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Strategies for the analysis of highly reactive pinacolboronate esters

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ABSTRACT

Pinacolboronate esters (or boronic acid, pinacol esters) are widely used in the Suzuki coupling reaction to connect organic building blocks for the total synthesis of complex molecules. The 2-aminopyrimidine-5-pinacolboronate ester was used as a starting material in the synthesis of a development compound, necessitating a chromatographic purity method to assess its quality. This aryl pinacolboronate ester posed unique analytical challenges due to its facile hydrolysis to the corresponding boronic acid, which is non-volatile and poorly soluble in organic solvents. This made GC and normal-phase HPLC analysis unsuitable. In reversed-phase mode, typical sample preparation and analysis conditions promoted rapid sample degradation to the boronic acid. To overcome these challenges, unconventional approaches were necessary in order to stabilize 2-aminopyrimidine-5-pinacolboronate ester, adequately solubilize its boronic acid, and produce acceptable separation and retention. The final method employed non-aqueous and aprotic diluent, and a reversed-phase separation using highly basic mobile phases (pH 12.4) with an ion pairing reagent. These strategies were successfully applied to several other reactive pinacolboronate esters for purity analysis, demonstrating broad applicability to this unique class of compounds.

1. Introduction

Since its discovery in 1979, the Suzuki–Miyaura cross-coupling reaction (or Suzuki coupling) has become one of the most efficient methods for carbon–carbon (C—C) bond formation [1–3], especially for biaryl construction [4,5]. The palladium-catalyzed Suzuki coupling is a reaction of organoboron compounds with organic halides or pseudohalides such as triflates. In Suzuki coupling, a wide variety of functional groups can be tolerated under relatively mild reaction conditions. The starting materials containing a broad range of building blocks for Suzuki coupling are commercially available. Furthermore, the boron-containing by-products, which show only low toxicity, are easy to purge from the final products [6]. Due to all these key features, Suzuki coupling stands out as a highly attractive tool for the synthesis of pharmaceuticals or fine chemicals, not only in research laboratories but also in industrial manufacturing [4,7].

Boronic acids have been used as the nucleophilic reagent for Suzuki coupling [8]. However, the boronic acid functionality is vulnerable to a number of degradation pathways, such as oxidation, protodeboronation, and polymerization [5,9,10]. Thus, boronic acids have been masked by replacing the hydroxyl ligands with more electron donating groups, such as pinacol, to make the corresponding boronate esters. The pinacol-masked boronic acids (i.e., the pinacolboronate esters) are easy to purify, relatively stable to air and moisture, and do not require a formal deprotection step for Suzuki coupling [5,11–13].

The analysis of pinacolboronate esters brings unique challenges. These compounds degrade in aqueous solution primarily to the corresponding boronic acids, to an extent dictated by the electron donating/withdrawing capabilities of the attached aryl or alkyl functionalities [14] and the solution pH [15–17]. The boronic acid products are generally less volatile and less soluble in organic media compared to boronate esters. These factors greatly restrict the options available for developing a single method that is capable of determining both analytes simultaneously. This is evident in the limited reports of effective purity or stability-indicating methods available in the literature, despite the enormous popularity of Suzuki coupling in organic synthesis. A fast reversed-phase HPLC method (5-min run time), which mitigated the on-column hydrolysis of several pinacolboronate esters, has been reported [18]. However, this approach may be ineffective for the analysis of pinacolboronate esters with higher reactivity or a more complex impurity profile. Additionally, the acetonitrile diluent used in that study may be inadequate to solubilize hydrophilic impurities, such as the corresponding boronic acid.

Recently, the 2-aminopyrimidine-5-pinacolboronate ester (**1**, see Fig. 1) was used as a starting material in the manufacture of a developmental active pharmaceutical ingredient (API), requiring the development of an effective purity method to assess its quality. Unconventional approaches in sample preparation and HPLC analysis were implemented in order to analyze this highly reactive aryl pinacolboronate ester. The selection of suitable analytical

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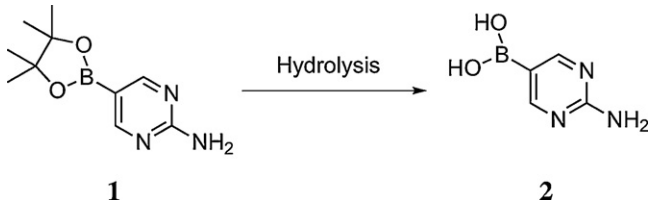

1 2

Fig. 1. The hydrolysis of 2-aminopyrimidine-5-pinacolboronate ester (**1**) to its primary degradant 2-aminopyrimidine-5-ylboronic acid (**2**).

technologies, the method development and optimization, and the application of this methodology to the analysis of other reactive pinacolboronate esters are discussed in detail.

2. Experimental

2.1. Chemicals

Potassium phosphate tribasic (K_3PO_4, ACS grade), tetrabutylammonium bisulfate (Bu_4NHSO_4, ACS grade), sodium hydroxide pellets (NaOH, HPLC grade), *N,N*-dimethylformamide (DMF, anhydrous, HPLC grade), and dichloromethane (DCM, anhydrous, HPLC grade) were from Sigma–Aldrich (St. Louis, MO, USA). Deionized water was from an in-house Milli-Q (Millipore, Billerica, MA, USA) water purification system. Acetonitrile (ACN, HPLC grade) was from EMD Chemicals (Gibbstown, NJ, USA).

The 2-aminopyrimidine-5-pinacolboronate ester and 2-aminopyrimidin-5-yl boronic acid were from Boron Molecular (Research Triangle Park, NC, USA). The 2-methyl-3-aminophenylpinacolboronate ester and 2-methyl-3-aminophenylboronic acid were internally synthesized at Genentech (South San Francisco, CA, USA). The 1-methyl-1*H*-pyrazole-4-pinacolboronate ester and 1-methyl-1*H*-pyrazole-5-pinacolboronate ester, 2-(ethoxycarbonyl)vinyl pinacolboronate ester and allenyl pinacolboronate ester were from Frontier Scientific, Inc. (Logan, UT, USA).

2.2. Sample preparation

The sample diluent DMF/DCM (50/50, v/v) was prepared by mixing the 1:1 volume ratio of anhydrous DMF and DCM and dried with molecular sieve desiccants (Sigma–Aldrich, St. Louis, MO, USA) overnight before use. The pinacolboronate ester solutions were prepared in the sample diluent at the concentration of ~2.0 mg/mL.

2.3. Mobile phases

Mobile phase A was 5 mM Bu_4NHSO_4 and 10 mM potassium phosphate buffer in water at pH 12.4. The Bu_4NHSO_4 (3.40 g) and K_3PO_4 (4.25 g) were dissolved into 2.0 L of purified water. The pH was adjusted to 12.4 ± 0.2 with sodium hydroxide pellets (~4.5 g). Mobile phase B was acetonitrile/water (80/20, v/v).

2.4. Instrumentation and chromatographic conditions

Separations were conducted on an Agilent 1200 Series HPLC-DAD coupled with a PLRP-S 100 Å HPLC column (150 mm × 4.6 mm, 5 μm, or 150 mm × 4.6 mm, 3 μm, both from Agilent/Varian, Inc., Santa Clara, CA, USA). The chromatographic conditions in Table 1 were followed unless otherwise noted.

The ACD/pK_a DB (Product version: 12.5, Build 32846, Advanced Chemistry Development, Inc., Toronto, Canada) was used for the prediction of the pK_a for 2-aminopyrimidin-5-yl boronic acid.

Table 1
The chromatographic conditions for the analysis of **1** by HPLC.

Parameter	Condition		
Column	PLRP-S 100 Å column, 150 mm × 4.6 mm, 5 μm.		
Column temperature	10 °C		
Flow rate	1.0 mL/min		
Injection volume	5 μL		
Autosampler temperature	10 °C		
Sample diluent	*N,N*-dimethylformamide/dichloromethane (50/50, v/v, anhydrous)		
Nominal concentration	2.0 mg/mL		
Detector wavelength	293 nm		
Mobile phases	A: 5 mM tetrabutylammonium bisulfate and 10 mM potassium phosphate in water, pH 12.4 B: acetonitrile/water (80/20, v/v)		
Gradient program	Time (min)	A (%)	B (%)
	0.0	99	1
	2.0	99	1
	20.0	40	60
	20.1	99	1
	25.0	99	1

2.5. Boronic acid solubility measurement

The 2-aminopyrimidin-5-yl boronic acid (~20 mg) was dissolved in 100 mL ACN/10 mM phosphate buffer at pH 12 (20/80, v/v). This served as an external standard. The saturated boronic acid in diluent DMF/DCM (50/50, v/v) solution was obtained by mixing excess amount of boronic acid with the diluent overnight and filtering through 0.45 μm disk filter. The solubility of boronic acid in the diluent was determined by HPLC analysis (see Table 1 for chromatographic conditions) against the external standard.

2.6. Quantitative NMR

The quantitative nuclear magnetic resonance (qNMR) measurements were carried out on a Bruker Avance 3, 600 MHz spectrometer equipped with a 5 mm, TCI, Z-gradient CryoProbe. Quantitative NMR (qNMR) measurements were made using the internal standard method and proton NMR. The samples were prepared by accurately weighing the sample (~20 mg) and internal standard, dimethylaminopyridine (DMAP ~15 mg), to an accuracy of 0.01 mg and dissolving them in 0.6 mL of DMSO-d6 (D, 99.8%, Cambridge Isotope). The samples were transferred to a 5 mm NMR tube (Wilmad screw-cap 535-PP-7), purged with nitrogen and sealed. The sample temperature was maintained at 28 °C and spectra were acquired using the standard Bruker pulse sequence, zg30 and processed with Bruker TopSpin software, version 3.0, and patch level 3. The proton spectra were collected with 32 acquisitions, a 10 ppm spectral width, a 10 s relaxation delay, 65,536 time domain data points (5.44 s acquisition time) and 65,536 frequency domain data points. The data were Fourier transformed, baseline corrected and integrated. The integrals were not further adjusted or corrected. The DMAP aromatic protons at 6.53 ppm were assigned an integral value of 2.00 and the boronic ester protons at 1.23 and 8.34 ppm were integrated and the values recorded. The weight percent purity of the boronic ester sample was calculated using the following equation:

$$\%\text{Purity} = \frac{\#\text{Protons\_IS}}{\#\text{Protons\_S}} \times \frac{\text{MW\_S}}{\text{MW\_IS}} \times \frac{\text{Wt.\_IS}}{\text{Wt.\_S}} \times \frac{\text{Intensity\_S}}{\text{Intensity\_IS}} \times \text{Purity\_IS}$$

where IS is the internal standard; S is the sample; intensity is the integral values; Wt. is the weight; and MW is the molecular weight.

3. Results and discussion

3.1. Analytical technology screening

The pinacolboronate ester **1** was used as a starting material for a development API, requiring an analytical method for the purity assessment of this starting material. However, this compound proved to be inherently unstable and readily hydrolyzing to its corresponding boronic acid (**2**, see Fig. 1) in aqueous media.

Several analytical technologies were evaluated with limited success. The ^{1}H qNMR was initially relied upon to estimate the purity of **1**. While qNMR is an excellent quantitative tool for the determination of the major component as a weight-based assay, it is not a sensitive technique for impurities due to the fact that only a small percentage of the analyte nuclei can be excited in the magnetic field and seen by NMR [19]. For this reason, qNMR is inferior to chromatography methods for purity assessment.

Gas chromatography may be feasible for the analysis of typically nonvolatile (for example, the melting point of **2** is over 200 °C) boronic acids which are derivatized to boronate esters [20]. However, derivatization may change the response factors and/or compete with the analyte pinacolboronate esters, and thus skew the impurity profile. Also, elevated temperatures promote the loss of water in boronic acid, producing boroxin, the cyclic trimer of boronic acid anhydride [9,21,22]. Due to these reasons, GC is not suitable for the purity analysis of **1**.

Non-aqueous HPLC was also ineffective as **2** is insoluble in most normal phase and polar organic phase elution solvents. Hydrophilic interaction liquid chromatography (HILIC) was also considered. However, the pK_a of boronic acids is generally ~9 [15,16], and the calculated pK_a of **2** is 10.90 by ACD/pK_a DB. To stabilize the boronate esters, mobile phases with pH ≥ 11 are preferred as boronate esters are favored in high pH media [15]. Most HILIC stationary phases are silica based and not compatible with strong basic mobile phases. Therefore, subsequent method development efforts focused on reversed-phase HPLC with high pH mobile phases.

3.2. Method development and optimization

3.2.1. Column screening and mobile phase pH selection

A few commonly used reversed-phase HPLC columns (various C18, phenyl-hexyl, CN, and mixed-mode Dionex WCX-1 phases, etc.) were screened with traditional conditions (2 < pH < 8). The analyte **1** rapidly hydrolyzed under these conditions to **2**, which eluted out at the dead time. The screening then turned to high pH mobile phases (pH ≥ 10) with the hope that high pH mobile phases may favor the pinacolboronate ester and thus slow its hydrolysis [15]. Using a Luna C18 column at pH 10, the maximum pH limit for this stationary phase, a significant baseline elevation between the peaks for **1** and **2** was observed, indicating severe on-column hydrolysis and the need for more basic conditions.

Considering that the aforementioned calculated pK_a of **2** is 10.90, the mobile phase pH was further raised to 12. This pH extreme greatly limited the choices of suitable columns to Zirconium or polymer based stationary phases. On the Discovery Zr-PBD column, **1** was not retained, whereas the Zr-CARB column produced some retention for **1** along with unacceptable peak tailing for **2**. The most promising chromatography was achieved using the Varian PLRP-S column (Fig. 2A), which consists of polystyrene-divinylbenzene resin. The PLRP-S column exhibited excellent retention for **1** with retention factor k of 6.3 and separation from **2**. While the separation at pH 12 using the Varian PLRP-S phase was further evaluated, two complications emerged, including the selection of an appropriate sample diluent to adequately stabilize **1**, and the need to improve the retention of the highly hydrophilic **2**.

3.2.2. Sample preparation

The instability of **1** prepared in aqueous media was evident even in buffer at pH 12. After 5 h, the reinjection of **1** prepared in ACN/10 mM phosphate buffer, pH 12 (25/75, v/v) diluent (Fig. 2B) showed that the analyte was completely decomposed to boronic acid and another degradant (t_R ~9.5 min), which could be the half-hydrolyzed pinacolboronate monoester. This led to evaluating neat solvents that could stabilize **1**. Using neat anhydrous methanol as sample diluent, **1** fully degraded after 4 h (Fig. 2C and D) to boronic acid and a different degradant eluted at ~8.8 min. This peak may be the boronic acid methyl ester, which may be formed by a reaction with excess methanol that competes with pinacol. Some other neat solvents were evaluated, including protic but bulky and thus much more inert isopropyl alcohol (IPA), and aprotic solvents like tetrahydrofuran (THF), 1,4-dioxane, and ACN. Each produced peak splitting even with a 5 μL injection volume (Fig. 3). These findings demonstrated that **1** is especially prone to decomposition in aqueous and/or protic solvents, and that its separations are sensitive to diluent strength. Note that neat acetonitrile was used as diluent in the fast LC approach for sample solution stability reasons [18].

Therefore, the determination of a weaker, aprotic diluent mixture was pursued. Combinations of methyl *tert*-butyl ether (MTBE), dichloromethane (DCM), and *n*-heptane with the above strong solvents were tested. The dilution solvent mixture ACN/DCM (25/75, v/v) did not degrade **1** and gave acceptable peak shape for both **1** and **2** (tailing factors ~1.1, see Fig. 4A). However, **2** has very limited solubility in this solvent mixture compared to **1** (Fig. 4B). If impurities do not have enough solubility in the diluent, they may be underestimated in the testing. Based on these findings, it became clear that the ideal diluent for **1** should be: (1) non-aqueous; (2) aprotic; (3) not too strong so that peaks are not skewed; and (4) enough solubility for **2**.

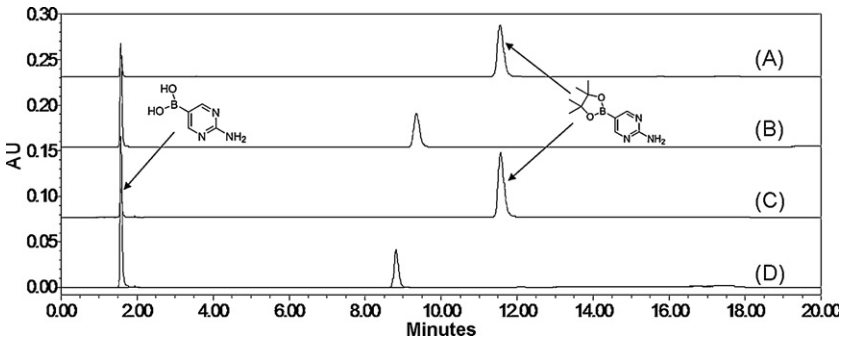

Fig. 2. The separation of **1** on PLRP-S phase at pH 12. In the diluent of ACN/10 mM phosphate buffer at pH 12, (25/75, v/v): (A) initial separation; (B) reinjection after 5 h. In the diluent of pure anhydrous methanol: (C) initial separation; (D) reinjection after 4 h.

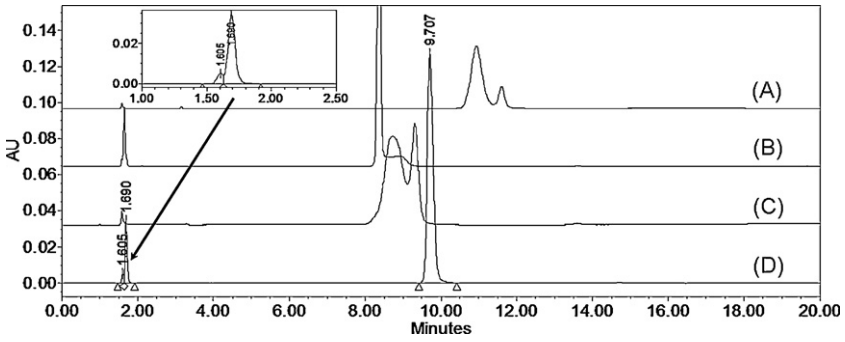

Fig. 3. Peak splitting was observed when (A) ACN, (B) 1,4-dioxane, (C) IPA and (D) THF were used as diluent. Injection volume was 5 μL.

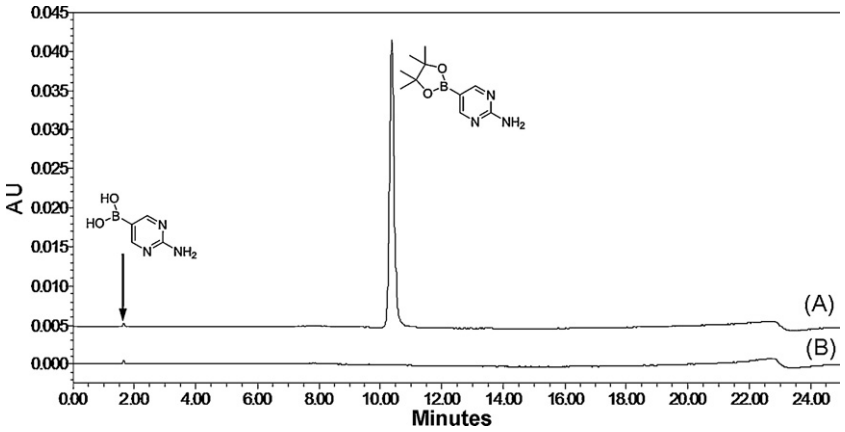

Fig. 4. (A) The analyte **1** (0.1 mg/mL in ACN/DCM 25/75, v/v) was stable and showed no peak distortion. (B) Saturated **2** in ACN/DCM (25/75, v/v) showed very limited solubility.

The final diluent DMF/DCM (50/50, v/v, anhydrous) satisfies all four above criteria. The analyte **1** did not degrade in this diluent and a 5 μL injection produced acceptable peak shape. The solubility of **2** in this diluent was determined to be 0.28 mg/mL, which is equivalent to ~14% of **1** in the nominal sample concentration of 2.0 mg/mL. Since the solubility limit for **2** is well above the total impurities specification for this starting material (≤5.0%), there was no concern that the level for **2** would be underestimated and the diluent was considered to be acceptable.

3.2.3. Ion pair chromatography

Unlike its pinacol ester, **2** is very hydrophilic. Even when the gradient started with only 1% ACN or less, **2** is barely retained. At pH 12, **2** will be negatively charged. A positively charged hydrophobic ion present in the mobile phase may form "ion pairs" with **2** and result in better interaction with the stationary phase thus better retention. A few positively charged ion pair reagents, such as tetramethylammonium bisulfate (Me_4NHSO_4), tetramethylammonium chloride (Me_4NCl), tetrapropylammonium bisulfate (Pr_4NHSO_4), tetrabutylammonium bisulfate (Bu_4NHSO_4), and hexadecyltrimethyl-ammonium bromide (C16-Me_3NBr), were screened for this purpose. With 5 mM Bu_4NHSO_4 added in the 10 mM potassium phosphate tribasic buffer at pH 12.4, the retention of **2** increased from ~1.57 min to ~1.86 min. The ion pair chromatography helped to increase the retention of **2**, which would assure the reliable determination of this impurity of interest.

3.3. Method qualification and purity assessment for lot release testing

The final HPLC parameters are summarized in Table 1. The specificity of the method is demonstrated in Fig. 5, which

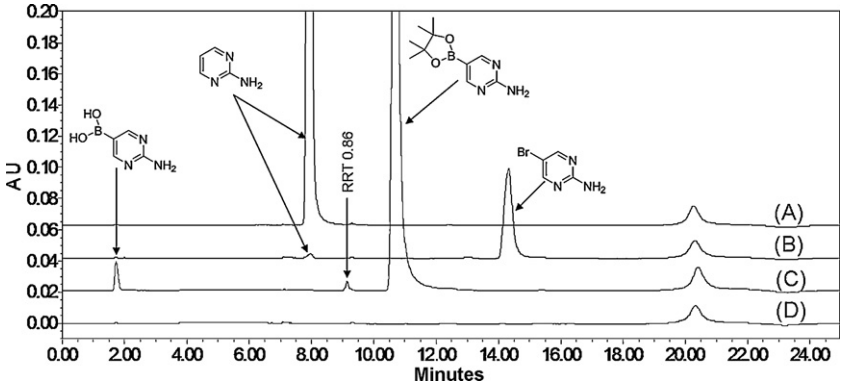

Fig. 5. Chromatographic separations of (A) 2-aminopyrimidine; (B) 2-amino-5-bromopyrimidine; (C) the compound **1**; and (D) diluent blank DMF/DCM (50/50, v/v), with chromatographic parameters listed in Table 1.

Table 2
The solution stability of **1** (Lot A) in DMF/DCM (50/50, v/v) at room temperature.

Time point	Purity by %area	Boronic acid	2-Amino-pyrimidine	RRT 0.86	RRT 1.08
Initial	98.24	1.03	<LOQ	0.47	0.20
Day 1	98.23	1.04	<LOQ	0.47	0.21
Day 2	98.21	0.99	<LOQ	0.46	0.28

shows that possible impurities 2-aminopyrimidine, 2-amino-5-bromopyrimidine, an unidentified impurity RRT 0.86, and **2** were well separated from the main component **1**. The blank injection of diluent DMF/DCM (50/50, v/v) showed no significant interference to peaks of interest. The method demonstrated good linear response for the analyte concentration from 1.99 μg/mL to 2.84 mg/mL with correlation coefficient $R = 1.000$. The limit of quantitation (LOQ) is 1.99 μg/mL (0.1% of nominal 2.0 mg/mL) with signal-to-noise ratio (S/N) > 10. The limit of detection (LOD) is 0.7 μg/mL (0.04% of nominal 2.0 mg/mL) with S/N ≥ 3. The system precision was demonstrated by %RSD of the main component in 6 consecutive injections (%RSD = 0.06, $n = 6$). The sample (Lot A) solution was stable for two days at room temperature (Table 2). After qualification, this method was used for lot release testing of **1** (Lot B). The 99.5% purity of Lot B (HPLC area normalization) determined by this method was consistent with the qNMR result (98.2%, weight based assay). Besides main component purity, this HPLC method also gave the impurity profile, which is not feasible by qNMR.

3.4. Applications to other pinacolboronate esters

The strategies for the method development of **1** may also be applied to a broad range of pinacolboronate esters. As shown in Fig. 6, 2-methyl-3-aminophenyl-pinacolboronate ester was resolved from its impurities. The corresponding boronic acid had enough solubility in the diluent DMF/DCM (50:50, v/v) and good retention in the chromatography. Compared to its 5 μm version (150 mm × 4.6 mm), which was used in the initial method development, the 3 μm PLRP-S column (150 mm × 4.6 mm, 100 Å) produced better sensitivity as expected. No on-column hydrolysis of the analyte was observed during analysis. The ion pair reagent Bu_4NHSO_4 was not used as the corresponding 2-methyl-3-aminophenylboronic acid was well retained.

The 1-methyl-1*H*-pyrazole-4-pinacolboronate ester and 1-methyl-1*H*-pyrazole-5-pinacolboronate ester are positional isomers, which were well separated in the given chromatographic conditions (Fig. 7). These two positional isomers have very similar

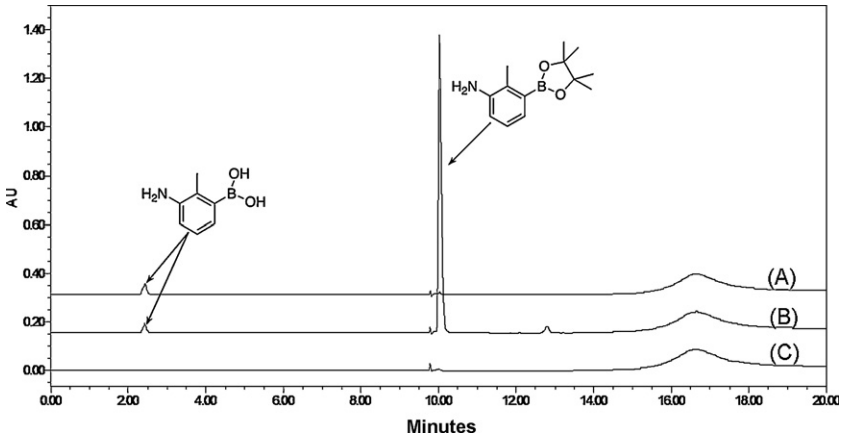

Fig. 6. Chromatographic separations of (A) 2-methyl-3-aminophenylboronic acid; (B) 2-methyl-3-aminophenylpinacolboronate ester; and (C) DMF/DCM (50/50, v/v) as sample diluent on 100 Å PLRP-S column (150 mm × 4.6 mm, 3 μm) with 10 mM potassium phosphate in water at pH 12.4 as mobile phase A, column temperature at 18 °C, flow rate at 0.7 mL/min, and UV detection at 270 nm. Other chromatographic conditions see Table 1.

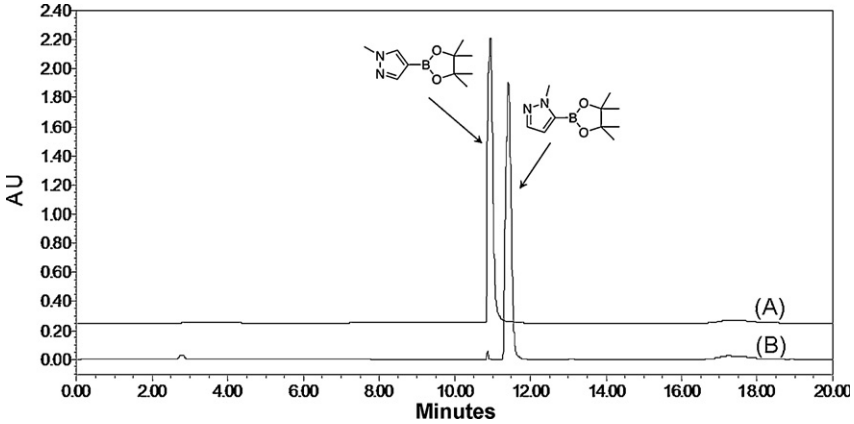

Fig. 7. Chromatographic separations of two positional isomers (A) 1-methyl-1*H*-pyrazole-4-pinacolboronate ester and (B) 1-methyl-1*H*-pyrazole-5-pinacolboronate ester on 100 Å PLRP-S column (150 mm × 4.6 mm, 3 μm) with ACN/DCM (50/50, v/v) as sample diluent, 10 mM potassium phosphate in water at pH 12.4 as mobile phase A, column temperature at 18 °C, flow rate at 0.6 mL/min, and UV detection at 230 nm. Other chromatographic conditions see Table 1.

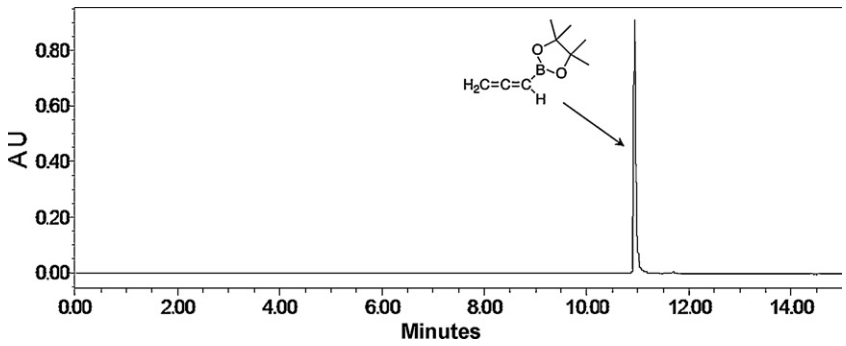

Fig. 8. Chromatographic separation of allenylpinacolboronate ester in ACN/DCM (50/50, v/v) as sample diluent, flow rate at 0.6 mL/min, and UV detection at 230 nm, on 100 Å PLRP-S column (150 mm × 4.6 mm, 3 μm) with 10 mM potassium phosphate in water at pH 12.4 as mobile phase A, and column temperature at 18 °C. Other chromatographic conditions see Table 1.

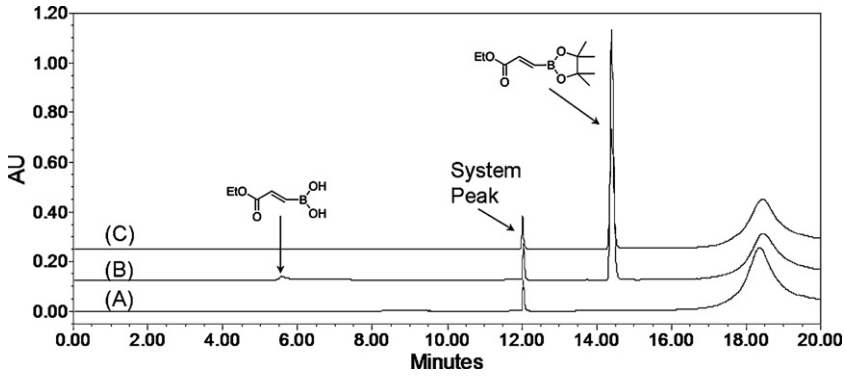

Fig. 9. Chromatographic separations of (A) sample diluent ACN/DCM (50/50, v/v); (B) marker solution showing both 2-(ethoxycarbonyl) vinylpinacolboronate ester and its corresponding boronic acid; and (C) 2-(ethoxycarbonyl) vinylpinacolboronate ester, flow rate at 0.5 mL/min, UV detection at 226 nm, sample concentration of 0.25 mg/mL, on 100 Å PLRP-S column (150 mm × 4.6 mm, 3 μm) with 10 mM potassium phosphate in water at pH 12.4 as mobile phase A, and column temperature at 20 °C. Other chromatographic conditions see Table 1.

Table 3
The solution stability of 2-(ethoxycarbonyl) vinylpinacolboronate ester in ACN/DCM (50/50, v/v) at room temperature.

Time point	Purity by %area	Boronic acid	RRT 0.96
Initial	99.61	0.19	0.20
Day 1	99.70	0.14	0.16
Day 2	99.66	0.17	0.17

hydrophobicity. The separation mechanism may involve π-π stacking of the analytes and the PLRP-S stationary phase, which is composed of π-election-rich polystyrene-divinylbenzene. The main components of allenylpinacolboronate ester (Fig. 8) and 2-(ethoxycarbonyl) vinylpinacolboronate ester (Fig. 9) were well retained without on-column hydrolysis. The 2-(ethoxycarbonyl) vinylpinacolboronate ester solution in ACN/DCM (50/50, v/v) was stable for two days at room temperature (Table 3). Unlike previously tested compounds, both of these two pinacolboronate esters do not contain an aromatic ring but still have π electrons and were well retained under similar chromatographic conditions. The fact that a number of structurally very different pinacolboronate esters were well separated by using similar chromatographic conditions clearly demonstrates that the analytical strategies described in this work are widely applicable to the broad range of this unique class of compounds.

4. Conclusions

A reversed-phase HPLC method has been developed for the purity assessment of **1**. High pH mobile phase is needed to minimize the on-column hydrolysis of this pinacolboronate ester. PLRP-S column is stable at the working pH and provides excellent retention for the analytes. An anhydrous DMF/DCM (50:50, v/v) solvent mixture provides sufficient solubility for the boronic acid, while stabilizing the pinacolboronate ester. The ion pair reagent tetrabutylammonium bisulfate further increases retention of the boronic acid. The method was successfully qualified and used for lot release testing of **1**. Compared to available alternative approaches for purity determinations, the strategies described in this work provide more flexibility for method optimization, and were successfully used to analyze several other pinacolboronate esters. The general principles outlined in this work, especially, the high pH mobile phase, the polymeric column, and the nature of the diluent, can be applied to analyses of similar pinacol boronate esters.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2012.01.050.

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