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Applications of 2, 2, 2 trifluoroethanol as a versatile co-solvent in supercritical fluid chromatography for purification of unstable boronate esters, enhancing throughput, reducing epimerization, and for additive free purifications

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ABSTRACT

Analysis and purification of boronic acid pinacol esters by RPLC is very challenging due to their degradation in aqueous and alcoholic solvents. These compounds are difficult to purify by SFC too as they are equally sensitive to traditional co-solvents like methanol, ethanol, and 2-propanol. 2,2,2 trifluoroethanol (TFE), which has been reported for the purification of a few alcohol sensitive compounds, was evaluated as a co-solvent in this study for the purification of chiral and achiral boronate esters by SFC. Examples of twelve compounds were presented in this paper where degradation of boronic acid pinacol esters was successfully controlled by replacing methanol with TFE as the co-solvent in SFC. A separate study showed that TFE can also control the epimerization of the enantiomers of 3 substituted 1,4 benzodiazepine analogues during the purification process. In addition to above benefits, 2,2,2trifloroethanol showed improved selectivity and resolution for most of the compounds. With its stronger solvent strength compared to other alcohols, TFE could also be used to reduce the co-solvent percentage needed for elution and to shorten retention time of highly polar samples which did not elute even in 50% of other co-solvents in SFC. A case study of compound B demonstrated that TFE provided a reduced co-solvent percentage and a shorter cycle time with much improved resolution as compared to methanol, thus resulting in higher loading and throughput with reduction of total solvent consumption.

1. Introduction

Boron-containing organic compounds have found widespread use in synthetic organic and medicinal chemistry [1]. More recently, boronic acid-containing polymers have proven valuable in a variety of biomedical applications, including the treatment of HIV, obesity, diabetes, and cancer [2]. Interactions of boronic acids with diols and strong Lewis bases such as fluoride or cyanide anions lead to their utility in various sensing applications [3,4].

Boronic acids are widely used in Suzuki-Miyaura reaction as nucleophilic agent for the C—C bond formation [5–8]. Suzuki coupling is becoming an attractive tool for synthesis as boron compounds are easy to use and their by-products are less toxic, easy to purge from reaction mixtures and ultimately converting into environment-friendly boric acid [9]. However, boronic acids are unstable and undergo degradation through the pathways like oxidation, photodeboronation, polymerisation and tendency to exist as oligomeric anhydrides [10,11]. To overcome this instability, boronic acids have been masked by electron donating group like pinacol to make the corresponding ester. Pinacol masked boronic acids are relatively stable to air, moisture and do not require any deprotection step in Suzuki coupling [10,12,13].

Analysis and purification of boronic acid pinacol ester are challenging by HPLC due to its ready conversion to boronic acid in

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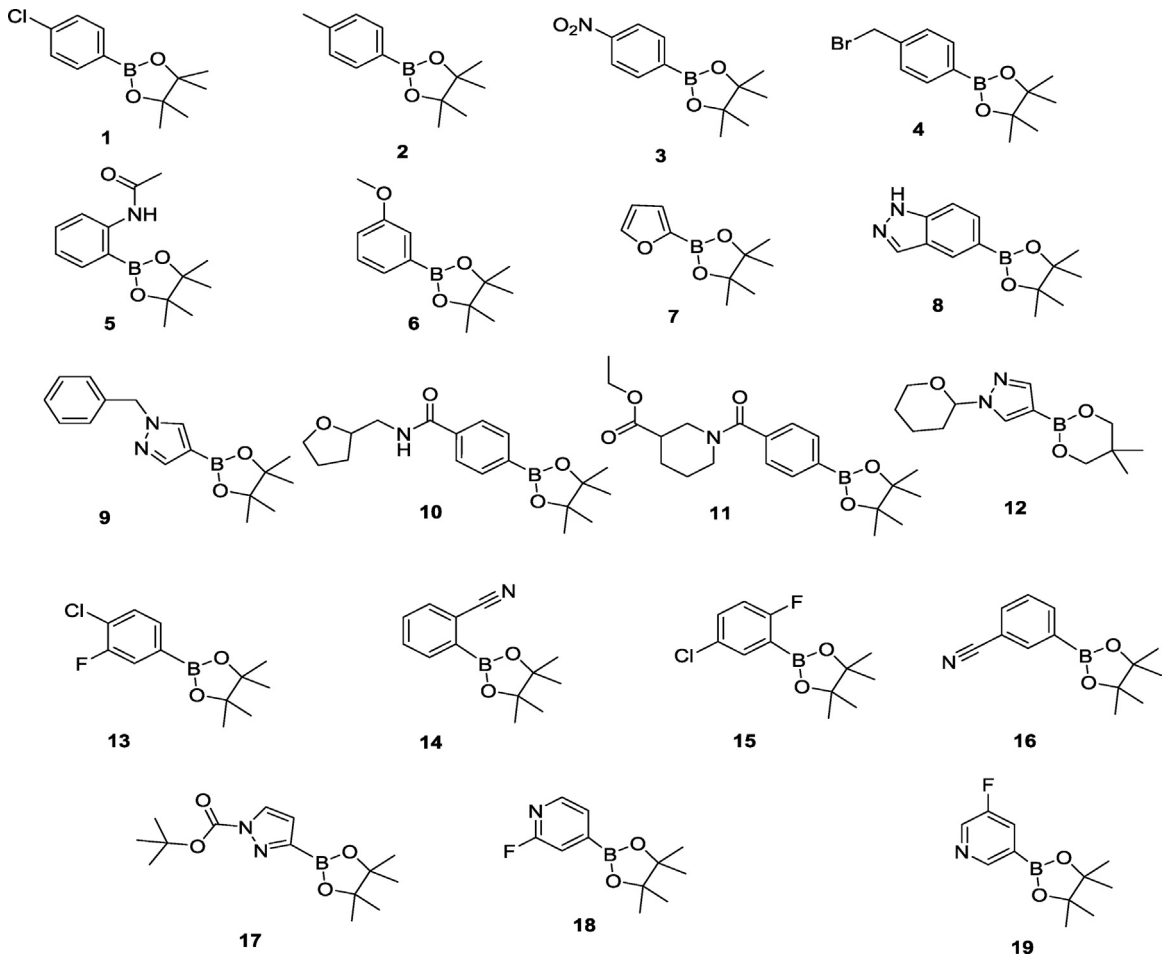

Fig 1. Structures of selected boronate esters for stability and purification studies in methanol and TFE.

aqueous solution. The extent of conversion depends on the electron donating or attracting power of the attached groups and the pH of the solution [14–17]. Xu et al. have attempted to determine the kinetics for on-column hydrolysis of boronic acid pinacol ester using a short column in reverse phase HPLC [18]. One more attempt has been made for the analysis of boronic acid pinacol ester using reverse phase HPLC [19] at high pH (~12) to minimize on-column hydrolysis. Forst et al. used capillary electrophoresis [20] for the separation of boronate esters from its corresponding acids and validated the method for qualitative and quantitative analysis. Even though there are few reported methods for the analysis of boronic acid pinacol ester, there are no reported methods for the preparative purification of these compounds due to associated instability.

Supercritical fluid chromatography (SFC) is becoming a preferred technique for the purification of chiral as well as achiral compounds at small and large scale [21–24]. Major advantages of using SFC for purification are attributed to its high throughput, low solvent consumption due to the low viscosity, high diffusivity of CO_2 in its super or sub-critical state, and the convenient evaporation of CO_2 from the SFC eluent during the fraction collection. SFC is also considered as a green technique as it uses non-toxic, non-flammable CO_2 as the main mobile phase constituent [25]. Use of modifiers like methanol, ethanol, propanol and acetonitrile etc. improves the application of SFC for the purification of polar samples in short run times. Though these solvents are convenient and efficient for separation and elution of compounds in SFC, they also induce the degradation of the ester functionalities via reactions like ester exchange, nucleophilic cleavage or substitution reactions [26]. Common acyl derivatives such as simple esters, anhydrides, thiol esters, phenol esters and certain amides often undergo significant reactions with solvents like methanol during the separation and post-column workup process [26]. Another problem observed during the purification of some chiral compounds by SFC is epimerization. During the isolation process of the enantiomers sometimes chiral inversion is observed in presence of mobile phase additives which leads to epimerization [27–29]. Byrne et al. reported the use of 2, 2, 2, trifluoroethanol (TFE) as a co-solvent in SFC for the purification of some alcohol sensitive compounds [26]. They suggested that nucleophilic reactivity of the oxygen lone pair in TFE might be weak due to electron withdrawing effect of three β substituted fluorine atoms. Therefore it serves as a polar hydrogen bond donor and being less reactive as a nucleophile compared to methanol and ethanol. Kagan et al. also reported the use of fluorinated solvents (Ethoxynonafluorobutane) for chiral separation without any detrimental effects on stationary phases [30].

Based on these reports the use of TFE for the SFC purification of chiral as well as achiral boronic acid pinacol esters were evaluated in the present study. Ten achiral and two chiral boronic acid pinacol esters were subjected to SFC purification using TFE and methanol as co-solvent in separate experiments and the stability was monitored throughout the purification process by HPLC. Collected fractions were concentrated by rotary evaporation and characterized by MS and NMR. A few case studies of co-solvent reduction, enhanced throughput and elimination of epimerization are also discussed below to showcase the additional advantages of TFE as a co-solvent in SFC.

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