Millennial Scientific

Tannic Acid Extraction Employing NanoPak-C All-Carbon Microbeads-Packed 1 mL SPE Columns, and Quantification Using a UV Microplate Reader

Michael Jack Parente, Balaji Sitharaman

Millennial Scientific, 25 Health Science Drive, Stony Brook, NY 11790, USA.

Abstract.

Background. Tannic acid, an amphiphilic polyphenolic compound found in plant-based products, is a well-established and ubiquitous nuisance compound in plant extracts. Herein, we report an optimal solid phase extraction method to extract tannic acid present in microliter volumes, using an all-carbon reverse phase material.

Method Used. Tannic acid dissolved in methanol was extracted using NanoPak-C all carbon graphite microbeads (100 mg bed weight), packed into 1 ml SPE columns. A UV microplate reader was used to characterize and quantify µL volumes of tannic acid.

Results. The optimized tannic acid extraction protocol allowed recovery efficiencies of 94%.

Conclusion. All carbon microbeads as reverse phase media efficiently retain tannic acid. The results suggest this media is suitable for solid-phase extraction of polar polyphenolic tannin compounds.

Keywords. Tannin, NanoPak-C, All Carbon Microbeads, Solid Phase Extraction, Microplate Reader

1. Introduction.

Tannins are a class of amphiphilic polyphenolic compounds of molecular weight 500 - 3000 Da that naturally occur in vegetable matter and natural products. They are well established as ubiquitous nuisance compounds in plant extracts. A key feature that distinguishes them from other plant polyphenols is their non-specific binding to many different proteins, leading to these proteins' precipitation. Thus, they are considered inferior drug leads. This feature also reduces digestible proteins in food, and therefore, tannins are considered anti-nutritional. Over the years, their prevalence in cell-free and cell-based natural product screening assays had led to significant efforts to remove them.

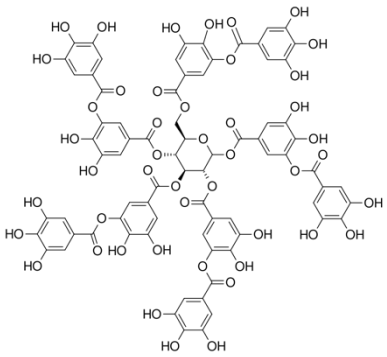

Figure 1: Structure of Tannic Acid.

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]

This study uses tannic acid (**Figure 1**), a representative member of hydrolyzable tannins. We present an optimal method to extract tannic acid employing the NanoPak-C graphite microbead SPE columns. Further, we share a protocol to quantify µL volumes of tannic acid using a UV microplate reader.

2. Materials and Methods.

2.1 Chemicals. NanoPak-C graphite microbeads (average diameter = 40 µm, Particle Size Distribution 40% of Average Diameter, Catalog # MT-12-MG-40-RR-11, Millennial Scientific, Stony Brook, NY USA), HPLC grade methanol (>99.9%), ultra-pure water, tannic acid (ACS reagent grade) were used as received.

2.2 Tannic acid solution. The tannic acid stock solution (1000 ppm) was prepared by adding mixture of 0.1g of tannic acid in 100ml of ultra-pure water. The working solution of tannic acid (100 ppm) was prepared from the stock solution by serial dilution. Additionally, 18 calibration standards between 0 and 25 pm of tannic acid were prepared.

2.3 SPE columns and Manifold. NanoPak-C graphite microbeads (bed weight = 100 mg) were manually packed into empty 1 mL straight barrel SPE columns. The microbeads were packed between a lower and upper frit (average pore size = 20 µm). A 12-port vacuum manifold was used for the extraction process.

2.4 SPE Extraction. Separate columns were used for the control group (n=3) and experimental group

(n=3) (see **Table 1**). All SPE columns were

Table 1. Experimental and Control Groups used for Tannic Acid Extraction Studies.

Name	*Definition*
Wash	The liquid collected after the wash step from the experimental SPE columns
Wash Control	The liquid collected after the wash step from the Control SPE columns
Elute	The liquid collected after the elution step from the experimental SPE columns
Elute Control	The liquid collected after the elution step from the Control SPE columns

conditioned with 3 mL of 100% methanol followed with an equilibration step. Fresh collection vials were installed for the load and wash steps. To each experimental column, 25 µL of tannic acid solution (100 ppm) was first applied. To each control column, 25 µL of acidfied ultrapure water(pH = 3.7) solution was first used. Next, a wash step of 500 µL acidified ultra-pure water (pH 3.7) was applied to both experimental and control groups. After this wash step, 200 µL of the liquid collected from the experimental and control SPE columns were labeled Wash and Wash Control, respectively. Next, 500 µL of organic solvent (100% methanol) was applied to each experimental and control SPE column. The control SPE columns were also spiked with 25 µL of tannic acid solution (100 ppm) bypassing the sorbent bed. After this elution step, 200 µL of the liquid collected from the experimental and control SPE columns were labeled Elute and Elute control, respectively.

2.5 Analysis. All samples were loaded into a UV transparent flat bottom 96 well microtiter plate and analyzed by a UV-VIS microplate spectrophotometer. The UV spectra of tannic acid in 100% methanol were obtained between 190 nm – 400 nm wavelength to identify peak UV absorbance wavelength λ_{max}. Next, 200 uL samples (n=3) of 18 tannic acid calibration standards at concentrations between 0-25 ppm were prepared. A UV absorbance vs. concentration calibration plot was generated (**Figure 2**). This plot's linear region (0-15 ppm) fits the Beer Lamberts equation using least-squares regression. Finally, the absorbance at λ_{max} of the experimental and control samples was measured. Their concentration was determined using the calibration plot. Average values from the triplicate sampling were calculated.
Recovery efficiency (RE) was calculated with the equation RE: $\frac{C0}{C1} \cdot 100\%$....(1); RE is the recovery efficiency, C0 and C1 are average concentration values for the tannic acid concentrations (ppm) for the Elute and Elute Control samples.

3. Results and Discussion. This study's overall objective was: (a) to present an optimal method to extract tannic acid using 1 ml SPE columns loaded with NanoPak-C graphite microbead SPE columns (bed weight – 100 mg). (b) Introduce a protocol to characterize and quantify tannic acid extraction efficiency loaded onto NanoPak-C graphite microbead SPE using a UV absorbance microplate reader. This setup is suitable for quantification of small volumes of the solution.

Our previous studies showed the breakthrough volume on 100 mg bed weight all carbon microbead media packed into 1 ml SPE columns to be 504 µL for tannic acid.[3] Thus, in this study, sample volumes lower than this upper limit were used to ensure acceptable recovery. We first characterized the UV spectra of tannic acid dissolved in methanol. The figure shows UV peak absorbance λ_{max} values of 278 nm. This value is similar to published λ_{max} values for tannic acid.[4]

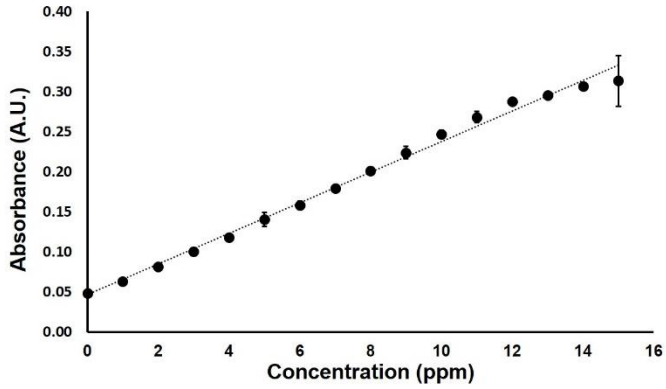

Figure 2. Linear Region of UV Absorbance vs Tannic acid Concentration used in Calculations

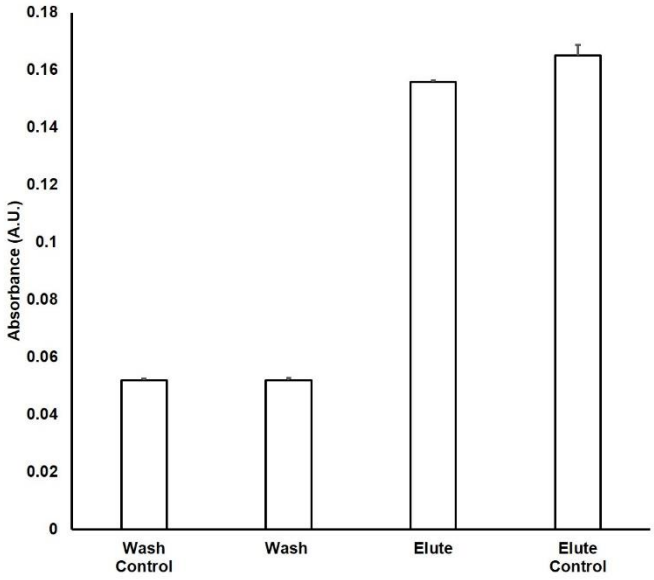

Figure 3: Elution samples from SPE show strong recovery.

Absorbance values at 278 nm were used for all calculations in each sample group. These

concentration values were determined through the linear model $y = 0.0191x + 0.0463$, $R^2 = 0.9928$, where y = Absorbance and x = tannic acid concentration (ppm) fit the prepared calibration standards.

Figure 3 presents the recovery absorbances for the experimental and control groups. Thus, substituting these values in equation 1, the recovery efficiency of tannic acid calculated to be 94%. The Elute group yielded back 5.7 ppm of tannic acid. The Elute Control group produced 6.2 ppm of tannic acid.

These results indicate that amphiphilic tannic acid compounds can be retained on all-carbon microbeads. The results suggest this media is suitable for solid-phase extraction of polar tannin compounds. These results also open avenues to extract and quantify tannic acid from more complex matrices. The extraction setup can be employed as an inexpensive method to remove tannic acid as nuisance compounds or impurities.

4. Conclusions. Tannic acid dissolved in simple organic solvents in small microliter volumes could be recovered efficiently and quantified with NanoPak-C All-Carbon Microbeads-Packed 1 mL SPE Columns. Taken together, the reduced sample sizes without affecting recovery efficiency could decrease solvent usages, bringing down analytical costs and processing times.

5. References.

1 Buszewski, B. & Szultka, M. Past, Present, And Future Of Solid Phase Extraction: A Review. *Critical Reviews In Analytical Chemistry* 42, 198-21, 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 (2013).

3 Parente, M. J. & Sitharaman, B. Breakthrough Volumes Of Tannic Acid Solution Loaded Onto Nanopak-C SPE Grade All-Carbon Microbeads. *Millennial Scientific White Papers On Nanopak-C Solid Phase Extraction Methods*, 1-3 (2021).

4 Tangarfa, M., Semlali Aouragh Hassani, N. & Alaoui, A. Behavior And Mechanism Of Tannic Acid Adsorption On The Calcite Surface: Isothermal, Kinetic, And Thermodynamic Studies. *ACS Omega* 4, 19647-19654 (2019).