Recovery and Purification Comparison of Glycinin Using Various pH Extraction Procedures on a Continuous Injection Gradient Gilson PLC 2020 System

Application Note FB0313

Keywords

Glycinin, soy protein, β-Conglycinin, PLC 2020 Personal Purification System, semi-preparative purification, extraction, HPLC, continuous injection, large volume loading, automated loading pump, gradient

Introduction

This application note was performed by Toni R. Hofhine, Gilson, Inc. in collaboration with Michael McGinley, Phenomenex (www.phenomenex.com).

Soy protein continues to be a cost effective and functional ingredient for safely increasing protein content in a variety of food products.[1] Soy from soybean is a complete source of protein, comparable to eggs, milk, fish, and other traditional protein food sources. Glycinin and β-Conglycinin are two macromolecule storage proteins found within soybeans that have been characterized and purified for new uses in foods, industrial adhesives, paper, and other commercial and industrial products.[2]

In this application, semi-preparative continuous HPLC purification of Glycinin from a commercial soy protein powder was performed using the Gilson PLC 2020 Personal Purification System to compare recoveries from three different Glycinin extraction solutions at pH 4.5, 7.0, and 11.0. Continuous purification allowed for manual automation of the injection process using a loading pump to provide a more consistent and efficient approach to load large volumes of macro molecule samples. The amount of Glycinin to β-Conglycinin conversion from within the collected fractions is discussed as it relates to the extraction solutions used. In addition, the PLC 2020 system temperature was varied to perform purification at room temperature (21˚C) and at cold room temperature (4˚C) to compare stability of chromatography and Glycinin retention time.

Materials & Methods

Materials:

Solvents

- Ammonium Acetate, >98%: Sigma-Aldrich
- Acetic Acid, 99.7+%: Sigma-Aldrich
- Acetonitrile, HPLC Grade: Burdick & Jackson
- NanoPure Water
- Sodium Hydroxide (NaOH) Pellets: EMScience
- Trifluoroacetic Acid (TFA): Sigma-Aldrich

Sample

- EAS Soy Protein Powder, Vanilla: Abbott

Standard

- Glycinin: Sigma-Aldrich

Equipment:

- Shaker - Gilson Orbital Shaker @ 650 RPM
- Centrifuge - Precision Scientific, Inc. @ Speed=50
- Filters - Pall Acrodisk CR 25 mm 0.45µm PTFE

Gilson PLC 2020 Purification System:

Mobile Phase: 18 mL/min

Time (minutes)	% Mobile Phase A Water + 0.1% TFA	% Mobile Phase B 60% ACN/40% Mobile Phase A
0.00	95	15
4.00	78	22
4.05	30	70
5.00	30	70
8.15	95	15
8.25	95	15
12.25	78	22
12.30	30	70
13.25	30	70
16.35	95	15
16.50	95	15
20.50	78	22
20.55	30	70
21.50	30	70
24.60	95	15
24.70	95	15

Gilson PLC 2020 Purification System, continued:

HPLC Column

- Phenomenex Synergi 4 µ Fusion-AP 80Å, 21.2x50 mm

Injection Volume

- 7 mL total injection volume using loading pump Gilson model 305 with 10WTi pump head @ 7 mL/min
- Continuous loading for three injections per PLC 2020 run with contact closures
  - Output 1 on PLC 2020 used to start and stop Gilson 305 pump keypad program
- Gilson GX rinse pump to automatically wash 10 WTi pump head piston seals
  - 24V contact & contact 2 on PLC 2020 used to automatically start and stop

Fraction collection

- Peak slope front and back = 20
- Subdivide volume per tube = 20,000 µL
- Peak width = 0.2 minutes

Extraction Method:

Soy Protein sample extractions were performed using the following extraction solvent solutions:

- Acidic: 20 mM Ammonium Acetate, pH 4.5
- Neutral: NanoPure water, pH 6.4
- Basic: 1M NaOH, pH 13.6

Procedure

- Weigh 10 g soy protein powder.
- Add 200 mL extraction solvent solution.
- Cap and shake for 30 minutes.
- Dilute 70 mL extract and 130 mL of 20 mM Ammonium Acetate, pH 4.5.
- Centrifuge for 30 minutes @ speed 50 RPM.
- Filter.

Results

Soy protein powder was extracted with acidic, neutral, or basic solutions prior to purification on the PLC 2020. Three continuous injections were performed per chromatogram run using a Gilson loading pump to increase the efficiency of manual injection purification on the PLC 2020. For each of the three continuous injections, approximately 58 mg of total soy protein was purified. Each acidic, neutral, or basic extraction solution chromatogram showed a significant and consistent Glycinin peak at retention times of 5.6, 13.8, and 22.1 minutes (Figures 1, 2, and 3).

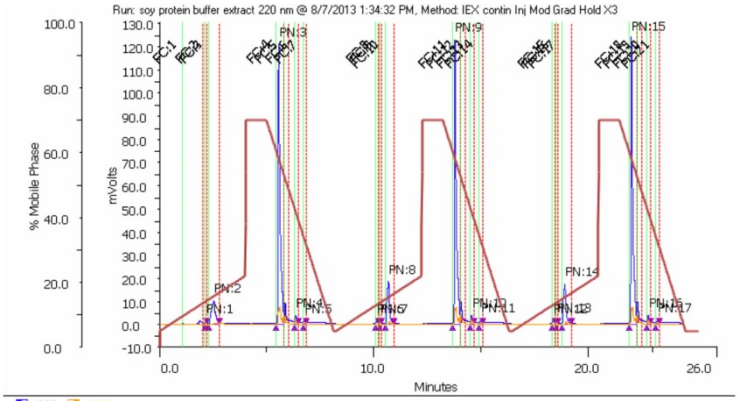

Figure 1:
Acidic (20 mM Ammonium Acetate, pH 4.5) Extraction Solution; Glycinin Fraction Tubes Collected: 4, 11, 17

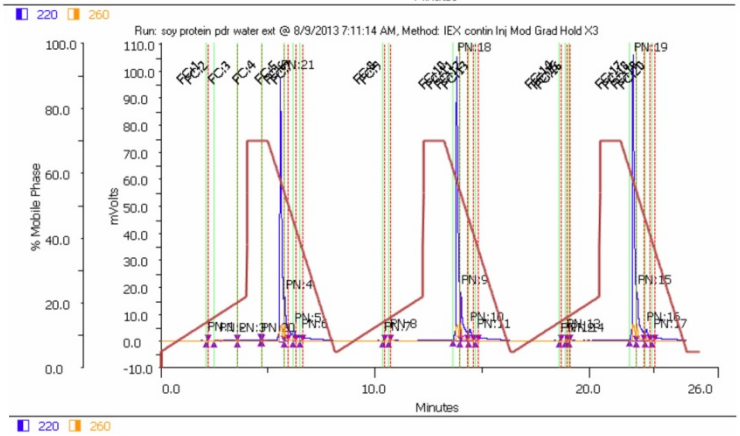

Figure 2:
Neutral (NanoPure water, pH 6.4) Extraction Solution; Glycinin Fraction Tubes Collected: 4, 10, 17

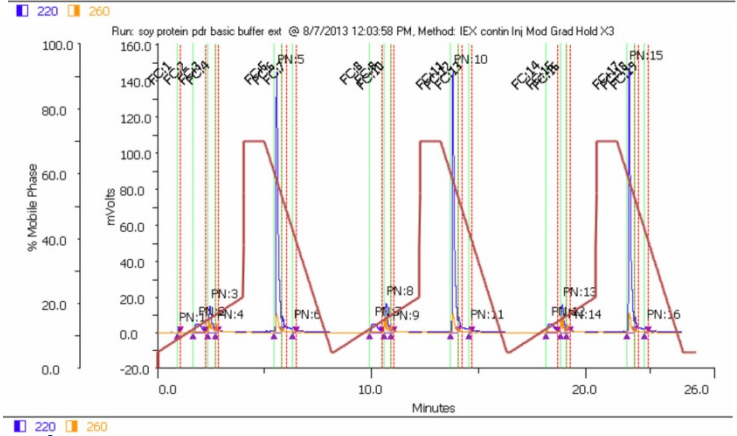

Figure 3:
Basic (1M NaOH, pH 13.6) Extraction Solution; Glycinin Fraction Tubes Collected: 5, 11, 17

For verification purposes, a Glycinin standard was prepared and injected to confirm the determined Glycinin peak retention time in the extracted samples. Fraction collection was performed using peak slope collection, with a 20 mL fraction volume collected per tube. Re-injections were performed for each of the collected Glycinin peaks using the same gradient for a single manual injection (Figures 4, 5, 6).

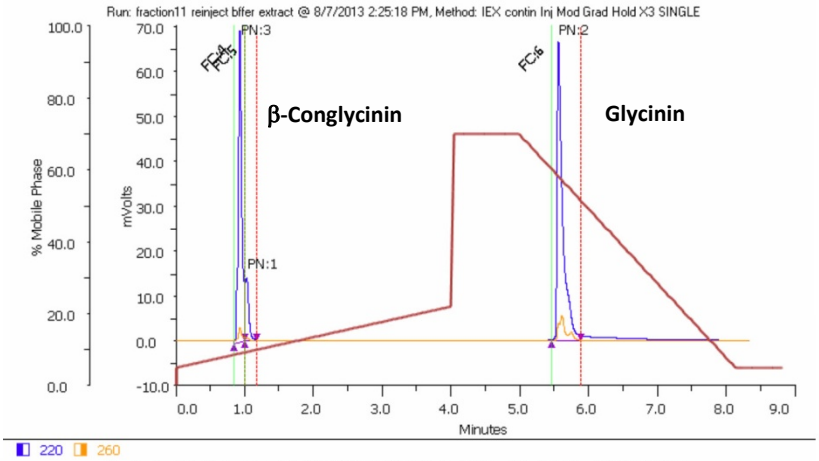

Figure 4:
Re-Injection of Glycinin Fraction Tube 10 From the Acidic (20 mM Ammonium Acetate, pH 4.5) Purification Run

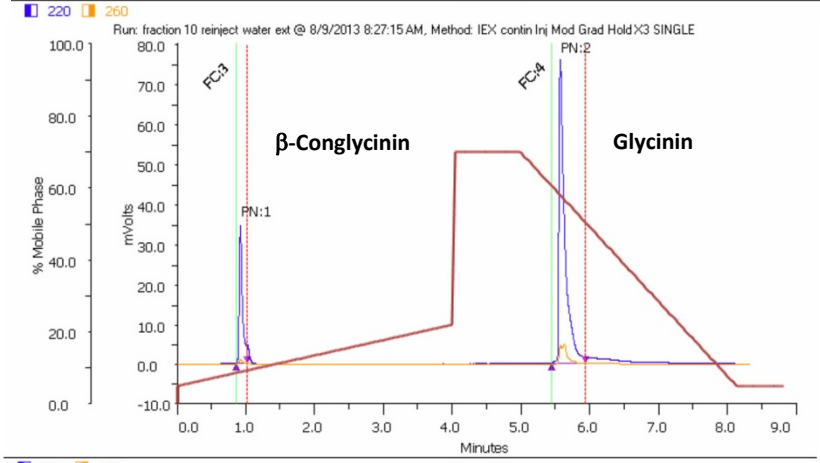

Figure 5:
Re-Injection of Glycinin Fraction Tube 11 From the Neutral (NanoPure water, pH 6.4) Purification Run

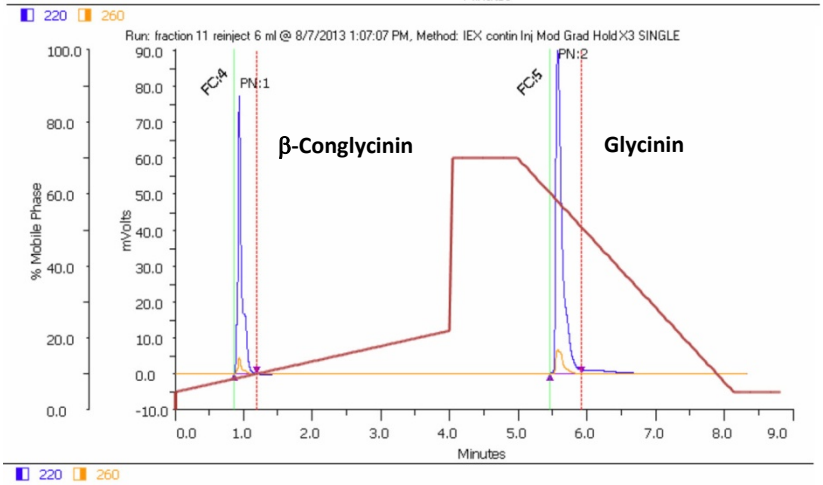

Figure 6:
Re-Injection of Glycinin Fraction Tube 11 From the Basic (1M NaOH, pH 13.6) Purification Run

The Glycinin fractions collected from the acidic, neutral, and basic purification runs was stored in ~ 50% acetonitrile (per the gradient in Figures 4, 5, and 6) until re-injected. Re-injections were performed immediately after each of the continuous purification runs was completed. The chromatograms for Figures 4, 5, and 6 indicated that extraction in different pH environments and subsequent storage in acetonitrile may slightly modify the soy protein structure.[3]

A changing structure is typically observed as an increase in β-Conglycinin formed from Glycinin. In each of the re-injected fractions, the Glycinin peak is prominent at 5.58 minutes, with the β-Conglycinin peak eluting at 0.92 minutes. The total recovered amounts of Glycinin and β-Conglycinin were calculated (Table 1).

Table 1: Total Average Glycinin Recovery and Glycinin to β-Conglycinin Conversion

Extraction Solution	% Total Recovery (Glycinin + β-Conglycinin)	% Conversion of Glycinin to β-Conglycinin
20 mM Ammonium Acetate, pH 4.5	94.5	79.1
NanoPure Water, pH 6.4	97.9	23.4
1M NaOH, pH 13.6	96.1	66.5

Based on stability of Glycinin in water at room temperature (19.7˚C), an additional water extraction was performed for purification at 4˚C to determine if there were any substantial visual chromatographic differences or retention time shifts with the Glycinin peak. Similar Glycinin peak shape and retention time (~5.6 minutes) were observed for samples purified at 4˚C (Figure 8) and at room temperature (Figure 9).

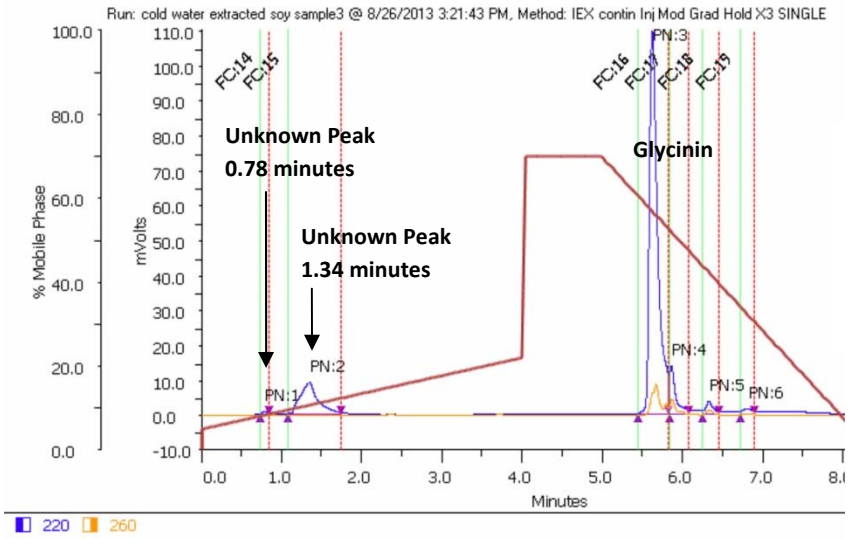

Figure 8:
Soy Protein Powder Sample Purification From Water Extraction at 4˚C.

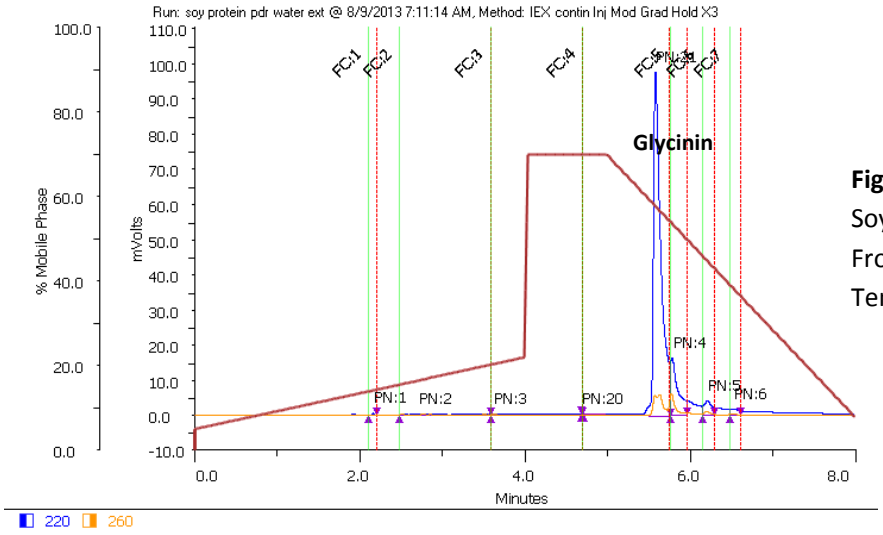

Figure 9:
Soy Protein Powder Sample Purification From Water Extraction at Room Temperature (19.7˚C).

Both soy protein powder samples were extracted on different days, with immediate purification performed following extraction. Purification at 4˚C showed two small peaks at 0.78 minutes and 1.34 minutes that were not present in the room temperature purification run. These retention times do not correlate with the β-Conglycinin peak, which consistently eluted at 0.92 minutes.

Summary

Purification of Glycinin from acidic, neutral, and basic extraction solutions demonstrated comparable recovery levels of 94.5%, 97.9%, and 96.1%, respectively. Continuous purification allowed for unattended automation of the injection process using a loading pump to provide a more consistent and efficient approach to large volume large molecule loading. For each of the three continuous injections, approximately 58 mg of soy protein was purified. Over a four hour period, it is estimated that 1.5 g of soy protein could be purified.

Glycinin did show some instability during the purification process. The extent of conversion from Glycinin to β-Conglycinin differed between the extraction solutions used. A neutral extraction solution resulted in the lowest Glycinin to β-Conglycinin conversion, 23.4%.

Purification of Glycinin from soy protein powder in a neutral (water) extraction solution was compared at 4˚C versus room temperature to compare stability of chromatography and Glycinin retention time. Glycinin peak shape was consistent between the 4˚C and room temperature chromatograms. Noticeable early eluting peaks present in the 4˚C chromatogram were not consistent with the known β-Conglycinin retention time.

References

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