Journal of Pharmaceutical and Biomedical Analysis 64–65 (2012) 49–55

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development and validation of non-aqueous capillary electrophoresis methods to analyze boronic esters and acids

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ARTICLE INFO

Article history:
Received 6 September 2011
Received in revised form 27 January 2012
Accepted 30 January 2012
Available online 14 February 2012

Keywords:
Nonaqueous capillary electrophoresis
NACE
Boronic ester
Boronic acid

ABSTRACT

Boronic esters and acids are potential intermediates in the manufacture of many active pharmaceutical ingredients (API). Accurate quantitation of the intermediate is necessary to assure the stoichiometry of the reaction. The analysis of these compounds is challenging due to their labile nature. For example, the boronic ester can hydrolyze to the acid during storage, when exposed to moisture in the air, during sample preparation and analysis, and thus give erroneous ester results. Traditional analytical techniques like gas chromatography (GC), normal phase chromatography (NPLC), hydrophilic interaction chromatography (HILIC), and reversed phase liquid chromatography (RPLC) have been utilized but with noted limitations such as poor peak shape, variation in retention times, and evidence of hydrolysis. All of these limitations impact accurate quantitation needed for selected situations. For the proprietary boronic ester evaluated here, these traditional techniques were insufficient for the accurate determination of assay and residual boronic acid. Non-aqueous capillary electrophoresis (NACE) is an accurate quantitative technique that can be used to analyze boronic esters and their corresponding acids without the limitations noted for traditional analytical techniques. The present study describes the development of methodology for the determination of the potency of a proprietary boronic ester as well as methodology for the determination of residual boronic acid in the ester. In addition, nine model boronic ester and acid pairs with a range in polarity, based on the electronic properties of the attached side group, were tested to evaluate and demonstrate the general applicability of these conditions. Under the conditions used for potency, all ten pairs had a resolution between the boronic ester and acid of greater than 1.5, acceptable peak shape for the boronic ester (tailing factor of less than 2.0), and a run time of less than 3 min. In addition, this work describes the development of methodology to determine residual levels of boronic acids in the corresponding boronic ester. Using the ten boronic ester and acid pairs, eight of the ten pairs were shown to have acceptable sensitivity (S/N of 10 or better at 0.5%) and spike recoveries (within the range of 80–120%). The potential for hydrolysis during analysis was also addressed by using a subset of the ten boronic ester and acid pairs and spiking water into the diluent. There was no observed conversion of the ester to the acid. The lack of hydrolysis during analysis and the high success in separating and validating these methods for the boronic ester and acid pairs supports the utility of NACE as a technique for the analysis of boronic esters and acids.

1. Introduction

Boronic esters and acids are potential intermediates in the manufacture of many active pharmaceutical ingredients (API). The recent Nobel Prize work by Heck, Negishi, and Suzuki highlights the utility of boron compounds in reactions such as the Suzuki coupling, where an organoboronic acid or ester is reacted with a halide compound in the presence of a palladium catalyst and a base in a carbon-carbon bond forming step [1–8]. Boronic acids are often considered for these types of reactions because they have good stability, are easy to use, have low toxicity, and are considered "green" compounds due to their ultimate degradation into environmentally friendly boric acid. The mild organic Lewis acidity and moderate chemical reactivity of boronic acids are additional properties that make this class of compounds attractive synthetic intermediates. The less appealing characteristic of boronic acids is their tendency to exist as mixtures of oligomeric anhydrides. Therefore, their corresponding boronic esters tend to be the preferred synthetic intermediates [9]. Boronic esters, however, can be easily hydrolyzed back to their acid form when exposed to trace levels of water. In spite of these concerns, boronic esters and acids are widely used in the pharmaceutical industry as coupling reagents in

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doi:10.1016/j.jpba.2012.01.042

the chemical synthesis of API. Unfortunately, the analytical techniques often used to assess the quality of those chemical reactions are often limited, which is the case for the work described here. Two analytical methods were needed for a proprietary boronic ester 'J' (see Table 1), which was being used in the chemical synthesis of an API: a potency method to determine reaction stoichiometry and a method to determine residual boronic acid in the boronic ester. The presence of water from the reaction or environment would result in the formation of boronic acid, which could impact quality and yield in subsequent steps of the synthetic route. Development of accurate quantitative methods is critical to the success of the chemical synthesis. Unfortunately, these methods proved challenging due to the tendency for boronic esters to hydrolyze to the boronic acid during sample handling, preparation, and analysis.

The literature devoted to the analysis of boronic acids and esters is limited. Haken and Abraham utilized gas chromatography (GC) with both a flame ionization detector (FID) and mass spectrometer (MS) to analyze alkyl borate and boronate esters. They found that even trace levels of water in the carrier gas could result in hydrolysis of the esters, especially acyclic boronate esters [10]. Rose et al. also used GC to analyze aromatic boronic acids by using propane-1,3-diol and an on-column derivatization technique. Though this method was successful, it had limitations such as peak tailing and variation in retention times [11]. Reversed phase liquid chromatography (RPLC) was utilized by Xu et al. to analyze pharmaceutical related boronic acid and boronic pinacol ester functionalized compounds. They found that by utilizing short columns and fast run times, the amount of on-column hydrolysis could be minimized [12]. Although GC and fast RPLC provide options for analysis of these labile compounds, the problem of hydrolysis, peak tailing, and variability, still limit their use for accurate quantitative analysis. Similar limitations were observed when evaluating the traditional analytical techniques (GC, RPLC, HILIC, NPLC) for the analysis of boronic ester 'J' (see Table 1); these techniques each had limitations such as on-column hydrolysis, solution instability, poor peak shapes, lack of resolution, and/or lack of sensitivity, that made them not viable for this analysis. Thus, for this boronic ester, an alternative technique was required. Non-aqueous capillary electrophoresis (NACE) is an alternative technique that can be used to evaluate water sensitive compounds like boronic esters.

The use of CE and non-aqueous background electrolytes (BGEs) was first demonstrated in 1984 by Walbroehl and Jorgenson and then again in 1986 in their separation of neutral organic molecules [13,14]. Since that time, NACE has continued to grow in popularity and been applied to a multitude of different compounds [15–17]. Typically, NACE utilizes a BGE consisting of ammonium acetate or formate in an organic solvent like methanol or acetonitrile. The separation of two analytes by CE is dependent on a difference in their electrophoretic mobilities. The electrophoretic mobility is defined as the ratio of the charge of the analyte (q) to the molecular weight or solvation size of the analyte (r) [15]. Organic solvents alter the q/r ratio of an analyte as well as the selectivity, by influencing the solvation size, modifying the pK_a value, or allowing ion pairing to occur [15,16]. NACE also has the potential for enhanced efficiency. High ionic strength BGEs allow for stacking opportunities in the injection zone and enhanced sensitivity [16]. In addition, the use of less viscous organic solvents allows higher voltages to be applied and analysis times to be reduced without the generation of a large electric current or subsequent Joule heating [15,16].

In those cases where the q/r ratio of two analytes cannot be differentiated, micellar electrokinetic chromatography (MEKC) is required. A pseudo stationary phase is formed via the addition of an additive to the BGE. Neutral analytes, which would not migrate on their own, will heteroconjugate with an additive, thus causing them to migrate toward the detector. The difference in the strength of the heteroconjugation results in the separation of the analytes [18]. The interaction of an additive and analyte in an organic solvent system is very complicated and multidimensional. A critical review of these type of interactions has been completed by Chen [19]. Despite the complexity of the interactions, additives have been utilized to separate a variety of compounds. For example, Tjornelund and Hansen successfully used cationic additives to separate neutral substances [20] and Li and Fritz were successful in separating non-ionic organic compounds using anionic additives [21].

The present study shows the utility of NACE for the determination of the potency of boronic ester 'J' (see Table 1) as well as to determine the residual amount of boronic acid in the boronic ester without the limitations noted with the traditional analytical techniques. In addition, nine model boronic ester and acid pairs with a range in polarity, based on the electronic properties of the attached side group (see Table 1), were tested to evaluate and demonstrate the general applicability of these conditions. An evaluation of linearity, reproducibility, sensitivity, and accuracy of the methods developed is reported.

2. Experimental

2.1. Chemicals

HPLC grade methanol (MeOH) and acetonitrile (ACN) were purchased from Burdrick and Jackson (Muskegon, MI). Solvents used were low in water content: MeOH contained 70 ppm water and the ACN contained 10 ppm water. Sodium hydroxide (1 N NaOH) was purchased from Red Bird (Batesville, IN). Glacial acetic acid (HOAc) was purchased from Mallinkrodt Baker (Phillipsburg, NJ). Ammonium acetate (NH_4OAc) and sodium dodecyl sulfate (SDS) were purchased from Fluka (Milwaukee, WI). Water was deionized and filtered through a Milli-Q^{TM} water purification system from Millipore (New Bedford, MA) and was used to rinse the capillary. Model boronic esters and acids were purchased from Sigma Aldrich (Milwaukee, WI), Fluka (Milwaukee, WI), and Boron Molecular (Research Triangle, NC). Proprietary boronic ester and acid were provided by Eli Lilly and Company (Indianapolis, IN).

2.2. Instrumentation

An Agilent HP3D capillary electrophoresis system with online diode array detector (Agilent Technologies, Santa Clara, CA) was used throughout this work. The optimum UV maximum for each boronic ester and acid pair was determined and used as the detection wavelength. The separation was performed in an Agilent uncoated fused-silica capillary that was 33 cm × 50 μm, with a length to the detector of 24.5 cm. The temperature of the capillary was kept constant at 25 °C. Samples were injected by applying a pressure of 50 mbar for 4 s or 25 mbar for 2 s. Applied voltages of −25 kV or +25 kV were utilized. Electropherograms were collected using a data collection rate of 10 points/s and plotted by Empower 2 chromatography data software (Waters Corporation, Milford, MA).

2.3. Electrophoresis media

Two BGEs were prepared. One BGE contained ACN with 2.5 mM NH_4OAc and 20 mM HOAc and the other BGE contained ACN:MeOH (90:10, v/v) with 2.5 mM NH_4OAc, 20 mM HOAc, and 10 mM SDS. The quality of the NH_4OAc and HOAc was found to be critical to achieving reproducible separations. It is therefore recommended that only reagents with the same quality be used once a separation is developed. Each BGE was filtered through a 0.45 μm syringe filter (Millipore Corporation, Bedford, MA).

New capillaries were conditioned and cleaned as necessary with Milli-Q^{TM} water and 0.1 N NaOH for 10 min each and then flushed with BGE for 20 min. The capillary was allowed to sit in BGE and

Table 1
A list of the boronic esters and acids separated using NACE.

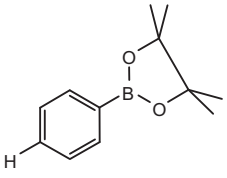
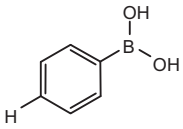
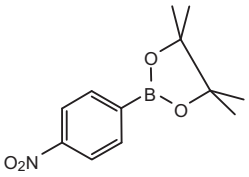
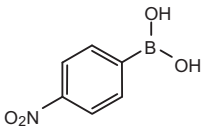
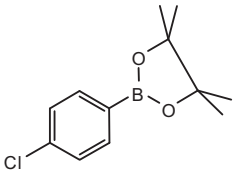
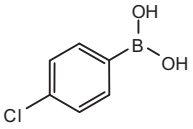
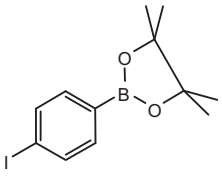
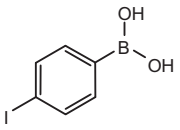
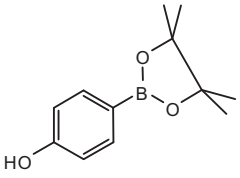
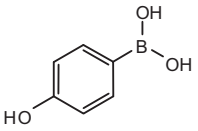
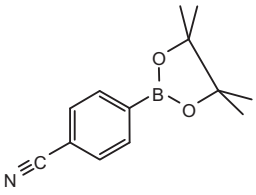
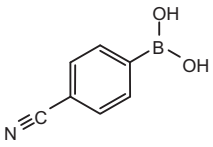
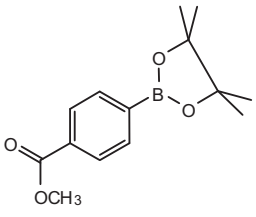
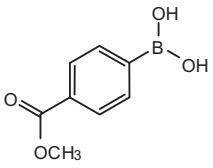
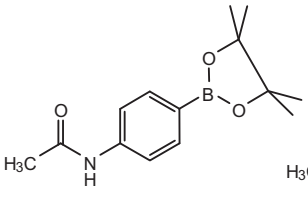
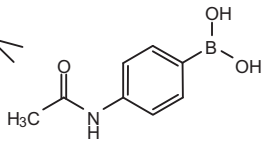
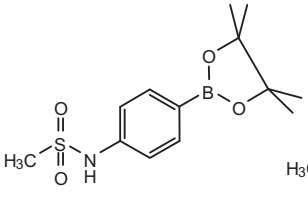
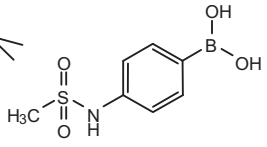
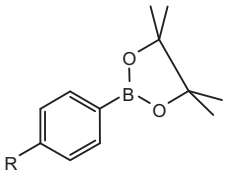
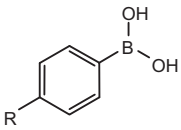
A		
B		
C		
D		
E		
F		
G		
H		
I		

Table 1 (*Continued*)

J		

where R is a proprietary structural group

Table 2
The results of the BGE screen are shown below. "Y" indicates that the resolution between the boronic ester and boronic acid was greater than 1.5. "N" indicates that the resolution between the boronic ester and boronic acid was less than 1.5. Conditions: 33 cm (total length)/24.5 cm (length to detector) × 50 μm uncoated capillary, 25 °C, 25 mbar × 2 s injection, +25 kV, UV at analyte maximum.

BGE	#1 1 mM NH_4OAc + 10 mM HOAc Resolution > 1.5	#2 2.5 mM NH_4OAc + 10 mM HOAc	#3 5 mM NH_4OAc + 10 mM HOAc	#4 2.5 mM NH_4OAc + 20 mM HOAc	#5 2.5 mM NH_4OAc + 30 mM HOAc
A	N	N	Y	Y	Y
B	Y	Y	Y	Y	Y
C	N	Y	Y	Y	Y
D	N	Y	Y	Y	Y
E	N	N	Y	Y	Y
F	N	Y	Y	Y	Y
G	N	Y	Y	Y	Y
H	N	N	Y	Y	Y
I	N	Y	Y	Y	Y
J	N	N	Y	Y	Y

equilibrate, as needed, for 30 min to overnight. Prior to the first injection, the capillary was flushed for 20 min with BGE and flushed for 1.5 min prior to each additional sample injection. Electrolyte vials were replenished after every six injections except for when SDS was included in the BGE and then the electrolyte vials were replenished after every injection.

2.4. Sample preparation

Stock solutions of the boronic esters were prepared at 1–2 mg/mL in ACN. Stock solutions of the boronic acids were prepared at either 1–2 mg/mL or 0.1–0.2 mg/mL in ACN. Stock solutions were mixed, when appropriate, and diluted to the desired concentrations using ACN.

3. Results and discussion

3.1. Development of boronic ester potency CE method

A method to separate the boronic esters from their corresponding acids (see Table 1) was needed in order to accurately determine the potency of the esters. In order to develop such a method, the appropriate BGE and separation parameters had to be determined. The hypothesis was made that a non-aqueous BGE with apparent pH greater than 3 would result in the silanols on the capillary wall to be predominately negatively charged. The positively charged cations from the BGE would then interact with the negatively charged silanols, and a positively charged voltage applied at the inlet would generate an electroosmotic flow (EOF) via cationic migration toward the detector. Likewise, it was also hypothesized that a BGE with an apparent pH greater than 3 would result in both the ester and acid being neutral, but the difference in their size would result in the boronic ester migrating faster than the boronic acid. A screening approach was used to determine an appropriate BGE. Five different BGE solutions were utilized that varied the ammonium acetate concentration from 1 mM to 5 mM and the acetic acid concentration from 10 mM to 30 mM. The 5 min run time and the instrument automation allowed for quick execution of the screen. All 10 compounds were screened in less than 13 h. The results of the screen are found in Table 2.

As the concentration of ammonium acetate or acetic acid was increased, the resolution for each ester and acid pair improved. When the BGE with the lowest ammonium acetate and acetic acid concentrations (BGE#1) was used, only 1 pair had a resolution greater than 1.5. On the other hand, when BGE #3, #4, and #5 were used, which had higher concentrations of ammonium acetate and/or acetic acid all the pairs had a resolution greater than 1.5. Resolution was not the only parameter considered when determining the optimum BGE. The overall analysis time and the peak shape of the analytes were also taken into consideration. As the concentration of ammonium acetate or acetic acid was increased, the migration time of each analyte also increased. The peak shape was directly affected by the amount of time the analytes spent in the capillary. Those analytes with longer migration times experienced more band broadening than those that spent less time in the capillary. Thus, the 2.5 mM ammonium acetate with 20 mM acetic acid (BGE #4) was chosen as the optimum BGE because it provided the best resolution between the ester and acid, best peak

Table 3
The reproducibility of the CE method as demonstrated by determining the %RSD (n = 6) for the migration time ratio of the ester versus the acid and time corrected peak area of the ester.

	%RSD of MT ratio (n = 6)	%RSD of time corr area (n = 6)
A	1.4	1.3
B	10.2	0.9
C	2.3	1.5
D	2.2	2.0
E	0.8	1.9
F	4.5	2.0
G	2.3	2.5
H	1.0	1.4
I	1.5	1.5
J	0.9	1.8

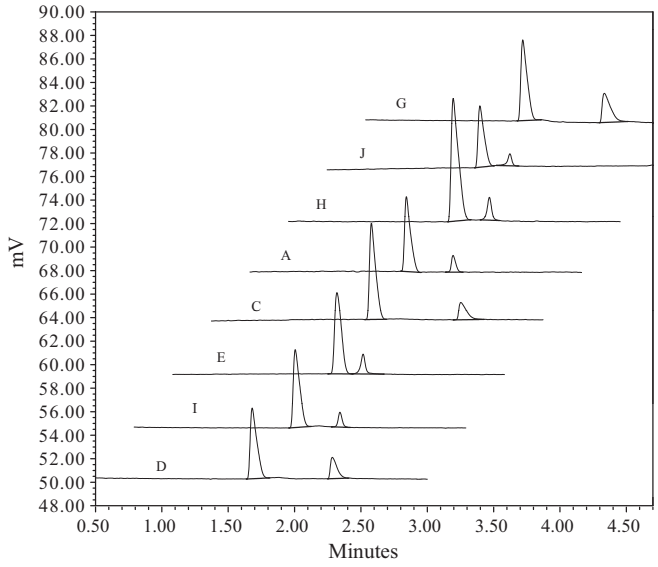


Fig. 1. An overlay of the boronic ester and acid pairs separated using the CE method. Conditions: 33 cm (total length)/24.5 cm (length to detector) × 50 μm uncoated capillary, 25 °C, 25 mbar × 2 s injection, +25 kV, UV at analyte maximum. BGE: 2.5 mM NH_4OAc + 20 mM HOAc in ACN. Ester @ 0.1 mg/mL, acid spiked at 0.01 mg/mL (10%).

shape for the ester, and with the shortest analysis time. For all ester and acid pairs tested, the resolution between the boronic ester and acid was greater than 1.5, the tailing factor for each boronic ester was less than 2.0, and the analysis time was only 3 min. Fig. 1 shows the separation for 8 of the 10 pairs. The 2 pairs not represented in Fig. 1 are compounds B and F. The boronic esters of these compounds displayed typical migration time and peak shape as those in Fig. 1 but their boronic acids had extremely long migration times, which resulted in peak shapes that were so broad that they were practically undetectable as shown in Fig. 2. The good resolution between the boronic ester and boronic acid, peak shape of the boronic ester, and short analysis time, supported the use of the 2.5 mM ammonium acetate with 20 mM acetic acid (BGE #4) for boronic ester potency determinations (see Section 3.3 for validation).

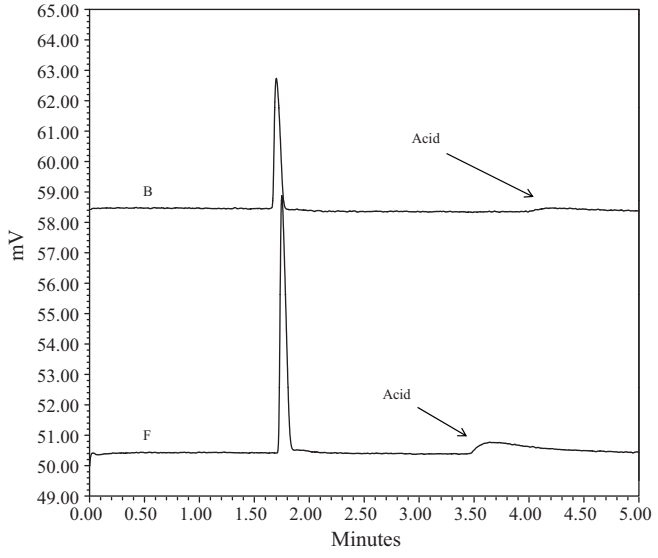


Fig. 2. An overlay of the boronic ester and acid pairs separated using the CE method. Conditions: 33 cm (total length)/24.5 cm (length to detector) × 50 μm uncoated capillary, 25 °C, 25 mbar × 2 s injection, +25 kV, UV at analyte maximum. BGE: 2.5 mM NH_4OAc + 20 mM HOAc in ACN. Ester @ 0.1 mg/mL, acid spiked at 0.01 mg/mL (10%).

3.2. Development of MEKC method to detect residual boronic acid

The ability to accurately determine the potency of a boronic ester is necessary in order to determine its quality. Water from the environment, sample handling, sample preparation, or the reaction itself can produce boronic acid and so the amount of residual boronic acid is another piece of data that is needed to determine the quality of the boronic ester. This determination can be challenging because the presence of water will cause the boronic ester to hydrolyze to the acid during analysis. In order to confirm that the boronic esters were not hydrolyzing due to trace levels of water in the solvents, a subset of the boronic esters from Table 1 were prepared using ACN that contained 10 ppm water that was used as-is or spiked with 50, 100, or 200 ppm water. Using the potency method conditions developed in Section 3.1, which can easily detect 10% boronic acid as shown in Fig. 1, the esters were analyzed immediately, 8 h, and 24 h after preparation. The results showed that there was no conversion of boronic ester to acid over a 24 h period for any of the boronic ester solutions tested. Thus, NACE does not have the propensity for the hydrolysis that is observed by other techniques within the range of 10–210 ppm water.

The water spiking experiment confirmed that during analysis, the boronic acid was not being formed in the predominately non-aqueous environment and that NACE was an appropriate technique for determining residual boronic acid. While a sample concentration of 0.1 mg/mL provides appropriate conditions for determining potency of the boronic ester, a higher sample concentration was needed to provide sufficient sensitivity to determine residual boronic acid. However, when the sample concentration was increased to 1 mg/mL, the boronic ester peak broadened such that the resolution for the boronic acid peak was no longer sufficient. Therefore, the best approach for being able to detect the acid at a low level was to reverse the migration order by reversing the polarity of the applied voltage. Reversal of the applied voltage also results in a reversal of the EOF in the direction of the inlet. The addition of a negatively charged additive, like SDS, was needed to overcome the reversed EOF. The negatively charged SDS forms a heteroconjugate with the analytes which migrate against the EOF away from the negatively charged inlet and toward the detector. As seen in Figs. 3 and 4, applying −25 kV at the inlet and adding 10 mM SDS and 10% (v/v) MeOH to the BGE (to aid with solubility of the SDS), resulted in the boronic acid migrating more quickly than the boronic ester. For all pairs tested, the resolution between the boronic ester and acid was greater than 1.5, the tailing factor for each boronic acid was less than 2.0, and analysis time was less than 15 min. Sensitivity was also assessed by determining the S/N of a 0.5% boronic acid solution. The S/N was greater than or equal to 10 for eight of the ten boronic acids tested (see Section 3.3 for validation). The good resolution between the boronic ester and acid, peak shape of the boronic acid, and S/N of a 0.5% boronic acid solution, supported the use of these MEKC conditions for residual boronic acid quantitation.

3.3. Validation

Due to its simplicity and speed of analysis, the CE method conditions (see Section 3.1; Figs. 1 and 2) were selected as the best system to determine the potency of the boronic esters. In order to validate these method conditions, the two most important parameters for potency determinations, reproducibility and linearity, were assessed. Linearity for each boronic ester was evaluated from 50% to 150% of nominal concentration (0.05–0.15 mg/mL) and the *R* square for each curve was calculated. The linearity was judged to be acceptable (*R* square greater than 0.999) for each boronic ester tested. *R* square values ranged from 0.9990 to 1.000. To evaluate the

Table 4
The accuracy of the MEKC method as demonstrated by calculating the recovery of the acid spiked at the concentrations of 0.001 mg/mL, 0.002 mg/mL, and 0.004 mg/mL, and the sensitivity of the MEKC method as demonstrated by calculating the S/N at 0.001 mg/mL. Compounds B and F were omitted because the S/N at 0.05% was less than 10.

	S/N @ 0.5%	Recovery @ 0.5%	Recovery @ 1%	Recovery @ 2%	Average recovery ($n = 3$)
A	17	111%	103%	124%	112%
C	12	107%	108%	113%	109%
D	16	91%	95%	94%	93%
E	27	111%	103%	107%	107%
G	10	98%	111%	87%	99%
H	10	116%	110%	103%	110%
I	17	98%	105%	106%	103%
J	10	98%	103%	114%	105%

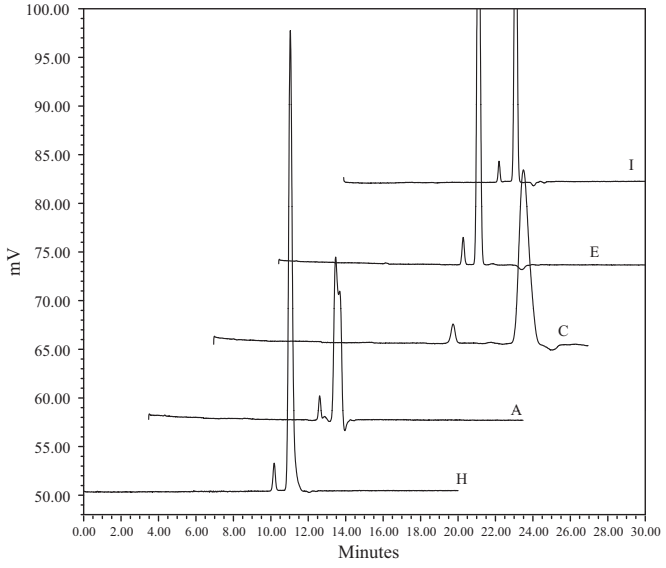


Fig. 3. An overlay of the boronic ester and acid pairs separated using the MEKC method. Conditions: 33 cm (total length)/24.5 cm (length to detector) × 50 μm uncoated capillary, 25 °C, 50 mbar × 4 s injection, −25 kV, UV at analyte maximum. BGE: 2.5 mM NH_4OAc + 20 mM HOAc + 10 mM SDS in 10/90 MeOH/ACN. Ester @ 0.2 mg/mL, acid spiked at 0.004 mg/mL (2%).

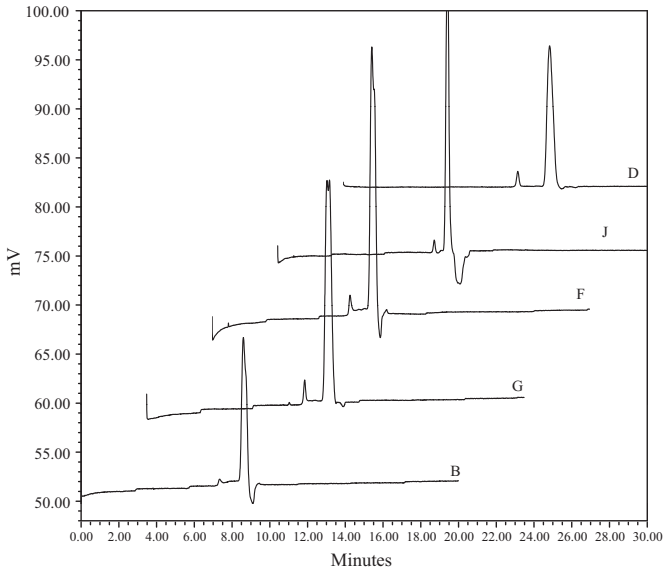


Fig. 4. An overlay of the boronic ester and acid pairs separated using the MEKC method. Conditions: 33 cm (total length)/24.5 cm (length to detector) × 50 μm uncoated capillary, 25 °C, 50 mbar × 4 s injection, −25 kV, UV at analyte maximum. BGE: 2.5 mM NH_4OAc + 20 mM HOAc + 10 mM SDS in 10/90 MeOH/ACN. Ester @ 0.2 mg/mL, acid spiked at 0.004 mg/mL (2%).

reproducibility, six replicate injections of a solution containing the boronic ester at nominal concentration (0.1 mg/mL) spiked with boronic acid at 10% of nominal concentration (0.01 mg/mL) were made. The time corrected area (area of the boronic ester divided by its migration time) of each injection was calculated and the %RSD determined. The %RSDs were found acceptable (%RSD ≤ 3.0%). As seen in Table 3, the %RSD ranged from 0.9% to 2.5%. The reproducibility of the migration time (MT) was also assessed. Since CE systems are known to experience high drift due to the BGE becoming depleted of ions over time, the boronic acid was added to the solution at 10% of nominal concentration (0.01 mg/mL) to serve as an internal standard. The ratio of the boronic ester MT to boronic acid MT was calculated and the %RSD determined. As shown in Table 3, the %RSD for the MT ratio ranged from 0.8% to 10.2%. The two highest %RSDs were for compounds B and F at 10.2% and 4.5%, respectively. The corresponding acids for compounds B and F have very poor peak shape (see Fig. 2), which prevented accurate integration. If you disregard compounds B and F, since their MT ratio cannot accurately be determined, then the %RSD for the MT ratio of the remaining 8 compounds ranged from 0.8% to 2.3%.

The CE method conditions lacked the sensitivity required for low level determinations and therefore the MEKC method conditions (see Section 3.2; Figs. 3 and 4), though more complex and with a longer analysis time, were determined to be the best system to determine the amount of residual boronic acid in its corresponding boronic ester. An external standard approach was utilized where a calibration curve was used to quantitate the amount of boronic acid present in the ester. The three most important parameters for low level determinations were used to validate these method conditions (linearity, sensitivity, and accuracy). Linearity for the boronic acid was evaluated from 0.5% to 5% of nominal concentration (0.001–0.004 mg/mL), and the *R* square for each curve was calculated. The linearity was acceptable (*R* square greater than 0.997) for each boronic acid tested; *R* square values ranged from 0.9980 to 0.9995. The S/N values for each boronic acid at 0.5% (0.001 mg/mL) were determined and are documented in Table 4. All S/N values were greater than or equal to 10, except for compound B, which was not detected, and compound F, which had a S/N of 9. The S/N values for these two boronic acids at 2% (0.004 mg/mL) were extrapolated to determine the concentration where the S/N value would be 10. The concentrations that would provide a S/N value of 10 for compounds B and F were 0.006 mg/mL (3%) and 0.0014 mg/mL (0.7%), respectively. Since the S/N value at 0.5% was less than 10 for compounds B and F, they were not included in the accuracy study represented in Table 4. The accuracy of the method was determined by spiking a known amount of boronic acid into a boronic ester solution and then using the calibration curve to determine the amount present. The calculated recoveries are shown in Table 4. The recoveries for each compound were averaged across the 3 levels and found to be acceptable (80–120%) for each boronic acid tested; average recoveries ranged from 93% to 112%.

4. Conclusions

The quality of API is often determined by the quality of the intermediates in the process, therefore the development of in-process control and quality indicating methods for boronic esters and acids is an important piece in the overall impurity control strategy that use these compounds. NACE has been shown here to be an accurate and quantitative technique for the analysis of boronic esters and acids. CE and MEKC conditions were developed to separate boronic esters from their corresponding boronic acids and in both separation modes, acceptable method performance was demonstrated. The CE conditions for potency determinations were shown to have acceptable linearity (*R* square greater than 0.999) and appropriate reproducibility for all boronic esters except compounds B and F, for which the peak shape of the boronic acid prevented accurate integration and calculations. The MEKC conditions for residual boronic acid determinations were shown to have acceptable linearity (*R* square greater than 0.997), sensitivity (S/N of 10 or better for 0.5% solution), and spike recoveries (80–120%) for 8 of the 10 compounds. The S/N at 0.5% for compounds B and F did not meet the required value of 10 or greater. Extrapolation of the data showed that a S/N of 10 could be achieved at 3% and 0.7%, respectively, for these two boronic acids. In addition, the lack of conversion of the boronic ester to acid over a 24 h period when up to 200 ppm of water was spiked into the diluent further supports that NACE does not have the significant hydrolysis issues observed by other analytical techniques.

The high success rate in separating all the boronic esters from their boronic acids and successfully validating the methods for 8 out of the 10 boronic ester and acid pairs supports the utility of NACE as a technique for the analysis of boronic esters and their corresponding boronic acids. NACE provides a general methodology for the determination of boronic esters and acids with good peak shape, resolution, sensitivity, stability, and accurate results.

Acknowledgments

The authors acknowledge discussion and input from Eric Jensen, Ph.D. and Steven Baertschi, Ph.D.

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