Millennial Scientific

NanoPak-C All-Carbon Microbeads-Packed Solid Phase Extraction (SPE) Columns – General Sample Preparation and Extraction Guide for Solid, Semi-Solid, and Liquid Samples.

Content Summary.

1. Introduction. Solid-phase extraction (SPE) is a widely employed sample preparation technique in chromatography. This document provides general guidance on establishing a rugged method to extract acidic, neutral, and alkaline compounds from solid, semi-solid, and liquid samples using NanoPak-C All-Carbon Microbeads-Packed Solid Phase Extraction (SPE) Columns.

2. Initial Sample Processing. This section discusses a few well-established methods for preparing your solid, semi-solid and liquid samples before SPE procedures.

3. SPE protocol. This section contains generic protocols to extract acidic, neutral, and alkaline compounds using NanoPak-C SPE columns.

4. Flowcharts of SPE Protocol and Optimization Tactics. The section presents the general sample preparation steps and tactics to optimize extraction protocol in a flowchart.

Keywords. NanoPak-C, All Carbon Microbeads, Solid Phase Extraction, Protocol, Guide

1. Introduction.

Solid-phase extraction (SPE) is a widely employed sample preparation technique in chromatography.[1] It is used to purify and concentrate analytes before introducing them into more expensive gas- or liquid- chromatography instrumentation. This process is now gaining recognition as a method for rapid fractionation of crude plant extracts.[2]

NanoPak-C All Carbon microbeads are universal SPE media for extracting and concentrating acidic, alkaline, and compounds. The microbead's porous carbon network allows reverse phase separation of large molecules, highly polar & closely related structures at extreme pHs. This feature facilitates better removal of nuisance compounds & impurities, improving quality control & regulatory compliance. Further, it allows higher retention capacity compared to traditional silica-based SPE sorbents like C18. Thus, more analytes can be extracted, concentrated, and retained, improving your SPE method's recovery and overall reproducibility.

The microbeads exhibit uniquely bioinert properties. These features increase column stability & durability at all pHs & high temperatures (up to 125°C). Thus, the retention capacity does not reduce even if the stationary phase bed is dried during conditioning or sample loading. Further, this feature reduces workflow time, solvent consumption. Finally, it allows cleaning & sanitization of the columns with alkaline washes & reusing of the SPE column.

The NanoPak-C All Carbon microbeads are available in different sizes (average diameter of 10 µm, or 40 µm), formats (columns, or cartridges of other volumes), and scalable. These options allow you to select the appropriate product based on your sample's volume, viscosity, and turbidity.

2. Preliminary Sample Processing.

A. Solid Samples*. Presented below is the general sample processing procedure for solid samples in the form of Soil, Food, or Biological Samples (e.g., Cells and Tissues) to get them ready for SPE procedures.*

1. The first step is to mix and homogenize the solid samples in an appropriate solvent to extract the analyte compound grossly. The solvent can be pure aqueous, organic, or a mix of aqueous and organic solvent. The aim is to find an appropriate solvent that maximizes the analyte's extraction and minimize the extraction of nuisance and other interference compounds present in the sample. The solvent itself may be insufficient for optimal extraction of the analyte compound. Thus, buffers, co-solvents, surfactants, and dispersive salts may be added to optimize analyte compound extraction.

If necessary, suspended solids present during the gross extraction analyte compound could also be centrifuged or filtered to remove before SPE procedures.

B. Liquid Samples: *Presented below is the general sample processing procedure for liquid samples in the form of aqueous (e.g., Water, Beverages), non-aqueous, or biological (e.g., plasma, serum, urine) Samples to get it ready for SPE procedures.*

1. The first step is to process the liquid samples to extract the analyte compound.

a. The aqueous samples such as water, or

beverages, if necessary, can be centrifuged or filtered to remove suspended solids to it ready for SPE procedure

b. Non-Aqueous samples can be mixed with aqueous buffers and organic solvents or co-solvents to improve their retention during the SPE procedure. If non-aqueous samples are diluted, they can be filtered to remove the suspended solids before the SPE procedure.

c. Biological samples such as plasma, serum, or urine, if needed, can first be clarified by centrifugation at 10,000-15,000 x g for 10-30 minutes. The supernatant can be decanted out and mixed or diluted in acidic or alkaline aqueous solutions to get it ready for SPE procedures.
To ensure consistency, prepare a large volume (100-500 mL of the acid or alkaline solution) for multiple samples.

The suggested acidic aqueous solution is 5% phosphoric acid. For example, to prepare 100 ml, take 5.8 mL of commercially-available 85% phosphoric acid and dilute it using distilled water to 100 mL final volume.

The suggested alkaline solution is 5% liquid ammonia (also known as ammonium hydroxide). For example, to prepare 100 ml, take 5 mL of commercially-available concentrated ammonia to dilute it using distilled water to 100 mL final volume.

Plasma, serum, or urine can be mixed with the acidified or alkaline aqueous solution in a 1:1 ratio. The samples can additionally be combined with buffers and organic co-solvents to improve their retention during the SPE procedure. If organic co-solvent is used, its concentration should be less than 10% total solvent amount. Above this concentration, proteins could precipitate. The precipitated protein can trap the analyte compound prevents its retention during SPE procedures.

3. SPE protocol.

Next, use this grossly exacted analyte solution and pass it through the SPE column to extract and concentrate the analyte compound finely. Additional procedures (pH, solvent adjustments) can improve extraction efficiency using SPE procedures (see section 4 below). Before starting the SPE procedures, add 10 to 50 µL of internal extraction standard to grossly extracted analyte solution. The SPE protocol is as follows.

1. Place the NanoPak-C SPE column on the vacuum manifold. Set the vacuum. Depending on the SPE column volume and stationary media bed weight, adjust the vacuum so that that liquid can flow through the bed. A suggested starting vacuum is 2 psi. You may then change it (increase or decrease) till discreet droplets are eluted. A prolonged flow rate will inordinately increase extraction time. A fast flow rate will lead to suboptimal analyte retention or elution. A flow rate to aim can be 1 mL/ min. This flow rate should allow the generation of discreet droplets and is recommended for loading and eluting the analyte. A higher flow rate can be used for other steps.

2. Place a waste collection device below the SPE column.

3. Pour methanol from the top to condition the SPE column. Next, equilibrate the column with the water. For each of these steps (conditioning and equilibration), add the solvent before applying a vacuum.

4. Before loading the sample, switch off or stop the vacuum. Next, load your diluted sample.

5. Switch on and gradually increase the vacuum as needed to load the entire sample onto the stationary media bed. Collect the waste liquid.

6. Switch off or stop the vacuum.

7. Introduce the wash solvent. For example, 5-10% methanol in water could serve as a wash solvent.

8. Again, switch on and gradually increase the vacuum to flow the wash solvent through the stationary bed. Collect the waste liquid.

9. Remove the waste collection device and discard waste liquid.

10. Place an analyte collection device below the SPE column.

11. Introduce 100% methanol in the SPE column as the elution solvent.

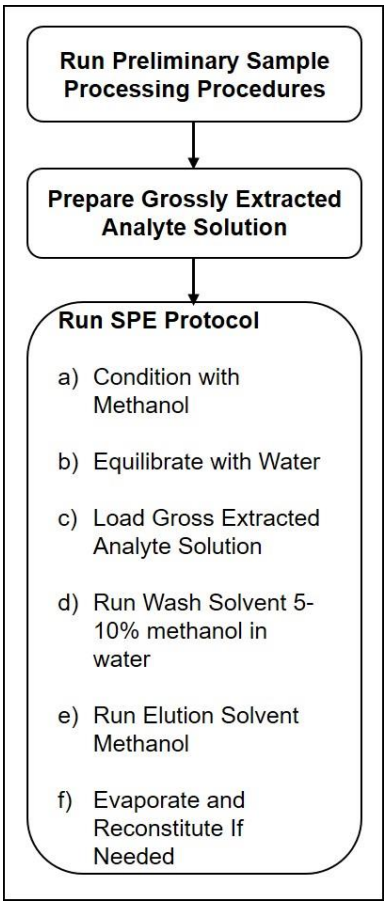
12. Let the elution solvent flow through the SPE under gravity before applying a vacuum. Gradually increase the vacuum to elute the solvent.

13. Remove analyte collection device, and evaporate, reconstitute or dilute the collected solvent as appropriate to get the analyte ready for analysis.

Additional Notes. i) The above procedure is a suitable sample preparation for liquid chromatography with MS or other detectors. It is also suitable for the preparation of samples for direct MS analysis.

ii) Do not use procedures developed for C18 or other silica-based stationary phase media. The washing and elution steps designed for C18 or other silica-based stationary phase media may not be optimal for NanoPak-C All Carbon media.

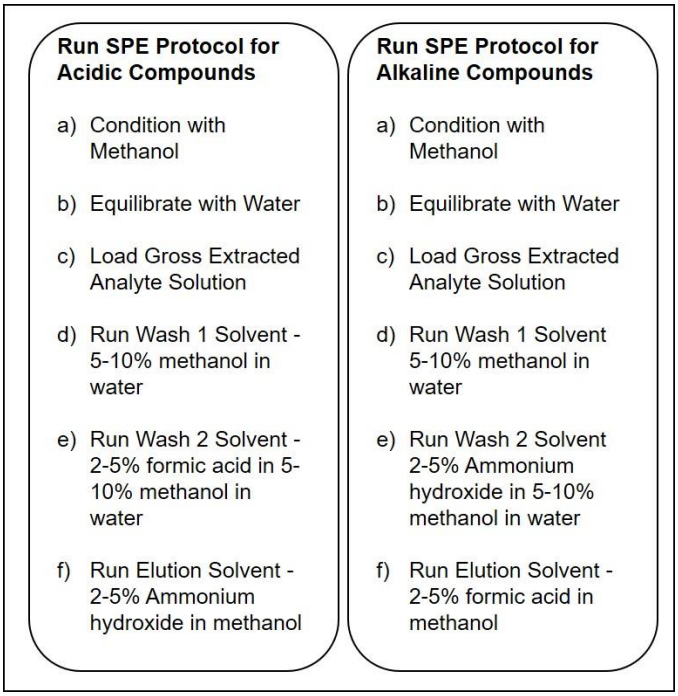
A. SPE protocol



4. Flowcharts of SPE Protocol and Optimization

B. Optimization Tips

The general SPE protocol works well for consistent extraction and concentration of a wide range of acidic, neutral, and alkaline compounds. The optimization tips below could be applied as part of the method development to remove any background interfering or nuisance compounds further.



B. Optimization Tips

Use the entire pH range (pH 1 to 14) and vary to organic solvent amounts to optimize the SPE protocol further.

1. The above flowchart presents the optimization tips. Run the general SPE protocol up to first wash. Then, to remove background interfering compounds, apply a second wash. The pH of this wash is adjusted to increase the retention of the analyte. For acidic analytes, the pH is decreased below the pKa of the compound. For alkaline analytes, the pH is raised above the pKa of the compound. A good thumb rule is pH should be adjusted two units below the pKa value for acidic compounds and two units above the pKa value for alkaline compounds.

To elute the retained analyte, change the pH of the elution solvent. For the acid analyte, adjust the pH to alkaline values. For alkaline analyte, adjust the pH to acidic values.

The optimal amount of organic solvent for wash 2 step can be identified by increasing the % methanol by 10% at each pH and analyzing the wash 2 and eluted solvents for the analyte compound.

Analyze the Wash 2 and Elute samples to determine optimum % methanol. First, choose the %methanol value that does not remove the analyte during wash 2. Next, choose the %methanol that removes the analyte during the elution step.

5. References.

1 Buszewski, B. & Szultka, M. Past, Present, and Future of Solid Phase Extraction: A Review. *Critical Reviews in Analytical Chemistry* 42, 198-213, doi:10.1080/07373937.2011.645413 (2012).

2 Bucar, F., Wube, A. & Schmid, M. Natural product isolation – how to get from biological material to pure compounds. *Natural Product Reports* 30, 525-545, doi:10.1039/C3NP20106F (2013).