Phenylboronic Acid (PBA) Solid Phase Extraction Mechanisms and Applications

Technical Overview

Introduction

Bond Elut PBA is a unique silica SPE sorbent containing a phenylboronic acid functionality that can retain analytes via a reversible covalent bond. This very strong covalent retention mechanism enables high specificity and cleanliness. The boronate group has a strong affinity for cis-diol containing compounds such as catechols, nucleic acids, some proteins, carbohydrates and PEG compounds. Aminoalcohols, alpha-hydroxy amides, keto compounds, and others can also be retained.

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PBA SPE Mechanism

Most solid phase extraction methodologies are based on non-polar, polar or ionic interactions. Phenylboronic acid (PBA) solid phase extraction media is unique in that it employs a reversible covalent interaction between the PBA and sample molecule(s). Covalent chromatography involves an interaction of considerably greater energy than other extraction mechanisms (Figure 1). This strong interaction allows for the development of extraction methods of much greater specificity.

The mechanism of boronate binding is illustrated in Figure 2. The immobilized phenylboronic acid is first equilibrated with an alkaline solution to obtain the reactive boronate form $RB(OH)_3^-$. The diol-containing compound is next applied and is covalently bound with the concomitant release of water. Once the compound of interest is retained, contaminants may be selectively washed from the bonded phase provided that an alkaline pH is maintained. Finally, the compound of interest is eluted by acidification of the boronate complex, which releases the diol-containing compound and renders the immobilized phenylboronic acid neutral ($RB(OH)_2$).

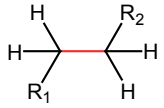
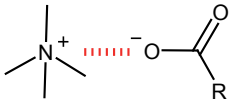
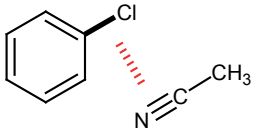
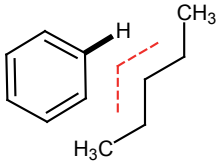
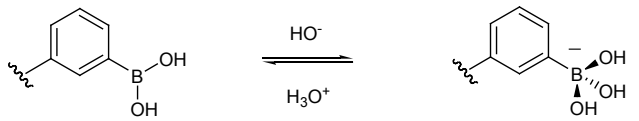
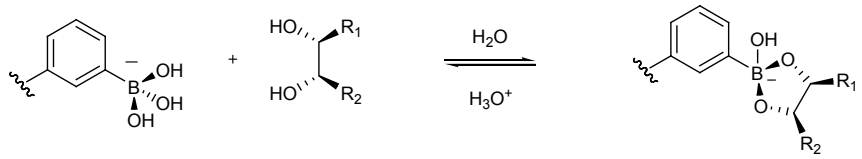
Interaction	Example	Energy [kcal/mole]
Covalent		100-300
Ionic		50-200
Hydrogen Bonding		5-10
Dipole-Dipole		3-10
Dipole-Induced Dipole		2-6
Dispersion non-polar or Van der Waals		1-5

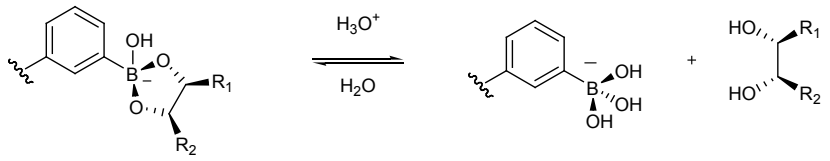
Figure 1. The relative energetics of various retention mechanisms emplyed for the purpose of bonded phase extraction



Equilibration



Retention



Elution

Figure 2. The three step process of borate binding including equilibration, retention and elution

Applicable Analytes

A variety of diol-containing and other compounds are retained by immobilized phenylboronic acid, including vicinal diols, 1,3-diols and 1,3,5-triols. Retention of vicinal diols requires that the diol maintains a coplanar or cis conformation. Intramolecular hydrogen bonding often renders 1,3-diols coplanar and they are retained. Carbohydrate rings that exhibit pseudorotation may be retained depending on other ring substitutions. Theoretically, any two or three hydroxyls spaced so as to fill the tetrahedral sites surrounding the trihydroxyboronyl functionality may be retained.

A variety of functional groups other than diols will be retained by immobilized phenylboronic acid through the combination of charge-transfer and covalent interactions. Compounds not yet mentioned include aromatic *o*-hydroxy acids and amides, α-hydroxy acids, 1,3-dihydroxy-, diketo-, triketo- and aminoalcohol-containing compounds.

PBA SPE has been used extensively for the extraction of catecholamines, nucleic acids, proteins and carbohydrates from a variety of matrices, as well as for the successful extraction of brassinosteroids, lipids and polyhydroxyflavones, and the successful separation of gycosylated proteins from non-gycosylated proteins and ribonucleotides from deoxyribonucleotides[1-16]. The cis-diol found in many biomolecules lends itself to PBA extraction. Unpublished work has shown that PEG (polyethylene glycol) metabolites can also be cleared from biological samples using PBA SPE through covalent bonding between PEG metabolites and PBA. The scope of molecular species that can interact with the di- or trihydroxyboronyl functionality is summarized in Table 1.

Table 1. Molecules expected to bind to phenylboronic acid

Compound Class	Examples
Polyhydroxy	Glycoproteins, monosaccharides
Aromatic o-hydroxy	Catecholamines, nucleic acids, ribavirin, glycoproteins, carbohydrates, SAMe, tannins
α-hydroxy acids	Lactic acid
Aromatic o-hydroxy acids and amides	Salicylic acid, salicylamide
1,3-Dihydroxy	Tris, pyridoxine
Diketo and triketo	Dehydroascorbic acid, benzil, alloxan
Aminoalcohols	β-Blockers
Other dihydroxys	Steroids, lipids
Others	Statins

Extraction Procedure

Cartridge conditioning

It is necessary to properly equilibrate the PBA bonded phase with an alkaline solution to obtain the active boronate form $RB(OH)_3^-$. The pKa of the immobilized phenylboronic acid is approximately 9.2. Recall that at pH 9.2 only half the immobilized groups will be reactive (ionized). A sample preparation column can be briefly equilibrated at pH 10 or 12 without damage to the silica substrate. For example, equilibrate PBA with 50-100 mM phosphate buffer, pH 10. If samples are base-sensitive, it can be effective to condition the extraction column with 0.05-1.5 M alkaline buffer, pH 10-12, followed by 0.01-0.50 M buffer at pH 8-8.5.

Secondary interactions

The secondary interactions anticipated for an immobilized phenylboronic acid are illustrated in Figure 3.

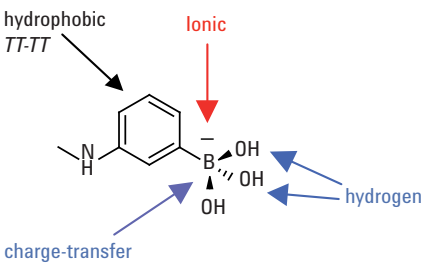


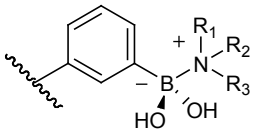
Figure 3. The anticipated secondary interactions exhibited by immobilized PBA

Below pH 7 these secondary interaction will generally predominate. Both the neutral and anionic forms of the immobilized functionality contain hydroxylated boron which functions as a hydrogen bond donor under appropriate conditions. Consequently, polar compounds including diols may be retained by normal phase mechanisms, if extracted from organic solvents. The propyl linkage and phenyl ring through which the boron functionality is immobilized may provide sites of nonspecific retention owing to Van der Waals and pi-pi interactions if compounds of hydrophobic character are present. Ionic repulsion may occur between the boronate anion and anions on the compound of interest. For example, because of the ionic repulsion between the boronate and phosphate groups of nucleic acids, it is necessary to add a divalent cation to the mobile phase in order to get good binding. Finally, cations may be retained by the anionic boronate functionality. If not removed in the wash step, these ions will coelute with the compound of interest when the bonded phase is acidified, and may subsequently interfere with detection, although most ionic species elute in the void volume of the analytical column. Ionic secondary interactions may also be

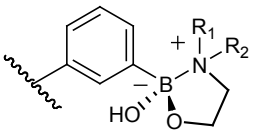
avoided by using buffers of higher ionic strength, between 50 and 500 mM, in the equilibration, sample application and/or wash steps.

Buffer selection

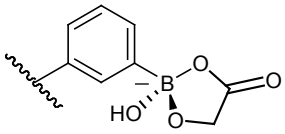
An additional secondary interaction of considerable importance in understanding immobilized phenylboronic acid is the potential of boron to form a charge-transfer complex with 1′, 2′, 3′ amines. Unprotonated amines readily form such complexes. Although complex formation is believed to involve only the neutral phenylboronic acid form, equilibrium considerations allow for complex formation under alkaline conditions. In the charge-transfer complex the amine obtains a positive charge and the boron a negative charge rendering the complex neutral (Figure 4). Charge-transfer complex formation with an immobilized phenylboronic acid can have both favorable and deleterious impact on a bonded-phase extraction.



A -Charge-Transfer complex with amine



B - Charge-Transfer complex further stabilized by covalent interaction of ß-hydroxyl group



C -Covalent interaction with aliphatic α-hydroxy carboxylic acid

Figure 4. Complexes resulting from interactions with immobilized phenylboronic acid

The effective pKa of an amine-organoboron charge-transfer complex is lower than that of the isolated organoboron. Consequently, complex formation can reduce the pKa of the immobilized functionality thus increasing the capacity of the bonded phase. Therefore, zwitterionic primary and secondary amine buffers such as HEPES can enhance binding. The zwitterionic character of these compounds has the added advantage of functioning as a local acid scavenger, removing protons associated with the sample matrix and thus ensuring that the organoboron retains its reactive ionic form. Buffers employed for this purpose include HEPES, glycine, diglycine and morpholine[17-18].

In selecting an appropriate amine for the purpose of charge-transfer complex formation, ß-hydroxyl amines should be avoided, as they form highly stable complexes in which the amine and ß-hydroxyl groups interact with the dihydroxyboronyl functionality to form an adduct with both charge-transfer and covalent components (Figure 4). Therefore, bicine, tricine, tris and 1′,2′,3′-ethanolamine buffers should not be used.

Removing interferences

It should be assumed that once the sample has been applied to a properly conditioned column, compounds are retained by both covalent and nonspecific mechanisms. It is at this stage of the extraction process that the advantages of covalent chromatography become apparent.

The energetics of the covalent retention mechanism are high as compared to those of reverse phase mechanisms. Consequently, compounds retained by nonspecific mechanisms may now be eluted while the compound of interest remains bound. Most contaminating species, including many ionic species, can be removed by washing the bonded phase with 50% methanol or acetonitrile. As long as the pH of the aqueous component of the wash solvent remains alkaline, the covalent complex will remain intact.

Elution

A variety of acids can be employed as elution solvents. In most cases, reduction to pH 5 is sufficient to effect elution and significant buffer capacity is not required. Acetic, formic, hydrochloric, phosphoric and trifluoroacetic acids (TFA) have been used. In many cases, HPLC mobile phase for reverse phase chromatography is an effective elution solvent.

The addition of up to 90% organic modifier is permissible and ensures that the compound of interest is not retained by secondary interactions. Charge transfer complexes involving amines require reduction to pH 3 to ensure complete elution. Competitive elution with a borate ion, competing diol such as sorbitol or mannitol, or an α-hydroxyl such as tris provide an alternative to acidification when extraction of proteins or acid labile compounds is required.

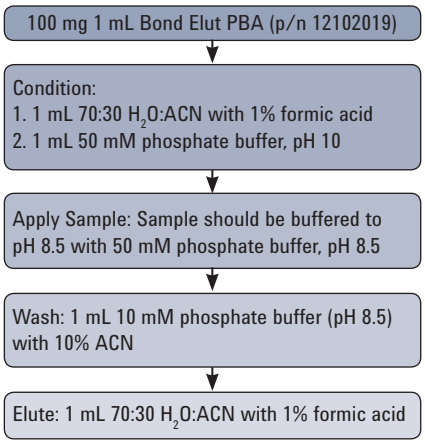


Figure 5. Generic method

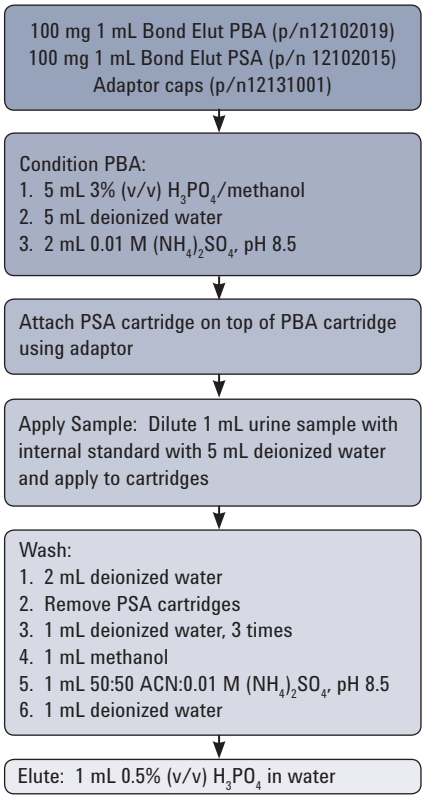


Figure 6. Extraction method for catecholamines from urine

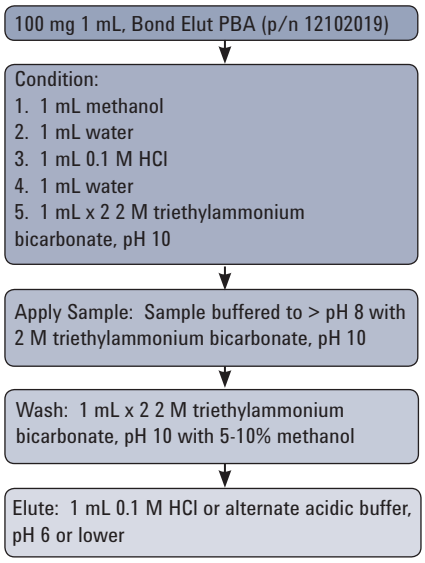


Figure 7. Method for separation of ribonucleotides from deoxyribonucleotides

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