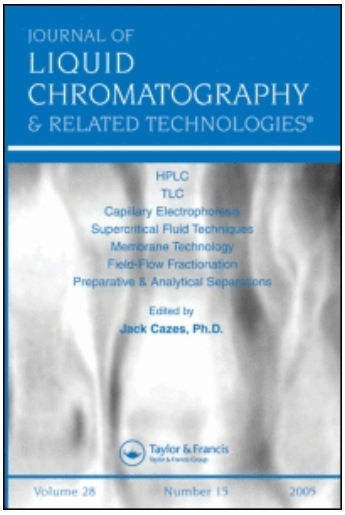

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Application of Fast Reversed Phase Liquid Chromatography for Analysis of Pharmaceutical Related Boronic Acid and Boronic Pinacol Ester Functionalized Compounds

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Abstract: Boronic acid and boronic pinacol ester functionalized compounds are the basis for Suzuki coupling and Petasis reactions that are widely used in pharmaceutical synthetic schemes. The purity analysis of these compounds utilizing traditional reversed phase liquid chromatography methodology is complicated by the potential of on-column hydrolysis. Therefore, a fast liquid chromatography method was developed to minimize on-column hydrolysis effects. The method was applied as an in-process control method for the separation of three process related impurities from a boronic pinacol ester functionalized intermediate with no appreciable on-column hydrolysis observed. The method was then optimized to successfully resolve ten different boronic acid and boronic pinacol ester functionalized compounds within five minutes. Thus, the wide range applicability of fast liquid chromatographic technology to this specific class of compounds was demonstrated.

Keywords: Boronic acid, Boronic pinacol ester, On-column hydrolysis, Fast liquid chromatography

INTRODUCTION

Within the past decade, fast reversed phase high performance liquid chromatography (RP-HPLC) has established itself as a viable separation technique for assay and purity analysis of key raw materials, process intermediates, final

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drug substances, and final drug products in the pharmaceutical industry.[2–5] Fast RP-HPLC analysis enables near real-time process control, as well as fast release of batches of final drug substance and/or drug product. However, when developing a fast RP-HPLC method, certain aspects relevant in the pharmaceutical industry, such as sensitivity, specificity, and ruggedness of the method, must be considered.[1]

In recent years, tremendous progress has been made in the area of fast RP-HPLC separations with particle packed columns. In the early 1990s, traditional 250 × 4.6 mm columns packed with 5 μm particles were used in most analytical HPLC applications. The simplest approach for significantly reducing HPLC run times is by using shorter columns with higher flow rates and/or fast gradients.[2] However, shorter columns with the same particle size (5 μm) results in fewer theoretical plate number, and thus, lower column efficiency. In most of the cases for pharmaceutical analysis, a certain level of column efficiency is essential to meet specificity requirements. Column efficiency may be improved dramatically by reducing the particle size. The preparation of porous and non-porous silica particles in the range of 0.2–2 μm has been reported.[3,4] Currently, column manufacturers provide short columns (≤50 mm of length) packed with particles less than 3 μm for fast RP-HPLC method development.[5] These columns usually produce 10000 – 20000 plates under typical operating conditions. Also, a reduction of column length from 250 mm to 50 mm obviates the dramatic increase of column backpressure caused by the smaller packed particle size according to the equation for column pressure:[6–8]

$$\Delta P = \Phi \eta L u / d_p^2$$

Where Φ is the flow resistance factor related to the column packing structure, η is the mobile phase viscosity, L is the column length, u is the mobile phase linear velocity, and d_p is the packed particle diameter.

Due to the reduced column volume when using short columns for fast RP-HPLC separation, the extra column volume effect requires significant consideration. The extra column volume, which includes the injection volume, the tubing connections to the column and from the column to the detector, and the detector cell volume, may result in broadening of the chromatographic peaks.[9] However, most of the commercially available HPLC systems today have sufficiently small extra column volume, provided that the column void volume is not less than 1 mL, enabling fast RP-HPLC separation to be performed.

Other approaches to fast RP-HPLC separation have also been explored. Extensive research has been done on high speed, high temperature liquid chromatography.[10–17] It was shown that by increasing the temperature to 175°C above ambient, the eluent viscosity decreased 5–10 fold.[18,19] Along with the increase in analyte diffusivity,[20] the limitations on column back pressure at high flows may be dramatically reduced and column efficiency may be improved concomitantly.[10,13,17] Therefore, a higher temperature

may play a central role in improving separation speed for RP-HPLC. The major concerns for the high temperature high speed liquid chromatography approach are analyte stability at high temperature and limited availability of stable columns.[21] Thus, pharmaceutical analysis using high temperature RP-HPLC for shorter analysis time is very limited.

Monolithic stationary phases have also been investigated for fast RP-HPLC separation. Monolithic stationary phases have a total porosity of over 80%.[22] Monolithic columns are constituted of large and small pores, which allow much higher flow rates without significant column pressure drop, while providing sufficient surface for the separation process.[23,24] The unique silica network structure of the monolithic columns provides excellent resolution at high flow rates due to enhanced mass transfer, which makes the separation process much faster.[25–28] However, an increase of the baseline noise and peak tailing are often observed when using monolithic columns. Also a major draw back of using monolithic columns is the very limited choices of the commercially available phases. Currently, only one manufacture provides monolithic columns with very limited dimensions.

This paper describes an in-process control method for separation of a boronic pinacol ester functionalized process intermediate (Figure 1), which undergoes on-column hydrolysis under traditional RP-HPLC conditions, from its three process related impurities using fast RP-HPLC. The nature of the separation and the range of applicability of this fast LC technique for this specific class of compounds are investigated, as well as the effect of varying such parameters as initial composition of organic modifier, mobile phase pH, and column temperature.

EXPERIMENTAL

Instrumentation

All LC experiments were performed with an Agilent 1100 series LC (Palo Alto, CA) equipped with dual pumps, a diode array UV detector, oven, and

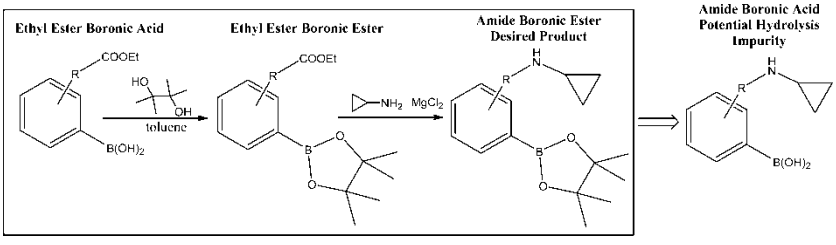

Figure 1. Structures of boronic pinacol ester functionalized process Intermediate and its process related impurities.

an autosampler. The instrument was controlled by the HP Chemstation software. The UV detection wavelength was set at 220 nm.

Four types of columns were investigated in the fast LC study, Waters Xterra MS C_{18} (50 × 4.6 mm; 2.5 μm particles), Zorbax SB-C_{18} (50 × 4.6 mm; 1.8 μm particles), Zorbax SB-C18 (50 × 4.6 mm; 5 μm particles), and Chromolith RP-18e (100 × 4.6 mm). An Ace C_{18} column was used for the traditional reversed phase LC method (250 × 4.6 mm, 5 μm particles).

Chemicals and Reagents

HPLC grade acetonitrile as organic modifier for LC method development was obtained from EM Science (Gibbstown, NJ). Phosphoric acid (0.1%) and DI water were used for the aqueous portion of the LC mobile phase. Five pairs of boronic acid and boronic pinacol ester functionalized compounds (Figure 2) were obtained from Aldrich Chemical Company, Inc. (WI, USA). The boronic pinacol ester functionalized process intermediate and its three process related impurities (Figure 1) were obtained from the Department of Process Research and Development, Merck & Co., Inc. (Rahway, NJ, USA). All samples were dissolved and diluted to the desired concentration with acetonitrile.

RESULTS AND DISCUSSION

On-Column Hydrolysis of the Boronic Pinacol Ester Functionalized Process Intermediate under Traditional Reversed Phase Conditions

Boronic pinacol ester derivatives are often used in place of boronic acid as an isolated process intermediate in the pharmaceutical synthetic scheme due to the complications of the boronic acid drying process. However, the ester derivatives are prone to hydrolysis back to acid derivatives under aqueous conditions. Initial steps toward development of an in-process control method for this specific ester intermediate (Figure 1) were performed under traditional reversed phase conditions. Under ambient conditions, significant baseline elevation between the amide boronic-acid impurity and amide pinacol-ester intermediate was observed, which indicated an on-column hydrolysis from the process intermediate to the amide boronic acid impurity (Figure 3).[29] The baseline could not be improved significantly by reducing the column temperature and/or increasing buffer pH (Data not shown).

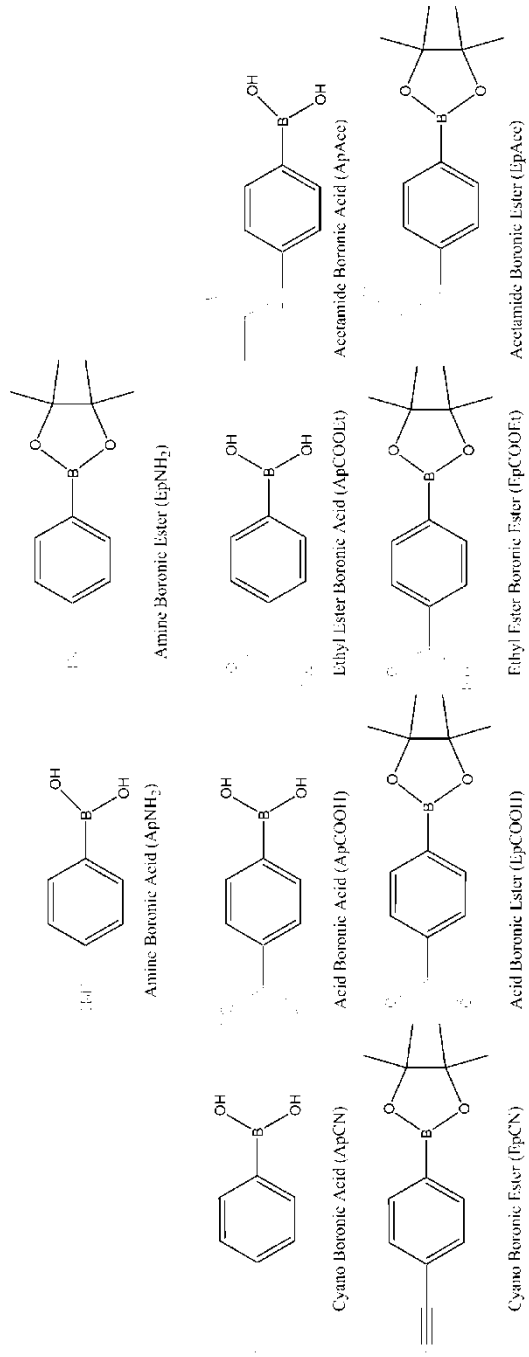

Figure 2. Ten boronic pinacol ester and boronic acid functionalized compounds.

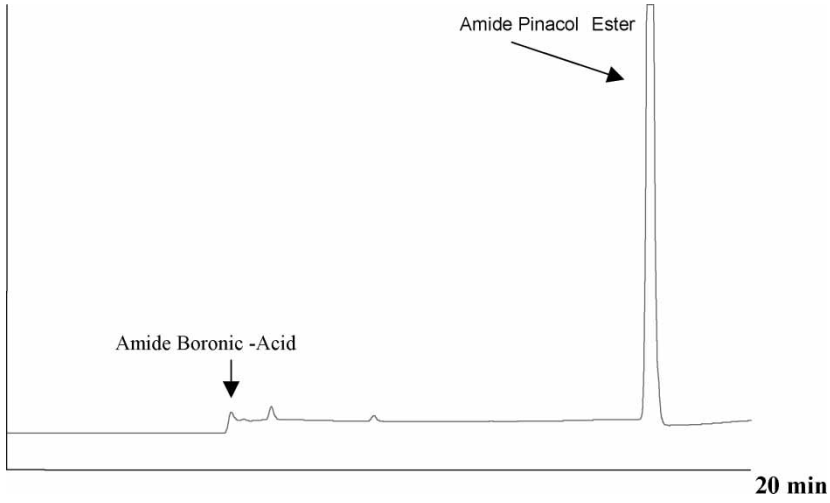

Figure 3. RP-HPLC chromatogram of amide boronic ester intermediate with traditional HPLC column. See Figure 1 for compound structures. Conditions: Ace C_{18} column, 250 × 4.6 mm, 5 μm; 1.0 mL/min flow rate, 10 μL injection, 210 nm UV detection, 20°C column temperature, linear gradient from 30:70 to 80:20 CH_3CN:0.1% H_3PO_4 (v/v) in twenty minutes.

Development and Optimization of Fast LC Method for Amide Pinacol Ester Intermediate

In cases where significant on-column degradation is observed with utilization of traditional reversed phase chromatography, fast LC chromatography approaches are considered as viable alternatives to minimize degradation during analysis. Different fast LC approaches were investigated in this case to achieve optimal resolution of amide pinacol ester intermediate and its three process-related impurities, as well as minimize on-column hydrolysis observed under traditional LC conditions. A short column with a standard particle size (Zorbax SB-C_{18}, 50 × 4.6 mm, 5 μm) and a fast gradient program was investigated as the first approach. No baseline elevation was observed between amide boronic acid impurity and the amide boronic ester intermediate. The elimination of baseline elevation may be attributed to the ultra fast separation run time relative to the on-column hydrolysis rate. However, the resolution between amide boronic ester intermediate and ethyl ester boronic ester impurity diminished due to the lower efficiency of the short column (Figure 4). Thus, a similar column with much smaller particle size (Zorbax SB-C_{18}, 50 × 4.6 mm, 1.8 μm) was used to address the resolution issue with no sacrifice of the faster separation time. The column efficiency was significantly improved with the smaller particle size and baseline separation was achieved for all four compounds within three minutes (Figure 4).

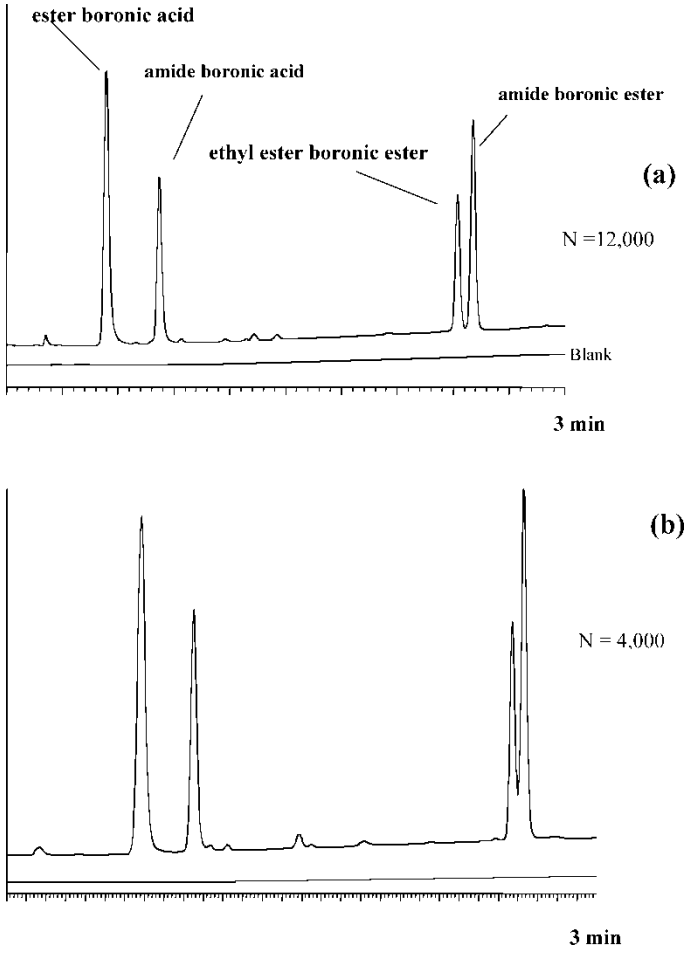

Figure 4. Fast RP-HPLC separation of amide boronic ester intermediate from its process related impurities with short particle packed columns. See Figure 1 for compound structures. (a) Zorbax SB-C_{18}, 50 × 4.6 mm, 1.8 μm, (b) Zorbax SB-C_{18}, 50 × 4.6 mm, 5 μm. Conditions: 1.2 mL/min flow rate, 2 μL injection, 210 nm UV detection, 20°C column temperature, linear gradient from 30:70 to 80:20 CH_3CN:DI water (v/v) in three minutes.

Column back pressure is always a concern when small particle packed HPLC columns are used, particularly with subambient column temperatures, which result in additional back pressure due to an increase of eluent viscosity.[11] Thus, in order to optimize the method, another type of short column with a particle size between 1.8 μm and 5 μm phases was studied in an effort to obtain a balance between a fast separation, optimal resolution, and acceptable column back pressure under relatively high flow rate and

low column temperature. A Waters Xterra MS C_{18} column (50 × 4.6 mm, 2.5 μm particles) was used for this purpose. The column demonstrated sufficient separation of all four compounds within four minutes under optimized fast LC conditions (Figure 5). The column back pressure was within an acceptable limit (<250 bar), even at a column temperature of 5°C.

The use of monolithic columns was also investigated. Chromolith RP-C_{18} column (100 × 4.6 mm) was used with high flow rate (4 mL/min) for fast separation to prevent on-column hydrolysis. However, the optimal resolution among the four compounds could not be achieved due to the peak tailing of the compounds (Data not shown).

To further optimize the method for minimization of on-column hydrolysis, the initial concentration of organic modifier, acetonitrile, was varied from 15% (v/v) to 40% (v/v) with column temperature at 20°C, DI water as aqueous phase, and total flow rate of 1.2 mL/min. The level of amide boronic acid impurity decreased from 3% to 1% when the initial acetonitrile concentration was increased from 15% (v/v) to 30% (v/v). The observation demonstrated that higher initial organic modifier composition might decrease the on-column hydrolysis rate of the boronic pinacol ester

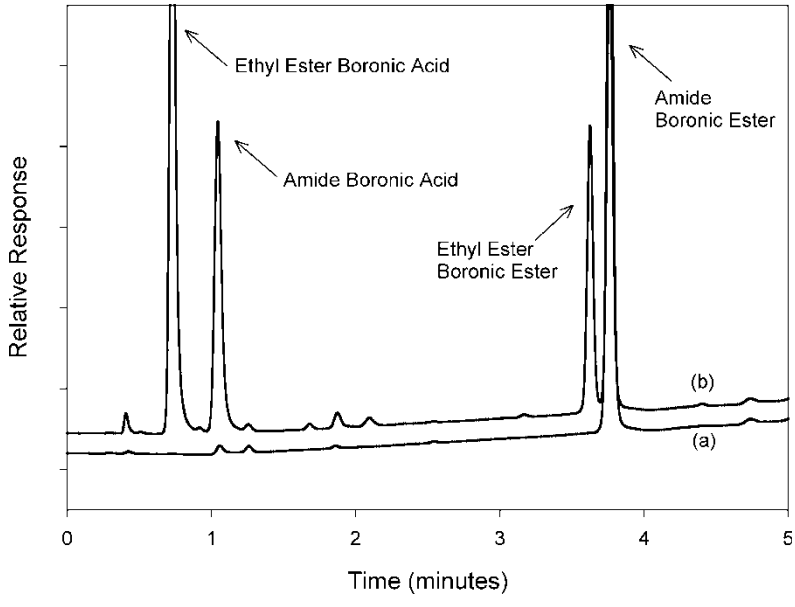

Figure 5. Fast RP-HPLC separation of amide boronic ester intermediate from its process related impurities with Waters Xterra MS C_{18} column (50 × 4.6 mm, 2.5 μm). See Figure 1 for Compound Structures. (a) Authentic sample of amide boronic ester intermediate, (b) Synthetic mixture of the four compounds. Conditions: 1.2 mL/min flow rate, 2 μL injection, 210 nm UV detection, 5°C column temperature, linear gradient from 30:70 to 80:20 CH_3CN:DI water (v/v) in five minutes.

derivatives. At an initial acetonitrile concentration of 40% (v/v), the column retention for both boronic acid impurities diminished. Thus, an initial modifier concentration of 30% (v/v) acetonitrile was chosen to sustain a balance between minimized on-column hydrolysis and adequate retention times for the compounds.

The range of mobile phase pH studied was 2–7 with initial concentration of 30% (v/v) acetonitrile as modifier, 1.2 mL/min total flow rate, and 20°C column temperature. As expected, the rate of on-column hydrolysis decreased significantly at higher pH. The pH dependence of on-column hydrolysis rate is due to facilitation of hydrolysis of boronic esters back to boronic acids under acidic aqueous conditions.[30] The optimal mobile phase pH for this work was found to be 7.

The effect of column temperature was investigated. The temperature was varied from 5°C to 30°C with 30% (v/v) initial acetonitrile concentration, DI water as aqueous phase, and 1.2 mL/min total flow rate. A significant decrease in on-column hydrolysis was observed at lower column temperatures.[31] However, when column temperature was decreased, a considerable increase in column back pressure occurred due to the higher viscosity of the mobile phase. Thus, 5°C was chosen as the optimal column temperature for this separation, which provides a balance between minimal on-column hydrolysis and acceptable column back pressure.

The optimum fast LC conditions for the boronic pinacol ester intermediate in-process control method were found to be at 5°C column temperature, DI water as aqueous phase, and a 5 minute gradient of acetonitrile from 30% v/v to 80% v/v with 1.2 mL/min total flow rate, using a Waters Xterra MS C18 (50×4.6 mm, 2.5 μm particles) column. Resolution of the pinacol ester intermediate and its three process related impurities was achieved within 4 minutes. The fast separation combined with low column temperature (5°C), high mobile phase pH (pH = 7), and a gradient program starting with a higher level of organic modifier (30% v/v acetonitrile), effectively minimized the on-column hydrolysis of the boronic pinacol ester intermediate. A fast LC chromatograph of the amide boronic pinacol ester intermediate and its impurities (~1 mg/mL) obtained under these conditions is shown in Figure 5. The limit of quantitation was found to be 0.10 wt%, based on the satisfaction of relative standard deviation (RSD) of injection precision less than 15% with a signal to noise ratio of 10:1, for boronic pinacol ester intermediate.

Fast LC Application for Separation of Other Boronic Pinacol Ester Functionalized Compounds

To demonstrate the wide range of applicability of fast LC technology to this specific class of compounds, a mixture of five pairs of commonly used boronic acid and boronic pinacol ester functionalized compounds (Figure 2)

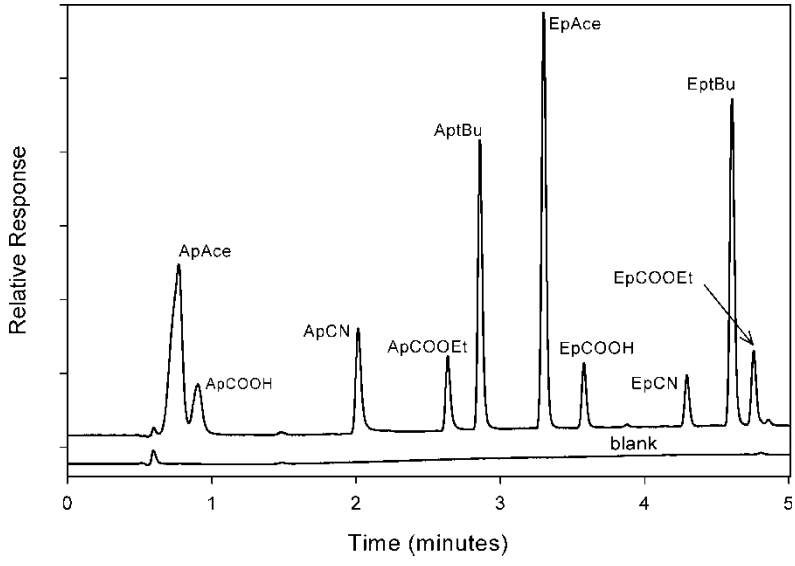

Figure 6. Fast RP-HPLC separation of ten boronic pinacol ester functionalized compounds with Waters Xterra MS C_{18} column (50 × 4.6 mm, 2.5 μm). See Figure 2 for compound structures. Conditions: 1.2 mL/min flow rate, 2 μL injection, 210 nm UV detection, 20°C column temperature, linear gradient from 15:85 to 90:10 CH_3CN:0.1% H_3PO_4 (v/v) in four minutes, and hold 1 minute at 90:10 CH_3CN:0.1% H_3PO_4 (v/v).

was analyzed under fast LC conditions. A Waters Xterra MS C_{18} column (50 × 4.6 mm, 2.5 μm particles) was applied to ensure fast analysis, acceptable resolution among all ten compounds, as well as reasonable column back pressure. A chromatogram of the sample mixture is shown in Figure 6. An acceptable separation was achieved for all ten compounds within 5 minutes and no on-column hydrolysis was observed. Most importantly, the fast reversed phase LC method can be considered as a good starting point for the analysis of all boronic pinacol ester derivatives. Further method optimization may be done from there to meet the requirements for a specific compound.

CONCLUSION

On-column hydrolysis of boronic pinacol esters to their corresponding boronic acids is a major issue for traditional reversed phase HPLC analysis of this class of compounds. Thus, a fast reversed phase HPLC method was developed to separate an amide boronic pinacol ester intermediate from its three process related impurities. Separation of all four compounds was achieved in less

than four minutes with minimal on-column hydrolysis. Moreover, the fast LC method was demonstrated to be applicable for separation of ten boronic pinacol esters and boronic acid functionalized compounds within five minutes, and thus, provides a general LC system for the purity analysis of this specific class of compounds.

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