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.
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).

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Example</th>
<th>Energy [kcal/mole]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covalent</td>
<td><img src="image" alt="Covalent" /></td>
<td>100-300</td>
</tr>
<tr>
<td>Ionic</td>
<td><img src="image" alt="Ionic" /></td>
<td>50-200</td>
</tr>
<tr>
<td>Hydrogen Bonding</td>
<td><img src="image" alt="Hydrogen Bonding" /></td>
<td>5-10</td>
</tr>
<tr>
<td>Dipole-Dipole</td>
<td><img src="image" alt="Dipole-Dipole" /></td>
<td>3-10</td>
</tr>
<tr>
<td>Dipole-Induced Dipole</td>
<td><img src="image" alt="Dipole-Induced Dipole" /></td>
<td>2-6</td>
</tr>
<tr>
<td>Dispersion non-polar or Van der Waals</td>
<td><img src="image" alt="Dispersion" /></td>
<td>1-5</td>
</tr>
</tbody>
</table>

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

**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, ribavirin, glycoproteins, carbohydrates, SAMe, tannins, aromatic o-hydroxy acids and amides, lactic acid, salicylic acid, salicylamide, tris, pyridoxine, dehydroascorbic acid, benzil, and alloxan, β-blockers, steroids, lipids, statins, and others.

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

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.
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.

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. 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 4. Complexes resulting from interactions with immobilized phenylboronic acid**

![Figure 4](image-url)
100 mg 1 mL Bond Elut PBA (p/n 12102019)
100 mg 1 mL Bond Elut PSA (p/n 12102015)
Adaptor caps (p/n 12131001)

Condition PBA:
1. 5 mL 3% (v/v) H3PO4/methanol
2. 5 mL deionized water
3. 2 mL 0.01 M (NH4)2SO4, pH 8.5

Attach PSA cartridge on top of PBA cartridge using adaptor

Apply Sample: Dilute 1 mL urine sample with internal standard with 5 mL deionized water and apply to cartridges

Wash:
1. 2 mL deionized water
2. Remove PSA cartridges
3. 1 mL deionized water, 3 times
4. 1 mL methanol
5. 1 mL 50:50 ACN:0.01 M (NH4)2SO4, pH 8.5
6. 1 mL deionized water

Elute: 1 mL 0.5% (v/v) H3PO4 in water

Figure 6. Extraction method for catecholamines from urine

100 mg 1 mL Bond Elut PBA (p/n 12102019)

Condition:
1. 1 mL methanol
2. 1 mL water
3. 1 mL 0.1 M HCl
4. 1 mL water
5. 1 mL 50:50 ACN:0.01 M (NH4)2SO4, pH 8.5
6. 1 mL deionized water

Apply Sample: Dilute 1 mL urine sample with internal standard with 5 mL deionized water and apply to cartridges

Wash: 1 mL x 2 2 M triethylammonium bicarbonate, pH 10

Elute: 1 mL 0.5% (v/v) H3PO4 in water

Figure 7. Method for separation of ribonucleotides from deoxyribonucleotides

References


