Quantification of Whey Protein Content in Infant Formula by CE-SDS

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Introduction

Milk is almost always the first source of protein for human growth and development. Mammalian milk includes two major groups of proteins: whey proteins, a family of globular proteins, and casein proteins, a family of phosphoproteins. They are found in a roughly 60:40 ratio in human milk and in other ratios in other mammalian milks. Whey proteins are also a standard component of most infant formulas. According to the People's Republic of China National Food Standard GB 10765, milk-based infant formula refers to liquid or powdered products made mainly from milk and milk protein products; with addition of adequate amount of vitamins, minerals, and/or other ingredients; and produced and processed by physical methods only, that is suitable for normal infants.

Figure 2. PA 800 Plus Pharmaceutical Analysis System

To verify the claims of infant formula vendors and ensure the quality, safety, and efficacy of infant formula, it is essential to have an accurate method for measuring whey content. Previously, however, there was no reliable method to separate the two major protein types and quantify the whey protein

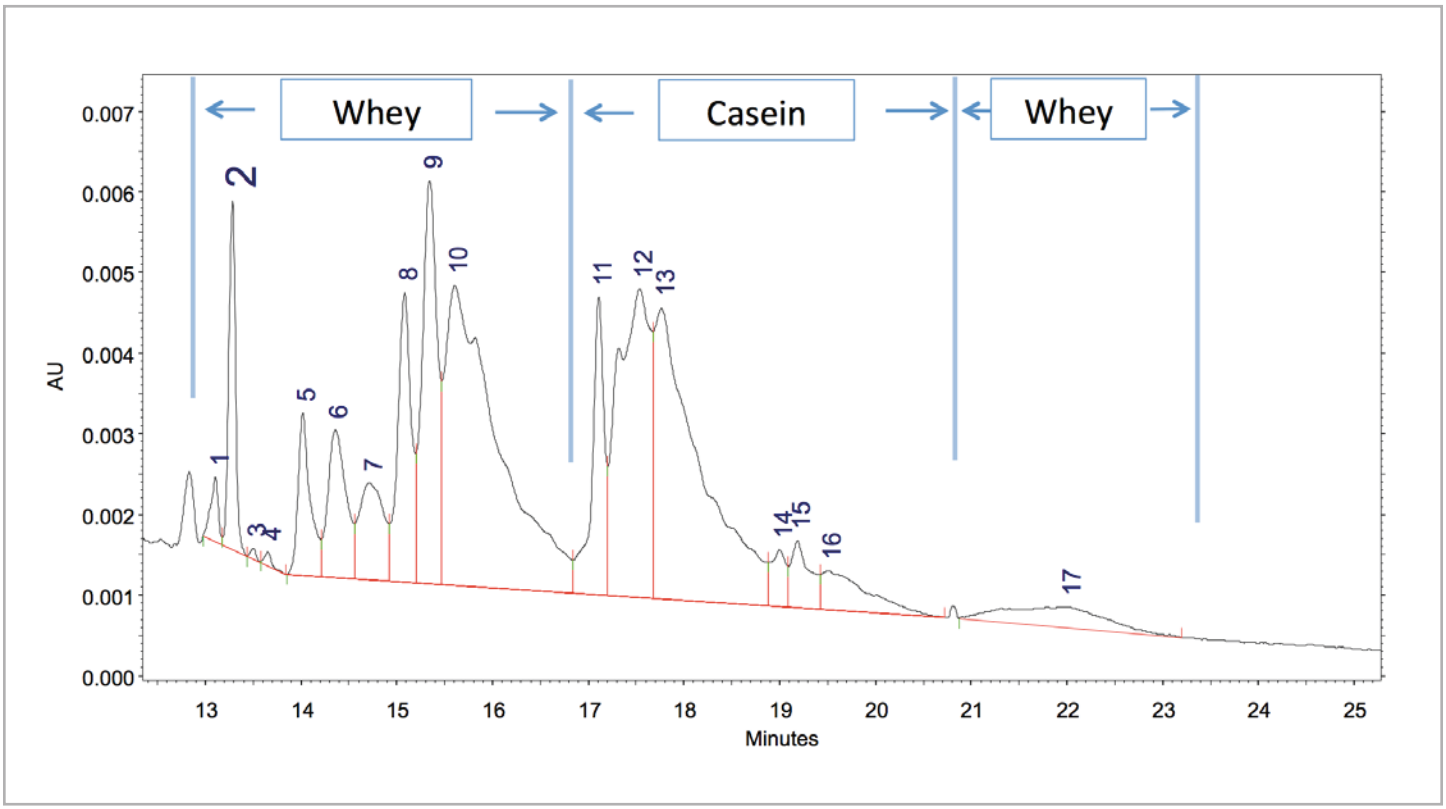

Figure 1. Electropherogram of infant formula.

content. The challenge was compounded by pasteurization, during which the Maillard reaction can cause proteins to form complexes with sugars. These complexes make effective separation of the various proteins even more difficult.

This note explores an effective, practical method based on capillary electrophoresis—sodium dodecyl sulfate (CE-SDS)—for separating and quantifying the whey protein content in infant formula. Analysis using the CE-based SCIEX PA 800 Series Pharmaceutical Analysis System clearly separated whey, casein proteins, and immunoglobulins (Figure 1), and provided accurate, reliable quantitation of the whey proteins. The method was validated across multiple laboratories and demonstrated to be reliable and reproducible.[1] This method was approved by the AOAC Expert Review Panel for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN).[1]

Key Features

- CE-SDS on the PA 800 Plus system provides excellent separation resolution, clearly separating the whey proteins, casein proteins, and immunoglobulins in both protein standard and infant formula samples
- Separations are relatively fast; under 30 minutes in the case of this analysis
- Validation across multiple laboratories found the method to be reliable and reproducible

Experimental

Reagents and Supplies

The SDS-MW Analysis Kit (SCIEX, P/N 390953) supplied the capillary, SDS gel buffer, 10 kDa marker, MW marker, and sample buffer. Contents are listed in Table 1. Other reagents and supplies included: β- mercaptoethanol (SIGMA P/N M7154 or M6250); milk protein standards (SIGMA: α-lactalbumin, L6010; β-lactoglobulin, L3908; IgG, I5506; BSA, A1933; β-casein, C6905; αS-casein, C6780; κ-casein, C0406. ALAR Ingredients: CGMP). Infant formula was provided by a local dairy manufacturer. Deionized water (Millipore).

Sample Preparation

Milk protein standard mixture

Milk proteins were weighed and dissolved in water according to the infant formula ratio.

Infant formula

Infant formula (100 mg ± 4 mg) was added to 1.5 mL centrifuge tubes. Water (1 mL) was added to each tube. Tubes were vortexed and oscillated until the infant formula was fully dissolved.

Sample running pre-solution

Sample pre-running solution was prepared by mixing sample buffer and the 10 kDa Marker (internal standard), both from the SDS-MW Analysis Kit, in an 84:1 ratio.

Denaturation

10 µL of each sample solution was pipetted into separate 1.5 mL microcentrifuge tubes. 85 µL of sample running pre-solution and 5 µL of β-mercaptoethanol were added to each microcentrifuge tube. Each tube was mixed well and then heated in a water bath at 100° ±5° C for 10 minutes. The tubes were allowed to cool to room temperature and then vortexed. Finally, each sample was transferred to a separate injection vial.

Items	Part Number
50 µm ID capillary column	338451
SDS Gel Buffer	A30341
10 kDa marker	A26487
MW marker	A22196
SDS sample buffer	
0.1N NaOH	
0.1N HCl	

Table 1. Items in SDS-MW Analysis Kit

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	10.00 min	BI:D1	BO:D1	forward	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	forward	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse - Pressure	20.0 psi	2.00 min	BI:F1	BO:F1	forward	ddH2O rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	SDS Gel rise to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, reverse polarity, both	SDS Gel for voltage equilibration
6								

Figure 3. Column conditioning method.

Separation and Detection

All separations were carried out on a capillary electrophoresis-based SCIEX PA 800 Plus Pharmaceutical Analysis System equipped with a photodiode array (PDA) detector operated at 220 nm, 2 Hz. Aperture was 2 (100 x 200 µm). The system was equipped with a fused-silica capillary column (50 µm ID, 30 cm total / 20 cm effective length). Sample storage and capillary temperatures were both 25° C. Conditioning and separation methods are show in Figures 3 and 4, respectively.

Data Analysis

Data analysis was performed using SCIEX 32 Karat Software.

Integration

Integration was carried out with Caesar integration off.

The electropherogram of the protein standard mixture was integrated. Migration of the whey and casein proteins are defined by the comparison to the E-grams of every single protein standard shown in Figure 5.

For the infant formula electropherogram, the autointegration start point was after the solvent peak and approximately 0.4 minutes before the 10 kDa marker peak. The auto integration end point was the end of the casein protein peaks group, the position of which was defined according to the standard mixture. Manual integration was performed for high MW whey proteins that eluted after the casein proteins.

Calculation

Whey protein content in the infant formula was calculated using the following equations:

$$Aw.c. = 1.4 \times Aw \quad (1)$$

where: Aw.c. = corrected integrated area of the whey components

Aw = total integrated area of the whey components

1.4 = correction factor

$$\text{Whey protein \%} = Aw.c./(Aw.c.+Acn)x100 \quad (2)$$

where: Acn = integrated area of the casein components

The correction factor is used to adjust the response factor difference of whey proteins and casein proteins when detected at UV 220nm, since caseins contain more aromatic amino acids (t) which contribute higher response for the amount to area than whey proteins do.

Results and Discussion

Milk Protein Standard Mixture

The milk protein standard composed of whey proteins (α-lactalbumin, β-lactoglobulin, CGMP, IgG, and BSA) and casein proteins (β-casein, αS1-casein, αS2-casein, κ-casein) were analyzed by CE-SDS. All the casein proteins migrated between the IgG light chain and IgG heavy chain. The whey proteins and casein proteins were completely separated from each other (Figure 5).

Infant Formula

The electropherogram of the infant formula sample was previously shown in Figure 1. Whey protein content was calculated to be 61.97%, in line with the whey protein content of natural human milk.

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D1	forward, In / Out vial inc 8	0.1 N NaOH rinse to clean capillary surface - Automatic increment every 8 runs
2		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 8	0.1 N HCl rinse to neutralize capillary surface silanol group - Automatic increment every 8 runs
3		Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	forward, In / Out vial inc 8	Water rinse to remove the acid residue - Automatic increment every 8 runs
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	SDS Gel rinse to fill the capillary with SDS gel - Automatic increment every 8 runs
5		Wait		0.00 min	BI:A1	BO:A1	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs
6		Wait		0.00 min	BI:A4	BO:A4	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs
7		Inject - Voltage	5.0 KV	20.0 sec	SI:A1	BO:C1	Override, reverse polarity	Sample injection
8		Wait		0.00 min	BI:B4	BO:B4	In / Out vial inc 8	ddH2O, use for dipping to avoid sample carry over - Automatic increment every 8 runs
9	0.00	Separate - Voltage	15.0 KV	30.00 min	BI:C1	BO:C1	1.00 Min ramp, reverse polarity, both, In / Out vial inc 8	SDS Gel for separation - Automatic increment every 8 runs
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Figure 4. Separation method.

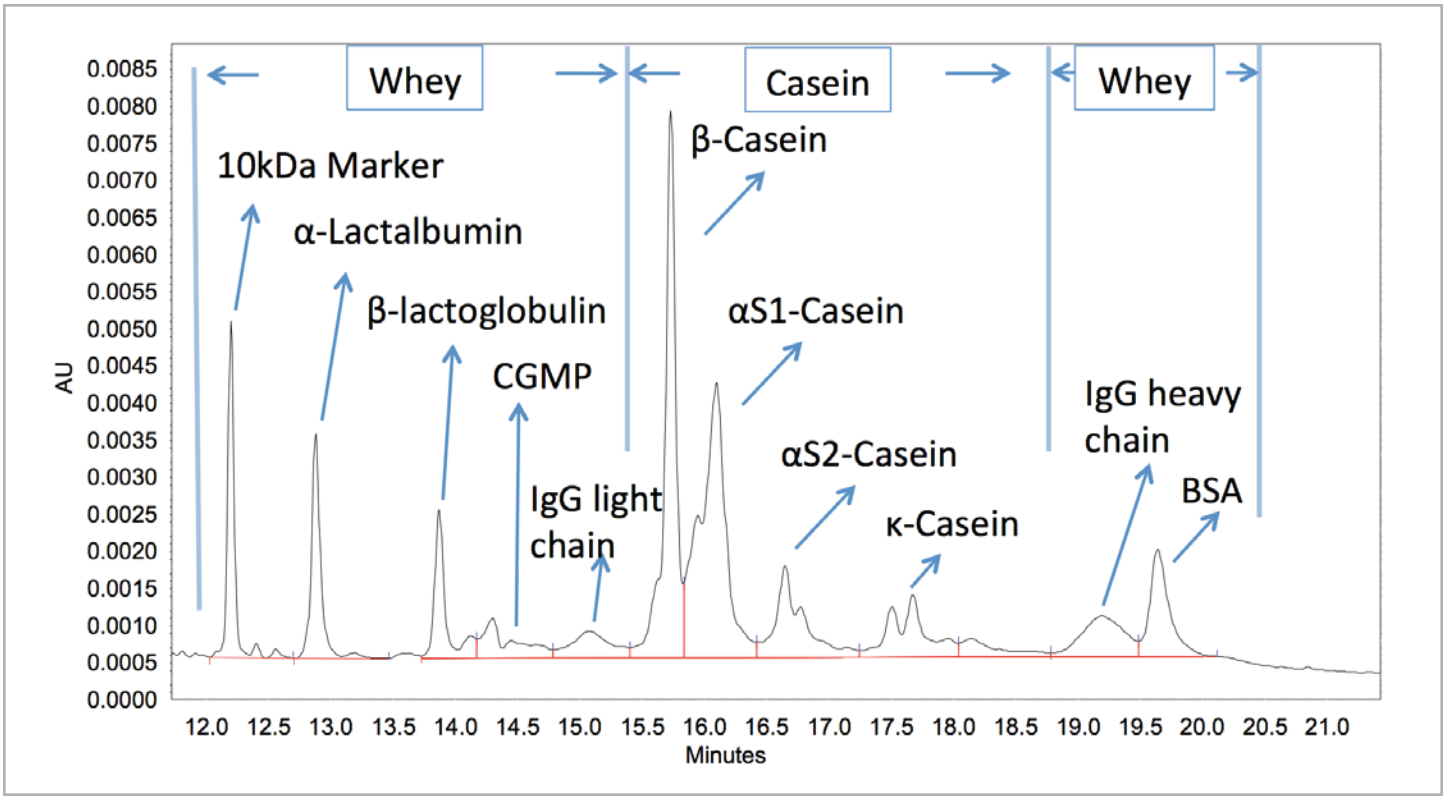

Figure 5. Electropherogram of protein standard mix. The first integrated peak is the 10 kDa marker.

Conclusion

Capillary electrophoresis with SDS was successfully applied to the quantification of whey protein content in infant formula. The CE-SDS method fully separated the whey proteins, casein proteins, and immunoglobulins, facilitating quantification of the whey proteins. Validation in multiple laboratories has shown the method to be an accurate, reliable solution to the challenging problem of verifying whey content.

References

1. Ping Feng, Christophe Fuerer, Adrienne McMahon. Quantification of Whey Protein Content in infant Formulas by Sodium Dodecyl Sulfate-Capillary Gel Electrophoresis (SDS-CGE): Single-Laboratory Validation, First Action 2016.15; *Journal of AOAC International*, Vol. 100, No. 2, 2017

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