand and John O Edwards, *J Org Chem*, **24**, 6, 769-774 (1959).
2. *Methods in Molecular Biology, vol. 147: Affinity Methods and Protocols*, Humana Press, Ed.: Pascal Bailon, George K. Ehrlich, Wen-Jian Fung and Wolfgang Berthold.
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