BENEFITS OF AN INERT SURFACE TECHNOLOGY IN THE ANALYSIS OF BOVINE LACTOFERRIN IN INFANT FORMULA AND PEDIATRIC/ADULT NUTRITIONAL FORMULA

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INTRODUCTION

Lactoferrin (LF) is an iron-binding and iron-transporting glycoprotein that is present in human milk at an average concentration of about 1 mg/mL, making up approximately 7% of the total breast milk protein. The addition of LF to infant formula is of interest to infant formula manufacturers in order to imitate the nutritional properties and biological functionality of human milk.

AOAC Method 2021.10 provides a reliable and accurate solution for the determination of bovine lactoferrin (bLF) in infant formula and pediatric/adult nutritional formula. In this method, bLF is isolated and purified from the whey using a heparin affinity column. Eluates from the heparin column are then collected for the RP-HPLC analysis using an XBridge™ Protein BEH C4 Column and detected at UV 280 nm.

MaxPeak™ High Performance Surfaces (HPS) is a new technology that has been developed to mitigate metal analyte adsorption in LC. Significantly improved LC performance, such as increased peak intensity, reduced peak tailing, and more consistent results have been demonstrated for various analytes, including peptides, oligonucleotides, phosphoglycans, sugar phosphates, organic acids, and phospholipids, etc.

OBJECTIVE

The goal of this work is to evaluate the potential benefits of MaxPeak HPS for the analysis of bLF in infant formula and pediatric/adult nutritional formula using the AOAC Method 2021.10.

EXPERIMENTAL

Sample preparation

The chemicals and analytical procedures that are recommended in AOAC Method 2021.10 were followed. Table 1 contains the sample ID and sample description.

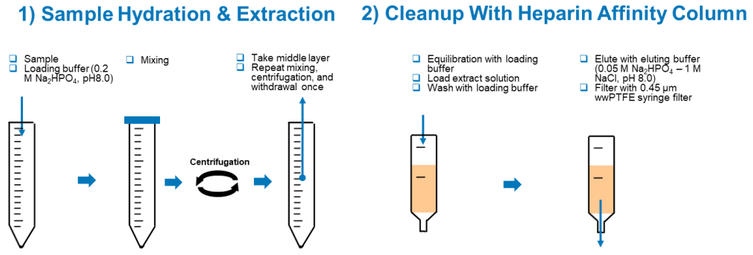

Figure 1. Schematic of sample preparation.

Table 1. Sample ID and description

Sample Identity	Sample Description
A	Milk-based infant formula powder, high bLF
B	Milk-based adult nutritional powder
C	Milk-based infant formula powder, partially hydrolyzed, high bLF
D	Milk-based infant formula powder, Low bLF
E	Milk-based Toddler powder
F	Milk-based infant formula powder, partially hydrolyzed, low bLF

LC conditions

LC System: Arc™ Premier System (BSM) with a 2998 PDA Detector
Detection: UV (280 nm)
Software: Empower™ 3 CDS
Column: XBridge Protein BEH C4 Column (3.5 µm, 4.6 mm X 150 mm); or XBridge Premier Protein BEH Column (2.5 µm, 4.6 mm X 150 mm)
Col Temp.: 35 °C
Inj. Vol.: 50.0 µL
Mobile phases: A: 0.1% Trifluoroacetic acid solution; B: 0.1% Trifluoroacetic acid in acetonitrile solution.
Run time: 16 min
Gradient program:

Time (min)	Flow (mL/min)	%A	%B
0	0.5	70.0	30.0
2	0.5	70.0	30.0
7.0	0.5	55.0	45.0
12.0	0.5	40.0	60.0
14.0	0.5	30.0	70.0
16.0	0.5	70.0	30.0

RESULTS

1) Chromatography optimization

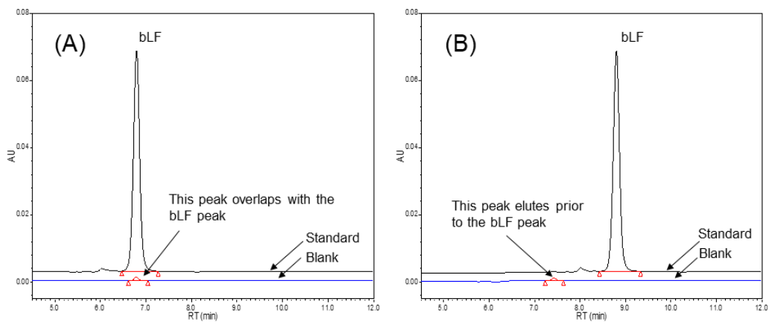

Figure 2. *Overlay of chromatograms of a blank and a bLF standard under the (A) original AOAC conditions and (B) modified AOAC conditions (additional initial gradient hold for 2 min).*

Potential interference

A small unidentified peak in solvent blanks eluted at the same retention time (RT) of bLF (see Fig. 2A). This issue was addressed by adding a 2-min hold at the initial elution program, allowing this small unknown peak to elute before the bLF peak (see Fig. 2B). This small unknown peak was found to have minimal impact on the sample analysis results, however, it is prudent to use the modified condition to eliminate potential interference. (see Table 2).

2) Benefits of MaxPeak HPS

Two new out-of-box columns, one was the XBridge Protein BEH C4 Column (3.5 µm, 4.6 mm X 150 mm) (conventional column), and the other one was XBridge Premier Protein BEH Column (2.5 µm, 4.6 mm X 150 mm) (MaxPeak HPS column), were compared. Significant differences were observed in the initial repeated injections of a bLF standard solution. (See Fig. 3).

The conventional column showed inconsistent results in the initial repeated injections of a bLF standard, while the MaxPeak HPS column showed consistent results from the very first injection. This indicates that a much shorter column conditioning time for the MaxPeak HPS columns are needed. Once the conventional columns are thoroughly conditioned, the results from the conventional column and the MaxPeak HPS column are comparable (see Table 2), however, the superb performance of the out-of-box MaxPeak HPS column makes it a better choice than the conventional column.

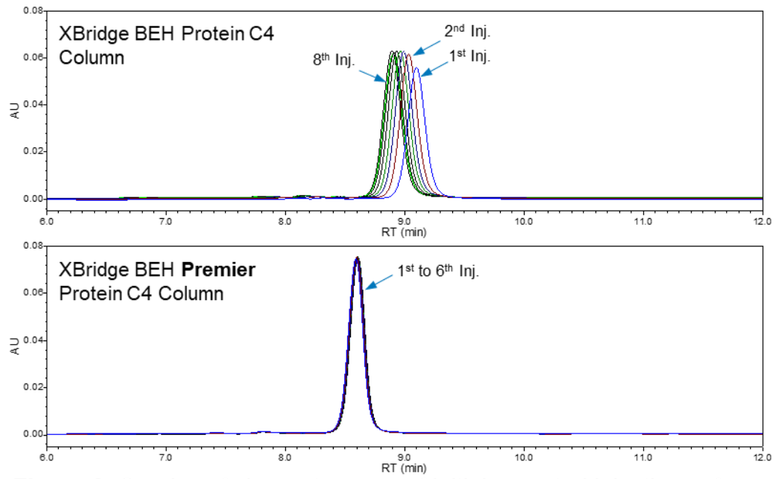

Figure 3. *Overlay of chromatograms of initial repeated injections of a bLF standard on a new XBridge Protein BEH C4 Column and a new XBridge Premier Protein BEH C4 Column.*

3) Sample analysis results under various conditions

Table 2. *Comparison of sample analysis results obtained under different conditions.*

	Original LC Conditions[1]	Modified LC conditions[2]		Premier Column and Modified Conditions[3]	
	Conc. (mg/100g)	Conc. (mg/100g)	Rel. to Original Condi.	Conc. (mg/100g)	Rel. to Original Condi.
Sample A	408	407	99.7%	410	100.4%
Sample B	1595	1577	98.9%	1589	99.6%
Sample C	403	400	99.3%	402	99.7%
Sample D	411	413	100.5%	409	99.4%
Sample E	390	391	100.1%	394	100.9%
Sample F	1606	1606	100.0%	1605	100.0%

Note: 1. The same LC conditions and column; 2. The elution program was modified with a 2-min isocratic elution at the same initioal MP composition; 3. Results obtained with an XBridge BEH Premier Protein C4 Column (300A, 2.5um, 4.6 x 150 mm) and the modified LC conditions.

CONCLUSION

- **The MaxPeak HPS column exhibited improved chromatographic performance in terms of peak shape and out-of-box performance consistency.**
- **Excellent linearity, precision, and accuracy were demonstrated in the analysis of bLF in representative samples (see details in app note, see link below) .**
- **The Arc Premier System coupled with a 2998 PDA Detector and using the XBridge Premier Protein BEH C4 Column provides an optimal and reliable solution for the determination of bLF in infant and pediatric/ adult nutritional formulas.**

For additional details, please scan the QR Code to download the PDF version of the application note at www.waters.com

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