Chromatography Solutions

Technical note #034

High Purity and Yield Purification of α-Lactalbumin from Cow's Milk using Preparative Liquid Chromatography

INTRODUCTION

α-Lactalbumin is one of the main proteins in human milk,[1] with bovine α-lactalbumin showing 72% amino acid sequence homology with human α-lactalbumin.[2] Applications of α-lactalbumin include its use in infant formula, as well as various nutraceutical and therapeutic supplements to help with dietary requirements, diseases, and/or disorders.[3] α-Lactalbumin extraction is therefore of high commercial value, particularly if the process is kept at a low cost.[4]

The extraction of targeted compound(s) from a sample is often performed using preparative-scale liquid chromatography (LC) under non-ideal overloaded conditions to maximize yield without compromising purity. In this technical note, the high yield fractionation of α-lactalbumin from cow's milk, via preparative LC using a wide-pore reversed-phase column, is demonstrated. The high purity of the extracts is then confirmed by non-overloaded analytical scale LC.

IDEAL NON-OVERLOADED SEPARATION CONDITIONS

The extraction of α-lactalbumin initially involves the separation of whey from bovine milk. Cow's milk (whole milk) was purchased from the local market and was curdled with the addition of a small amount of acetic acid, followed by filtration to remove the curds. A small aliquot of the filtered liquid (known as whey) was analysed by analytical LC, as shown in Figure 1. A wide-pore (300 Å) C4 reversed-phase column was used to ensure good chromatographic performance for the separation of the whey proteins.[5,6] The use of larger pore size ensures that the proteins have good access to the pore system within the stationary phase bead. The selection of the C4 stationary phase was based on the need for a weakly hydrophobic stationary phase, due to the hydrophobicity of the proteins. The three main whey protein components were identified as α-lactalbumin, β-lactoglobulin B and β-lactoglobulin A.

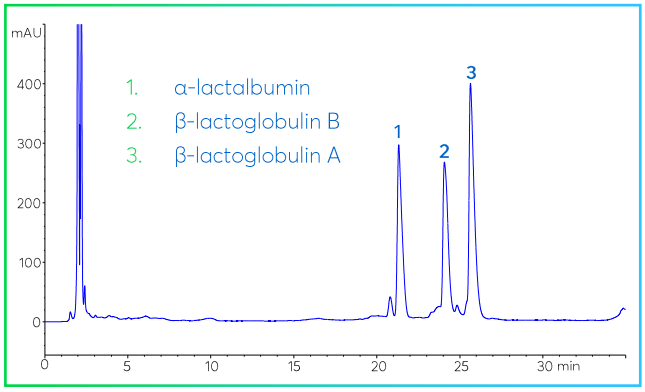

Figure 1: Separated whey proteins from cow's milk purchased at a local market. Column: Avantor® ACE® 3 C4-300, 150 x 2.1 mm; Mobile phase: A: 0.01% TFA (aq), B: 0.01% TFA in MeCN. Gradient: 33% B from 0-5 min to 43% B at 32 min. Flow rate: 0.2 mL/min; Injection volume: 1 µL; Detection: UV, 210 nm; Temperature: 45°C; Instrument: Agilent 1290.

HIGH YIELD EXTRACTION: NON-IDEAL OVERLOADED SEPARATION CONDITIONS

The systematic increase of the whey sample loading on both an analytical (4.6 mm) and preparative (21.2 mm) scale column is shown in Figure 2. This highlights the loss of peak shape and resolution that occurs when moving to non-ideal chromatographic conditions for both the analytical and preparative scale separations. In moving to non-ideal, overloaded conditions, the priority is to maximize the amount of α-lactalbumin extracted from cow's milk with high purity in a particular time frame.

HIGH PURITY CONFIRMATION

Three fractions were programmed for automated collection (green and blue bands shown in Figure 3) from the α-lactalbumin peak, using the same conditions established in Figure 2 for the preparative scale separation. The purity of each collected fraction was determined by taking a small aliquot from each fraction and subsequently analysing them using the analytical scale non-overloaded conditions established in Figure 1. The purity of the three α-lactalbumin fractions were confirmed at 89, 95 and 97%, respectively, and shown in Figure 3's insert.

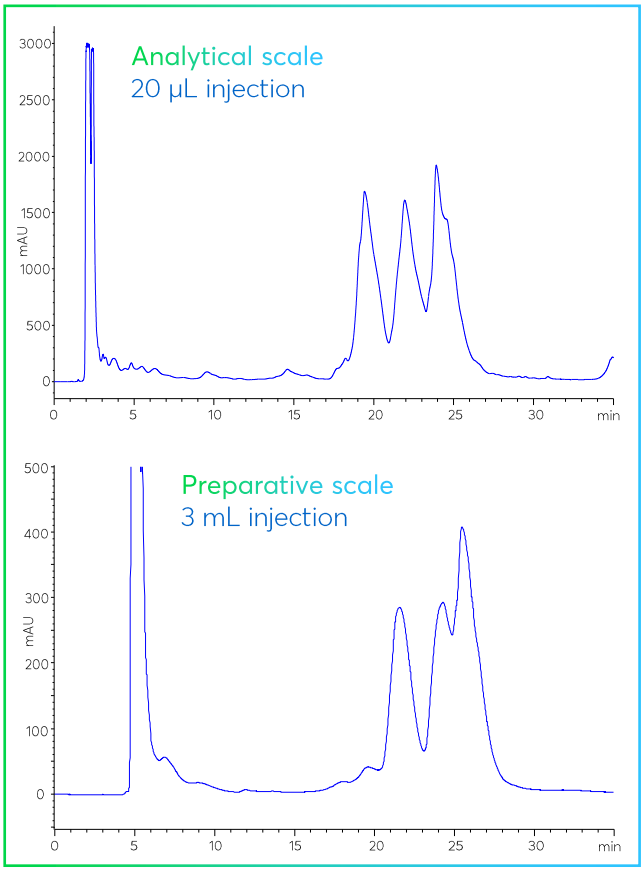

Figure 2: Analytical and preparative scale chromatograms highlighting the overloaded separation conditions of whey from cow's milk (see Figure 1 for conditions). Preparative scale separation conditions: Avantor® ACE® 5 C4-300, 150 x 21.2 mm; Mobile phase: A: 0.01% TFA (aq) B: 0.01% TFA in MeCN. Gradient: 33% B from 0-8.1 min to 43% B at 35.1 min (Sample loading 0-3.1 min; Injection at 3.1 min). Flow rate: 21.2 mL/min; Injection volume: 3 mL; Detection: UV, 210 nm; Temperature: Ambient; Instrument: Knauer Azura PrepLC Premier system.

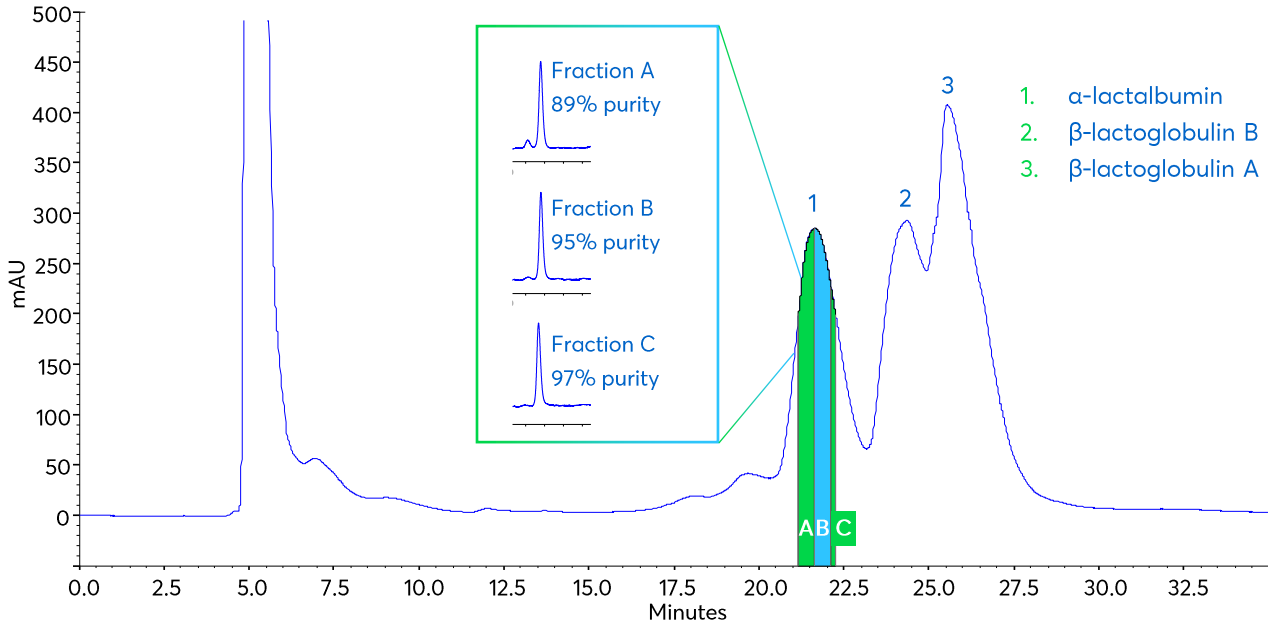

Figure 3: Preparative scale fractionation (conditions: see Figure 2) of α-lactalbumin with high purity analytical scale confirmation (conditions: see Figure 1). The fractions collected from the α-lactalbumin peak are highlighted in green and blue. Preparative Instrument: Knauer Azura PrepLC Premier system with Foxy fraction collector.

CONCLUSIONS

The high-yield purification of targeted compound(s) from a sample is typically performed under non-ideal overloaded preparative chromatography conditions. In this Technical Note, the high yield, high purity fractionation of α-lactalbumin from cow's milk is demonstrated. The fractionation was performed on a 21.2 mm preparative scale column to maximize the yield and amount, with purity confirmation using the analytical scale ideal chromatography conditions.

REFERENCES

1. D. E. W. Chatterton, G. Smithers, P. Roupas, A, Brodkorb *Int. Dairy J.* (2006) **16** 1229-1240

2. W. E. Heine, P. D. Klein, P. J. Reeds *J. Nutr.* (1991) **121** 277-283

3. D. K. Layman, B. Lönnerdal, J. D. Fernstrom *Nutr. Rev.* (2018) 76 444-460.

4. G. Konrad, T. Kleinschmidt *Int. Dairy J.* (2008) **18** 47-54

5. Avantor® ACE® Technical Note #003 "Avantor® ACE® 300 Å Wide Pore HPLC Columns for the Separation and Purification of Proteins" (https://av.cmd2.vwr.com/pub/apl/chrom/main?key=C-13237)

6. Avantor® ACE® Technical Note #028 "Analysis of intact proteins using Avantor® ACE® UltraCore BIO columns" (https://av.cmd2.vwr.com/pub/apl/chrom/main?key=C-13238)