



Technical Tip

How do I Extract Amino Acids?

Amino acids analysis is typically used to determine protein or peptide content, food composition/authentication, etc. Since their composition in proteins and food is of great interest to us, a simple and reliable extraction method for amino acids is in high demand.

For protein and peptide samples, an acid hydrolysis is a common pretreatment step to preparing amino acids. A pre-column or post column derivatization step is carried out with a reagent that enhances the ability of the detector being employed to qualify or quantify amino acids.

Excluding any side chain, each amino acid has at least one acid and one basic moiety. The acidic group has a pKa value of ~2.3 (± 0.5 depending on structure). The pKa values of the basic group range from 9.0-10.70. One would need to de-ionize one of the charges when an ion exchange SPE cartridge is employed for the extraction procedure to emphasize the other charge.

The best retention of amino acids occurs when the sample is at low to neutral pH using Strata™-X-C or Strata-SCX. When an amino acids sample at pH 3 on, the sorbent is fully charged and complete retention of the amino acids is observed. Even samples loaded at pH 7 are still fully retained. The amino acids can be recovered with an elution of 3M ammonium hydroxide solution. Typical recoveries are 75-99%, depending on the type of amino acid.

In conclusion, the following SPE procedure is recommended for the extraction of amino acid before any derivatization takes place.*

To a Strata-SCX or Strata-X-C cartridge**,

Condition with methanol and equilibrate with deionized water

Load sample*

Wash (if necessary) with 0.1N HCl followed by methanol

Dry cartridge under vacuum for 2-5 minutes

Elute with 5-10% ammonium hydroxide in methanol (if X-C is used); 5% ammonium hydroxide in water (if SCX is used)

*Arginine has pKa values of 2.17 (COOH), 9.04 (NH₂), and 12.48 (side chain). Due to the high pKa value of the side chain, its recovery may be lower than other amino acids

**Refer to the sorbent loading capacity guide to help with bed mass selection and volume of sample to be loaded