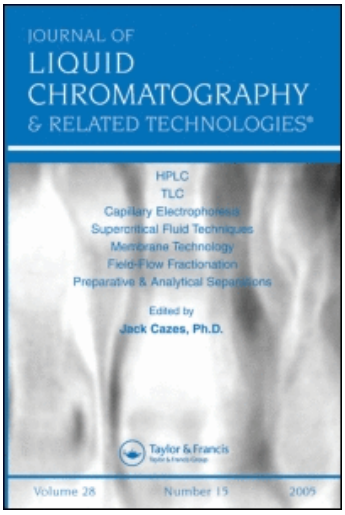

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On-Column Hydrolysis Kinetics Determination of Boronic Pinacol Ester Intermediates for Use in Optimization of Fast HPLC Methods

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On-Column Hydrolysis Kinetics Determination of Boronic Pinacol Ester Intermediates for Use in Optimization of Fast HPLC Methods

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Abstract: Boronic acid and boronic ester intermediates are the basis for Suzuki coupling and Petasis reactions that are widely used in pharmaceutical synthetic schemes. The analysis of these compounds utilizing traditional reversed phase liquid chromatography (RPLC) is complicated by the potential of on-column hydrolysis. In order to effectively develop and optimize RPLC methods for accurate analysis of these compounds, a better understanding of the potential on-column hydrolysis needs to be achieved. Kinetic studies of this type of on-column hydrolysis were performed utilizing a stop flow kinetic approach. The rate of on-column hydrolysis was determined as a function of initial organic composition, mobile phase pH, and column temperature. In addition, the Arrhenius activation energy was calculated. A fast reversed phase liquid chromatography method was then developed and optimized to minimize on-column hydrolysis effects based on the garnered information. The method was applied to successfully resolve ten different boronic acid and boronic pinacol ester functionalized compounds within five minutes. Thus, the wide range applicability of fast RPLC technology for accurate analysis of this specific class of compounds was demonstrated.

Keywords: On-column hydrolysis kinetics, Boronic acid, Boronic pinacol ester intermediates

INTRODUCTION

The utility of boronic acid and boronic pinacol ester intermediates in Suzuki coupling and Petasis reactions that are widely used in pharmaceutical

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synthetic schemes is well-established.[1–3] Boronic pinacol ester derivatives are often used in place of boronic acid as an isolated process intermediate in the pharmaceutical synthetic scheme due to complications of the boronic acid drying process. However, the ester derivatives are prone to hydrolysis back to its corresponding boronic acid derivatives under aqueous conditions.[1–3] Thus, purity analysis of boronic pinacol ester functionalized compounds utilizing reversed phase liquid chromatography methodology may be complicated by potential on-column hydrolysis of the compound.[4,5]

It has been long recognized that a chemical reaction occurring during a chromatographic process can give rise to distorted peaks and breakthrough curves.[6–8] Many such reactions have been reviewed by Jeng and Langer.[9] Clearly, determining the kinetics of those on-column reactions, which in our case is on-column hydrolysis, can help to establish proper chromatographic conditions to minimize the effect and maximize accuracy. The kinetics of on-column reactions can be controlled by optimization of the column temperature,[10] pH,[11] and addition of mobile phase additives.[12]

The influence of temperature on chromatographic separations has been discussed extensively. In general, temperature can have an impact on the mass transfer of the analyte between the mobile and stationary phases, and consequently, can lead to improved separations.[13] Improved separation of various steroids at subambient temperature using a non-aqueous mobile phase has been reported.[14] The effect of lowering the temperature on the separation mechanism in reversed phase chromatography was investigated.[15,16] The influence of column temperature on kinetics of on-column reactions has also been described in the literature. For instance, the inherent instability of active esters led to an on-column cyclization reaction under protic conditions, which give rise to distorted peaks.[10] The on-column cyclization was found to be extensive under ambient conditions. However, kinetic analysis demonstrated that insignificant on-column cyclization might be achieved utilizing subambient temperature chromatography. It has also been reported that lowering the separation temperature for proline based peptides under RPLC conditions retarded *cis-trans* isomerization of the amide functional group and enhanced their separation.[17,18] Moreover, lowering the temperature of chromatographic separations has served to retard the decomposition of unstable molecules.[19]

In this paper, we report the fast RPLC separation of a commonly used boronic pinacol ester functionalized compound, which undergoes extensive on-column hydrolysis under traditional RP-HPLC conditions. Kinetics of on-column hydrolysis of the compound was investigated utilizing a stop flow approach, and the rate constant was determined at variable column temperatures and mobile phase pH. Arrhenius plots can be generated from this data and the level of on-column hydrolysis can be predicted precisely under various RPLC conditions. Chromatography can then be optimized to minimize on-column hydrolysis resulting in more accurate RPLC analysis for the compound. Moreover, we utilize fast RPLC to separate ten commonly used boronic pinacol ester and boronic acid functionalized compounds.

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 an autosampler. The instrument was controlled by the HP Chemstation software. The UV detection wavelength was set at 220 nm. A Waters Xterra MS C18 column (50 × 4.6 mm; 2.5 μm particles) was used for kinetic studies and fast RPLC method development.

Chemicals and Reagents

HPLC grade acetonitrile as organic modifier for the 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 1) were obtained from Aldrich Chemical Company, Inc. (WI, USA). All samples were dissolved and diluted to the desired concentration with acetonitrile.

RESULTS AND DISCUSSION

Impact of Initial Organic Modifier Composition

A commonly used boronic pinacol ester functionalized compound and its hydrolysis product were used for the kinetic studies in this paper (Figure 2). It was found that under traditional RPLC conditions, significant baseline elevation between the boronic acid impurity and pinacol ester functionalized compound occurs and is due to continuous on-column hydrolysis of the ester.[4,5] Therefore, a method, which combines a fast 5 minute RPLC gradient program with a short Waters Xterra MS C18 column (50 × 4.6 mm; 2.5 μm particles) and DI water/acetonitrile mobile phase was used for purity analysis of the compound (Figure 3). The baseline elevation was eliminated under these fast LC conditions, which may be attributed to a shorter residence time in the column relative to the on-column hydrolysis rate.[4] The initial percentage of acetonitrile in the fast LC gradient program was varied from 15% to 28% v/v (Figure 3). The level of hydrolysis product at a different initial organic modifier (CH_3CN) composition was calculated (Table 1). As expected, a lower initial composition of organic modifier results in a higher level of hydrolysis product in the LC chromatography, since aqueous conditions facilitate on-column hydrolysis of boronic pinacol esters. An initial composition of 28% v/v acetonitrile was considered optimal, because at this composition minimal on-column

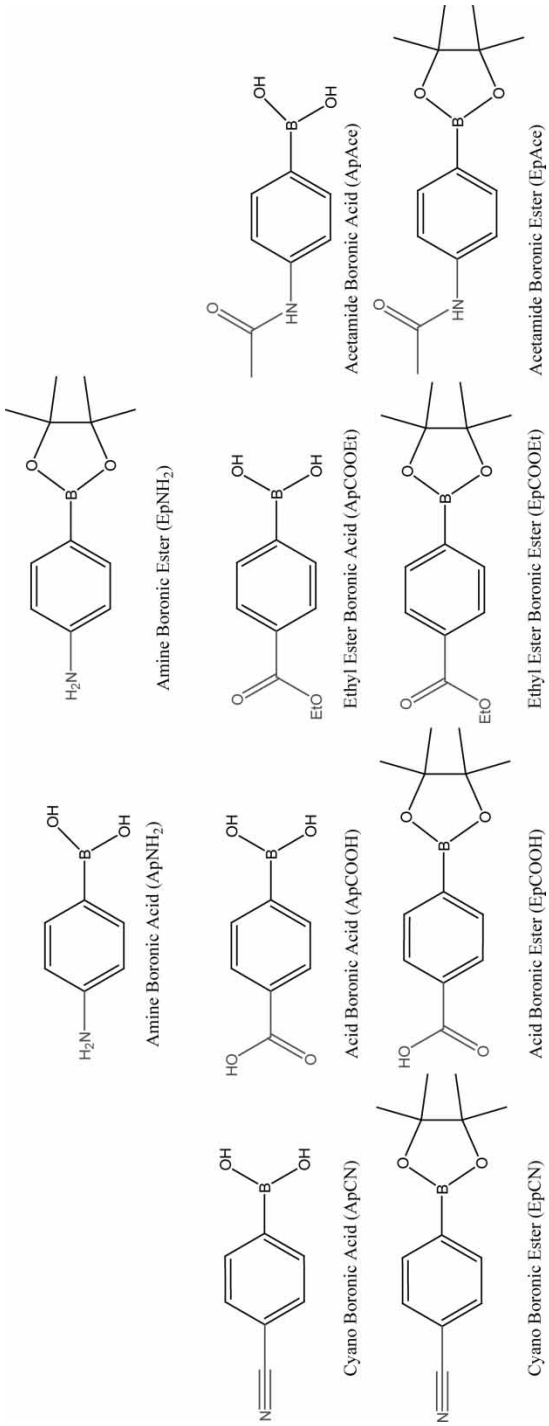

Figure 1. Ten commonly used boronic pinacol ester and boronic acid functionalized compounds.

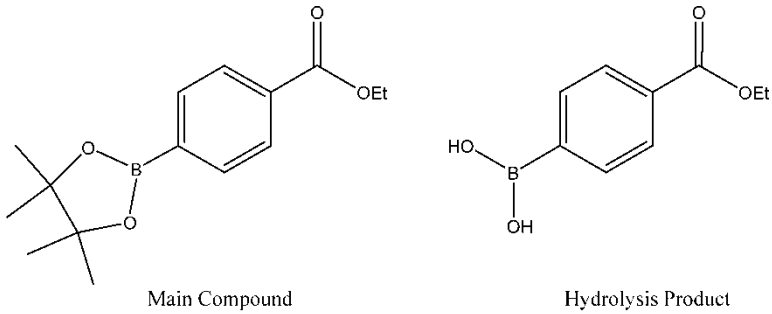

Figure 2. Model compound for kinetic studies.

hydrolysis occurs and significant retention of the ester and its hydrolysis product are achieved.

Kinetic Analysis of Boronic Pinacol Ester On-Column Hydrolysis Utilizing Stop-Flow Approach

To establish the kinetics of on-column hydrolysis, accurate levels of the boronic acid resulting from on-column hydrolysis need to be determined.

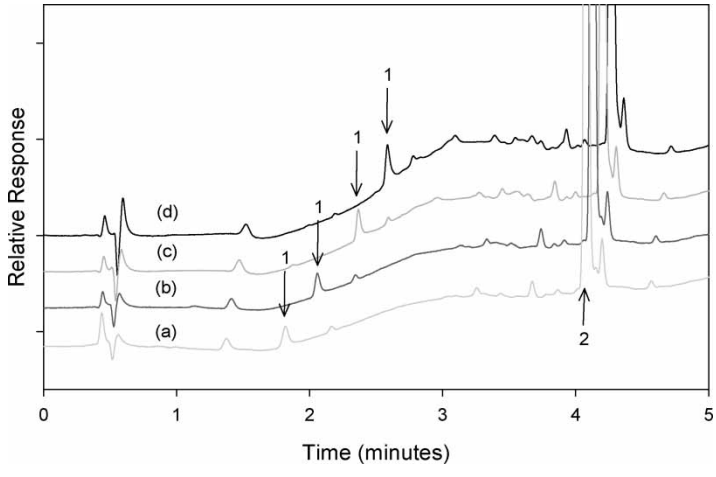

Figure 3. Fast RPLC chromatograms of the boronic pinacol ester functionalized compound with variable initial CH_3CN composition. (a) 28% v/v (b) 25% v/v (c) 20% v/v (d) 15% v/v. Other conditions: Waters Xterra MS C18 column, 50 × 4.6 mm, 3.5 μm; 1.25 mL/min flow rate, 2 μL injection, 210 nm UV detection, 20°C column temperature, linear gradient to 90:10 CH_3CN:DI water (v/v) in five minutes. 1: boronic acid impurity (ApCOOEt); 2: boronic pinacol ester functionalized compound (EpCOOEt); See Figure 2 for structures.

Table 1. Influence of initial composition of organic modifier on the formation of the boronic acid impurity

v/v% of initial CH_3CN	15	20	25	28
Area% of boronic acid imp.	3.1	2.0	1.8	1.8

For chromatographic conditions, see Figure 3.

A stop flow approach was used to separate the boronic acid originated by on-column hydrolysis from the pre-existing boronic acid (Figure 4). A boronic ester sample containing pre-existing boronic acid was injected with a flow rate of 1.25 mL/min at 28/72 v/v acetonitrile/water (pH = 7) for 0.5 minute. During this initial 0.5 minute, pre-existing boronic acid migrated along the column, while boronic ester was retained at the top of the column. The flow was then stopped for various periods of times, which allowed on-column hydrolysis of boronic ester to occur. The gradient program was then ramped up to 90% v/v of acetonitrile to elute out the boronic ester and all its impurities (Figure 5). The plot of ln $[(E + A')/E]$ versus the stop flow time was found to be linear ($R^2 = 0.998$) and, thus, consistent with pseudo-first-order on-column hydrolysis:

$$\ln [E/(E + A')] = -kt \tag{1}$$

where E represents the peak area of Boronic ester, and A′ as the peak area of bornic acid generated by on-column hydrolysis.

Applying the stop flow approach at various column temperatures in the range of 5–30°C, a series of plots were generated whose slopes represent the rate constants of on-column hydrolysis at respective temperatures (Figure 6).

Analysis of on-column hydrolysis kinetics under acidic mobile phase conditions (28/72 MeCN/0.1% H_3PO4, pH ∼ 2) was also performed using the same stop flow approach at column temperatures of 5 and 20°C,

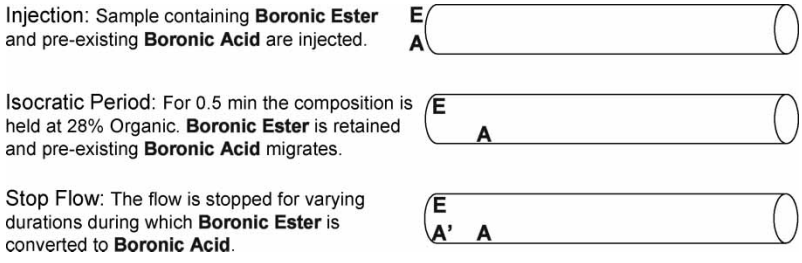

Figure 4. Illustration of stop flow approach for kinetic analysis of on-column hydrolysis.

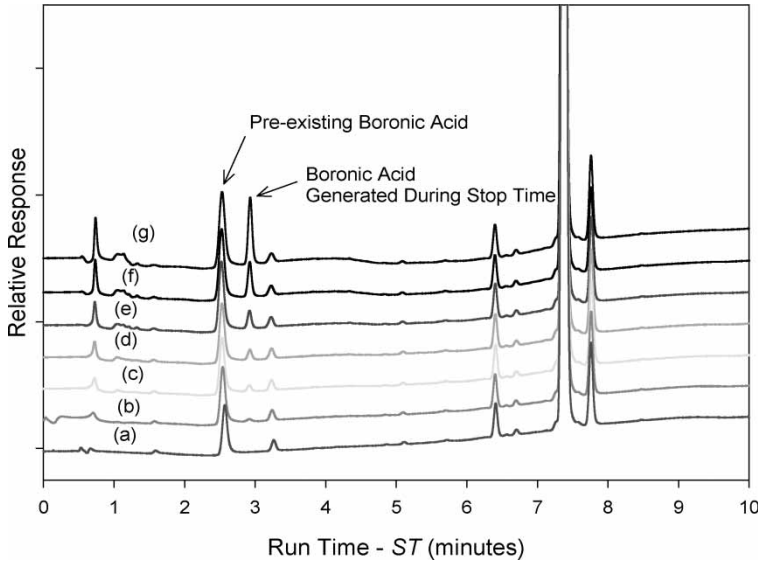

Figure 5. Stop flow RPLC chromatograms with variable stop times. Stop time (ST): (a) 0 min (b) 0.5 min (c) 1.0 min (d) 1.5 min (e) 2.5 min (f) 5.0 min (g) 10.0 min. Other conditions: Waters Xterra MS C18 column, 50 × 4.6 mm, 3.5 μm; 1.25 mL/min flow rate, 2 μL injection, 210 nm UV detection, 5°C column temperature, isocratic at 28:72 CH3CN:DI water (v/v) for 0.5 minute, stop flow with variable times, then linear gradient to 90:10 CH_3CN:DI water (v/v) in eight minutes, hold at 90:10 CH_3CN:DI water (v/v) for 2 minutes.

respectively. Plots of ln $[(E + A')/E]$ versus the stop flow time were generated and on-column hydrolysis rate constants were derived from the slopes.

The rate constants of on-column hydrolysis under different pH and respective temperature are summarized in Table 2. As expected, the on-column hydrolysis rate is pH dependent. Acidic mobile phase conditions will facilitate the on-column hydrolysis of boronic ester functionalized compounds (Figure 7). Moreover, column temperature plays an important role in minimizing on-column hydrolysis. The rate constant decreases as the column temperature decreases (Figure 7). Overall, the on-column hydrolysis rate of this boronic ester functionalized compound decreases 91% under optimal conditions (5°C, pH = 7), compared to that at higher temperature and in acidic mobile phase (20°C, pH = 2).

The relationship between the rate constant k and the temperature is given by the Arrhenius equation:

$$\ln k = -Ea/RT + \text{constant} \tag{2}$$

where Ea is the activation energy and R is the gas constant. According to the equation, a plot of ln k versus the reciprocal of the absolute temperature

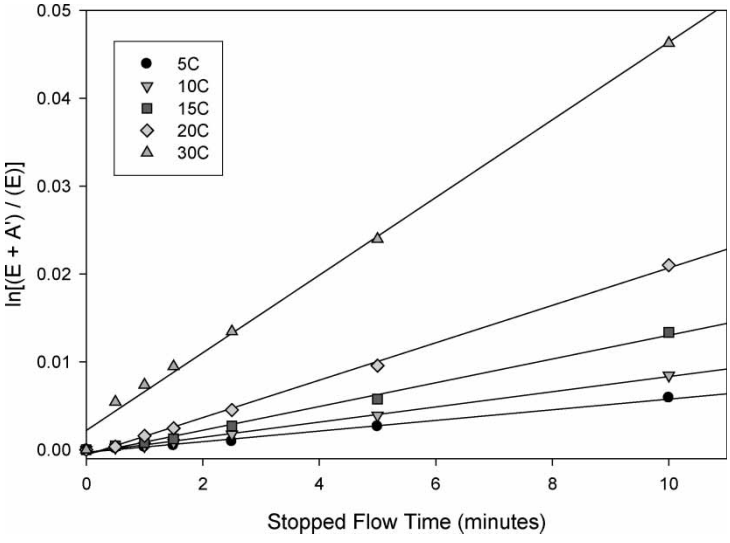

Figure 6. Pseudo-first-order on-column hydrolysis kinetics at variable temperatures. Other RPLC conditions: see Figure 5.

defines a straight line of slope $-Ea/R$. Indeed, a straight line was obtained in the range of 5°C to 30°C ($R^2 = 0.995$) with pH 7 mobile phase (Figure 8). The activation energy calculated from the slope of the graph was 13.1 Kcal/mol, which corresponds approximately to a doubling of the rate with every 10°C temperature increase.

The Arrhenius plot showed 0.16 area% of boronic acid produced from on-column hydrolysis at a column temperature of 5°C. By extrapolating the plot, it was calculated that insignificant on-column hydrolysis (<0.10 area% of hydrolysis product) might be achieved for this boronic ester functionalized compound at a column temperature of 0°C with initial 28% v/v

Table 2. Influence of pH and temperature on the rate constant of on-column hydrolysis

pH	Temp °C	1/Temp (K^{-1})	Rate constant (min^{-1})	R^2
7	5	0.00360	0.00063	0.998
	10	0.00353	0.00086	0.996
	15	0.00347	0.00135	0.994
	20	0.00341	0.00189	0.998
	30	0.00330	0.00442	0.995
2	5	0.00360	0.00300	1.000
	20	0.00341	0.00698	1.000

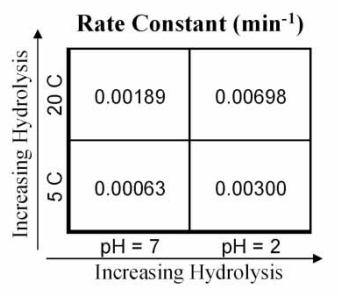

Figure 7. Comparison of on-column hydrolysis rate constants at variable pH and temperatures.

acetonitrile and pH 7 mobile phase system (Figure 8). Through the studies of on-column hydrolysis kinetics of the compound, optimal RPLC conditions for accurate purity analysis were obtained. At 0°C column temperature, DI water as aqueous phase and a 5 minute gradient of acetonitrile from 28% v/v to 90% v/v with 1.25 mL/min total flow rate, using a Waters Xterra MS C18 (50 × 4.6 mm, 2.5 μm particles) column, minimal on-column hydrolysis (<0.10 area% of hydrolysis product) of the boronic ester

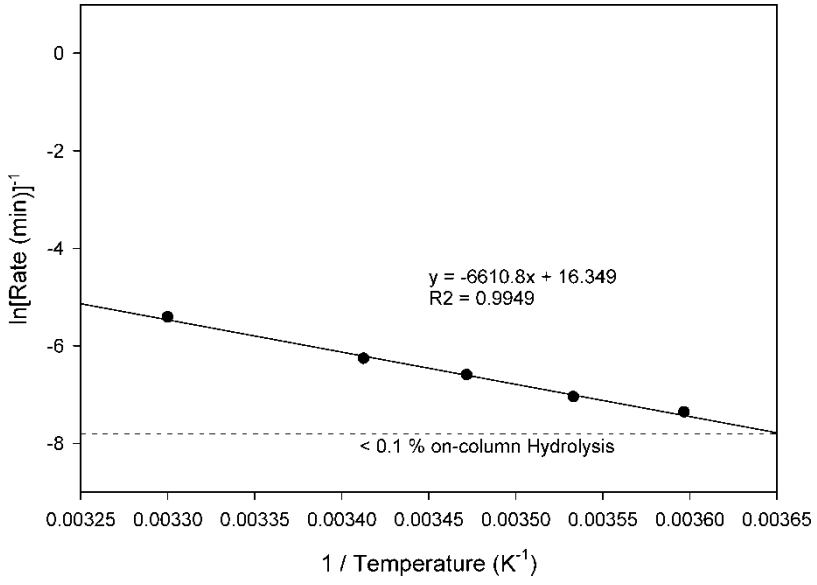

Figure 8. Arrhenius plot of hydrolysis rate constant vs. column temperature derived from stop flow approach. RPLC conditions: see Figure 5.

functionalized compound may be achieved with sustainable resolution between the compound and its process related impurities.

Fast RPLC Application and Stop Flow Approach for Analysis of Other Boronic Ester Compounds

The wide range applicability of fast RPLC technology to this specific class of compounds was investigated and discussed in detail in another paper. A mixture of five pairs of commonly used boronic acid and boronic pinacol ester functionalized compounds (Figure 1) was analyzed under fast LC conditions and an acceptable separation was achieved for all ten compounds within 5 minutes with no observed baseline elevation (Figure 9). Thus, the fast reversed phase LC method can be considered as a good starting point for the analysis of boronic pinacol ester functionalized compounds. The stop flow kinetics approach may be applied on each of the compounds, specifically to obtain accurate information of on-column hydrolysis. This information can then be used to optimize the RPLC method, so that accurate analysis of each specific boronic pinacol ester functionalized compound may be achieved.

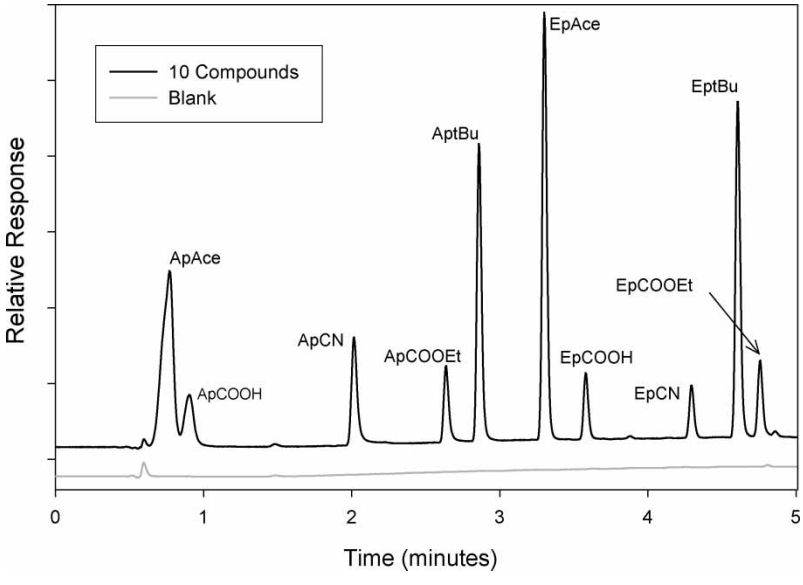

Figure 9. Fast RP-HPLC separation of ten boronic pinacol ester functionalized compounds with waters Xterra MS C18 column (50 × 4.6 mm, 2.5 μm). See Figure 2 for compound structures. Conditions: 1.25 mL/min flow rate, 2 μL injection, 210 nm UV detection, 20°C column temperature, linear gradient from 15:85 to 90:10 CH_3CN:0.1% H_3PO4 (v/v) in four minutes and hold 1 minute at 90:10 CH_3CN:0.1% H_3PO4 (v/v).

CONCLUSIONS

On-column hydrolysis of boronic pinacol esters is a major issue for accurate purity analysis of this class of compounds. Determination of hydrolysis kinetics of a commonly used boronic pinacol ester reveals that the rate of on-column hydrolysis depends on the initial composition of organic modifier, mobile phase pH, as well as column temperature. Pseudo-first-order rate constants were obtained utilizing a stop flow kinetic approach. An Arrhenius plot was constructed and the activation energy was calculated. From the Arrhenius plot, accurate levels of boronic acid produced from on-column hydrolysis may be obtained at varied column temperatures under certain RPLC conditions. After determining the hydrolysis kinetics under various conditions, a fast RPLC method may be developed that minimizes on-column hydrolysis and provides accurate determination of purity. Moreover, the fast RPLC method was demonstrated to be applicable for the separation of ten commonly used boronic pinacol ester and boronic acid functionalized compounds within five minutes. Combined with a stop flow kinetic approach, it provided a systematic resolution for the accurate purity analysis of this specific class of compounds.

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