

## OVERVIEW

The LC-ESI-MS/MS detection of the low molecular weight antifungal benzoxaborole AN2718 can be significantly enhanced by derivatization with pinanediol.

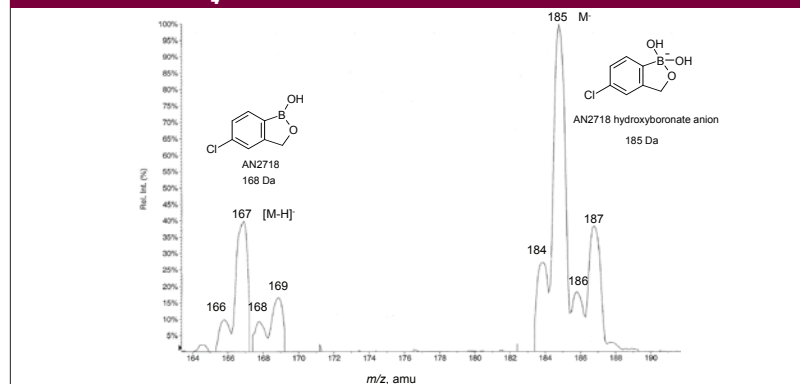
## INTRODUCTION

The benzoxaborole AN2718 (5-chloro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole) is derivatized with pinanediol at an alkaline pH that produces a negatively charged cyclic ester. Detection is by electrospray ionization operated in negative ion mode. AN2718, an antifungal agent, can be assayed directly in negative ion mode on a triple quadrupole mass spectrometer using the  $m/z$  167-43 transition. Due to the absence of a strong product ion the lower limit of quantitation using LC-ESI-MS/MS is approximately 20 ng/mL. Derivatization with pinanediol enhances the LLOQ to 1 ng/mL.

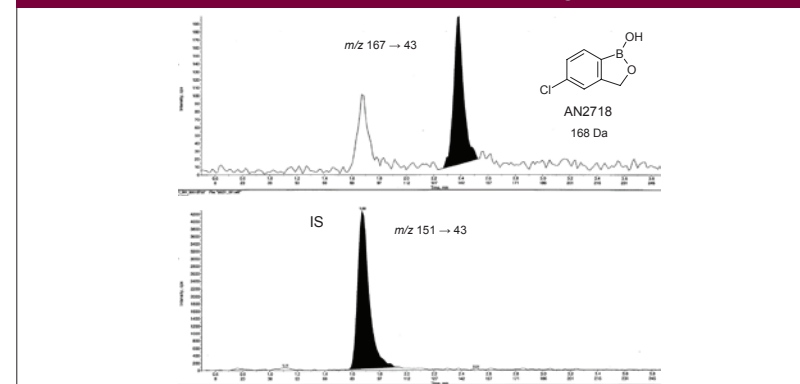
## METHODS

200  $\mu$ L of plasma was mixed with 25  $\mu$ L of the 5-F analogue internal standard, diluted with 100 mM  $\text{NH}_4\text{OAc}$ , pH 4.4, and applied to a 5 mg Oasis HLB SPE plate. The plate was washed with 300  $\mu$ L of 25:75 MeOH:10 mM  $\text{NH}_4\text{OAc}$ , pH 4.4, and eluted with 200  $\mu$ L of methanol. 30  $\mu$ L of 10 mg/mL (1S,2S,3R,5S) (+) pinanediol was added followed with 20  $\mu$ L of aqueous 7%  $\text{NH}_4\text{OH}$ , and then diluted with 250  $\mu$ L of 0.1%  $\text{NH}_4\text{OH}$ . 30  $\mu$ L was injected onto an XBridge Phenyl 2.1 x 100 mm column. MPA was 0.1%  $\text{NH}_4\text{OH}$ , MPB was MeOH with 0.1%  $\text{NH}_4\text{OH}$ , flow rate 0.6 mL/min, with a gradient of 35%B to 50%B over 2.5 minutes. A Sciex API-4000 using the TIS source was operated in negative ion mode using transitions  $m/z$  319-167 for AN2718 and  $m/z$  303-151 for the IS. Due to the relatively high pressure of 300 bar stainless steel tubing was used in front of the column.

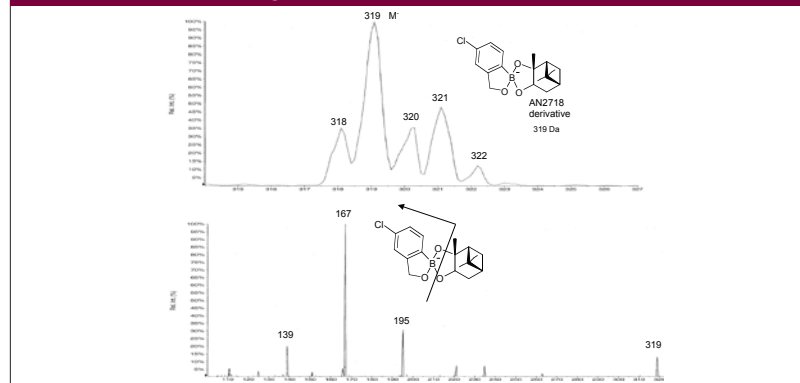
**Figure 2: Negative ESI background corrected mass spectrum of 900 ng/mL AN2718 in 10:1 MeOH:7%  $\text{NH}_4\text{OH}$ . DP = -20. Boron isotope abundance is 20%  $^{10}\text{B}$ , 80%  $^{11}\text{B}$**



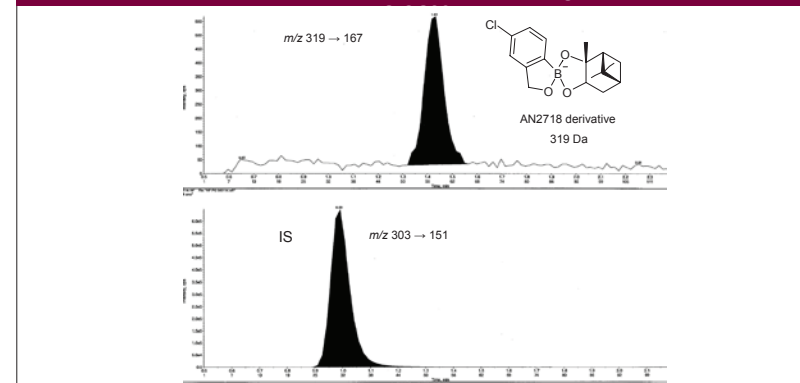
**Figure 5: Chromatogram of AN2718 at the 20 ng/mL LLOQ, 110 pg injected. The internal standard is the 5-F analogue**



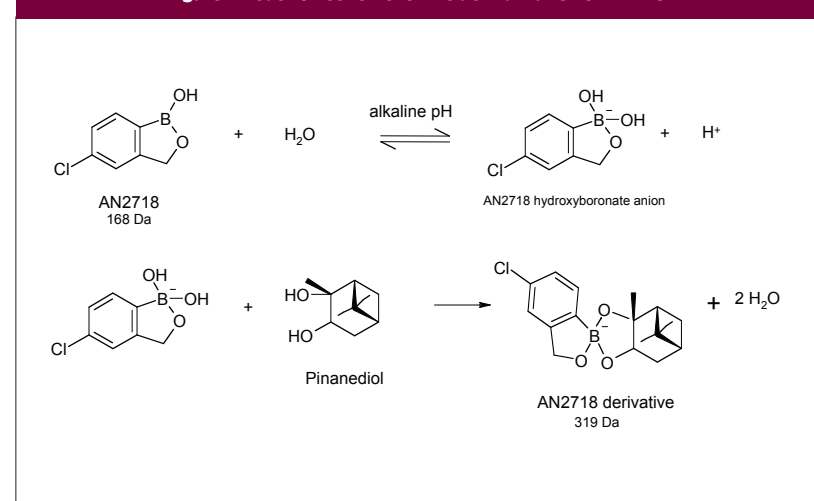
**Figure 3 Top: Negative ESI mass spectrum of the AN2718 derivative, DP = -100. Bottom: Negative product ion scan of  $m/z$  319, CE = -50**



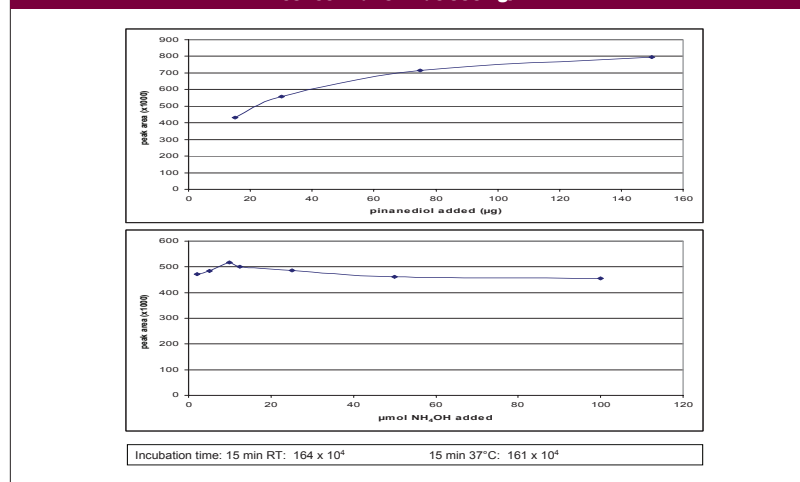
**Figure 6: Chromatogram of the AN2718 derivative at the 1 ng/mL LLOQ, 12 pg injected. The internal standard is the 5-F analogue**



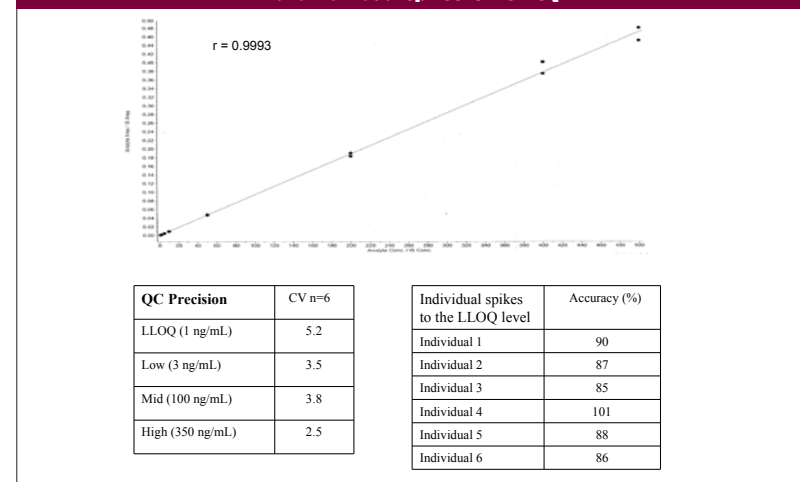
**Figure 1: Reaction scheme for the derivatization of AN2718**



**Figure 4: Effect of pinanediol,  $\text{NH}_4\text{OH}$ , and time/temperature on derivatization. AN2718 concentration was 500 ng/mL**



**Figure 7: Assay performance, standard curve of 1-500 ng/mL, QC precision, and individual spikes to the LLOQ**



## RESULTS AND DISCUSSION

Figure 1 shows the overall derivatization scheme.

Boronic acids, boronic esters and other oxygenated trivalent organoboron compounds are mild organic Lewis acids and at an alkaline pH will produce a negatively charged tetracoordinate hydroxyboronate anion<sup>1,2,3</sup> (Figure 1 top).

Figure 2 shows a negative ESI mass spectrum of this hydroxyboronate anion for the benzoxaborole AN2718. Note in Figure 2 that AN2718, in addition to Lewis acid character, also displays Brønsted acid character in an electrospray source operated in negative ion mode as noted by the deprotonated AN2718 ion at  $m/z$  167.

The tetracoordinate hydroxyboronate anion will react with diols to produce negatively charged cyclic esters<sup>1,2,3</sup>. Derivatization was tried with other diols including ethylene glycol, glycerol, serinol, tris(hydroxymethyl) aminomethane, 2,2-dimethyl-1,3-propanediol, and 2-amino-2-methyl-1,3-propanediol. The reaction was most complete using pinanediol, which has been shown to slow hydrolysis for boronic acid esters<sup>1</sup>.

Figure 3 top displays a negative ion ESI mass spectrum of the AN2718 pinanediol ester derivative. These diol derivatives of AN2718 are readily identified by both the boron isotope pattern which is 20:80  $^{10}\text{B}$ : $^{11}\text{B}$  and the  $^{35}\text{Cl}$ : $^{37}\text{Cl}$  isotope pattern. Figure 3 bottom is a product ion scan at  $m/z$  319 of the AN2718 derivative.

Figure 4 shows the effect of pinanediol,  $\text{NH}_4\text{OH}$ , and time/temperature on the derivatization. Note that the derivatization is insensitive to the amount of  $\text{NH}_4\text{OH}$  and to time/temperature, but is sensitive to the amount of pinanediol used.

Figure 5 shows chromatograms of non derivatized AN2718 at the 20 ng/mL LLOQ (110 pg injected) along with the internal standard, the 5-F analogue.

Figure 6 shows chromatograms of derivatized AN2718 and the derivatized 5-F analogue IS at the 1 ng/mL LLOQ (12 pg injected).

A one-day performance verification was performed and results are presented in Figure 7. The method was linear over a standard range of 1-500 ng/mL. Within-run CVs were 5.2% at the LLOQ of 1 ng/mL, and 3.5%, 3.8%, and 2.5% at the low, mid, and high QC level. There were no interfering peaks at the retention time of the analyte or the IS with pooled human plasma or with 6 individual human plasma lots. Matrix effects were assessed by spiking individual lots to the LLOQ level and the accuracy ranged from 85 to 101%. The S/N at the LLOQ was approximately 35:1 (peak to peak). The internal standard response was consistent throughout the run. Sufficient chromatography is required to resolve the derivatized AN2718 and the pinanediol. If the retention times of derivatized AN2718 and pinanediol overlap the sensitivity of derivatized AN2718 is increased but the signal varies with the degree of pinanediol retention time overlap, leading to imprecise results.

## CONCLUSIONS

The benzoxaborole AN2718 can be derivatized with pinanediol and the LC-ESI-MS/MS sensitivity is enhanced. The derivatization reaction proceeds via a tetracoordinate hydroxyboronate anion that reacts with pinanediol to produce a negatively charged cyclic ester.

## REFERENCES

- 1) *Boronic Acids*; Hall, Dennis G. Ed.; 2005, WILEY-VCH.
- 2) Springsteen, G. and Wang, B. *Tetrahedron*, 2002, 58, 5291-5300.
- 3) Yan, J., Springsteen, G., Deeter, S., and Wang, B. *Tetrahedron*, 2004, 60, 11205-11209.