Application Note

Is That Milk?
Use CE-SDS on Maurice™ and Find Out!

Introduction

Consumers of today have unprecedented access to information digitally. This, coupled with a global explosion of the health and fitness industry, has consumers increasingly scrutinizing the ingredients they consume. Consequently, the call for transparent labeling, backed by stringent regulatory guidelines, requires food manufacturers to accurately analyze and report the contents of their products.

Since milk and its various products are consumed by infants and adults alike - primarily for protein - identifying and quantifying those proteins is critical. Likewise, the growing industry of plant-based proteins and alternatives to animal-based food products must also provide accurate information on the quantity of proteins and other ingredients in its products.

Within the food industry, traditional methods like Kjeldahl and Dumas that measure total nitrogen, and UV-spectroscopy are frequently employed to estimate protein content[1]. However, these methods often don't provide a true representation of actual protein content and can be susceptible to errors[2]. To address these shortfalls, this application note introduces capillary electrophoresis sodium dodecyl sulfate (CE-SDS) on the Maurice™ system as a faster, automated alternative for milk protein analysis. Not only does CE-SDS offer accurate protein quantification, but it also minimizes inter-assay variabilities. The utility of the CE-SDS method is demonstrated for the analysis of protein content in infant formulas, commercial cow's and goat's milk, as well as popular non-dairy options such as soy and almond milk.

Key takeaways:

1. CE-SDS on the Maurice system offers a fast and automated approach for milk protein analysis.
2. The study shows protein quantification across infant formula, cow's and goat's milk, and popular non-dairy alternatives.
3. CE-SDS results from this study highlight the method's reproducibility and ability to detect major protein components and potential additives, enabling informed decision-making for product quality and regulatory compliance.

About Maurice

The Maurice system is a fully integrated capillary electrophoresis instrument that enables protein size and purity analysis (CE-SDS) and charge heterogeneity analysis with imaged capillary isoelectric focusing (icIEF). CE-SDS on the Maurice system confers several benefits over SDS-PAGE, including but not limited to:

- **Less time in the lab** as CE-SDS provides results as little as 5.5 minutes.
- **Making better decisions faster** with high-resolution separation.
- **High-quality data** with excellent reproducibility.
- **Versatility** with its ability to analyze complex proteins.

Materials and Methods

Table 1 lists the materials and reagents used in this study.

All protein standards were diluted with the Maurice CE-SDS 1X Sample Buffer to a final concentration of 0.5 mg/mL. Infant formulas were diluted with water to a final concentration of 50 mg/mL. All other milk samples were diluted with water at a ratio of 1:5. 10 µL of each sample was used for analysis. The Maurice CE-SDS Internal Standard (IS, 4%) was added to all samples, which were then treated with 5% of β-ME (14.2M) for analysis under reduced conditions. The samples were heated at 100°C for 10 minutes, cooled on ice for 5 minutes, and then subjected to centrifugation. The samples were then loaded onto the Maurice instrument for analysis with the CE-SDS PLUS cartridge. Samples were injected for 20 seconds at 4600 V and separated for 25 minutes at 5750 V. All data were analyzed using Compass for iCE software.

TABLE 1. A list of materials and reagents used in this study

Material	Vendor	Catalog #
Maurice CE-SDS PLUS Application Kit	ProteinSimple, a Bio-Techne brand	PS-MAK03-S
Maurice CE-SDS PLUS Cartridge		PS-MC02-SP
Maurice CE-SDS Molecular Weight Markers		046-432
Maurice CE-SDS IgG Standard		046-039
Maurice CE-SDS 25X Internal Standard		046-144
β mercaptoethanol (β-ME)	Millipore Sigma	M-3148
α-Lactalbumin		L6010
β-Lactoglobulin		L3908
IgG from Bovine Serum		I5506
Bovine Serum Albumin		A1933
β-Casein		C6905
α-S-Casein		C6780
κ-Casein		C0406
Infant Formula (2 brands)	NA	NA
Cow's Milk (2 brands)		
Goat's Milk		
Soy Milk		
Almond Milk		

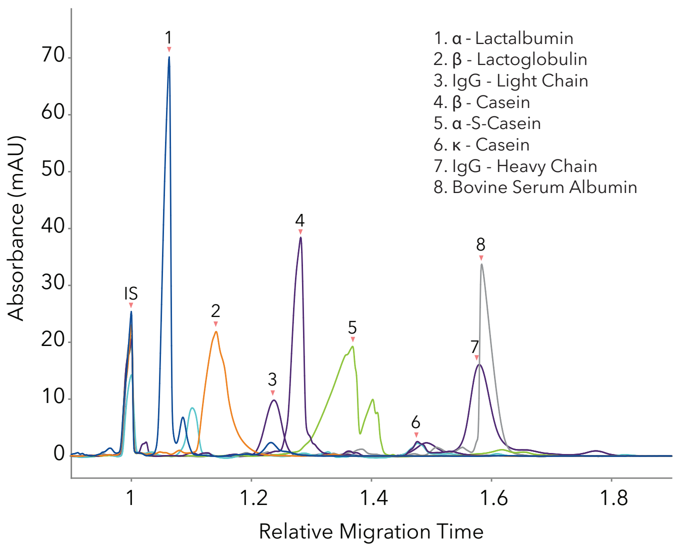
Results

Milk Protein Standards

The study began with the analysis of milk protein standards, which are utilized as a benchmark for consistency and accuracy during the analysis of proteins in milk products.

Figure 1 shows the electropherograms of different protein standards detected with Maurice CE-SDS, with percent peak area results summarized in **Table 2**.

FIGURE 1. CE-SDS PLUS analysis of milk protein standards using the Maurice system



Peaks represent identified proteins with numbers corresponding to the listed proteins on the right. The analysis also included comparisons with an immunoglobulin G (IgG) standard since the Maurice system has gained widespread recognition for its application in monoclonal antibody characterization. IS represents the Internal Standard.

TABLE 2. Relative quantification of milk protein standards on the Maurice system

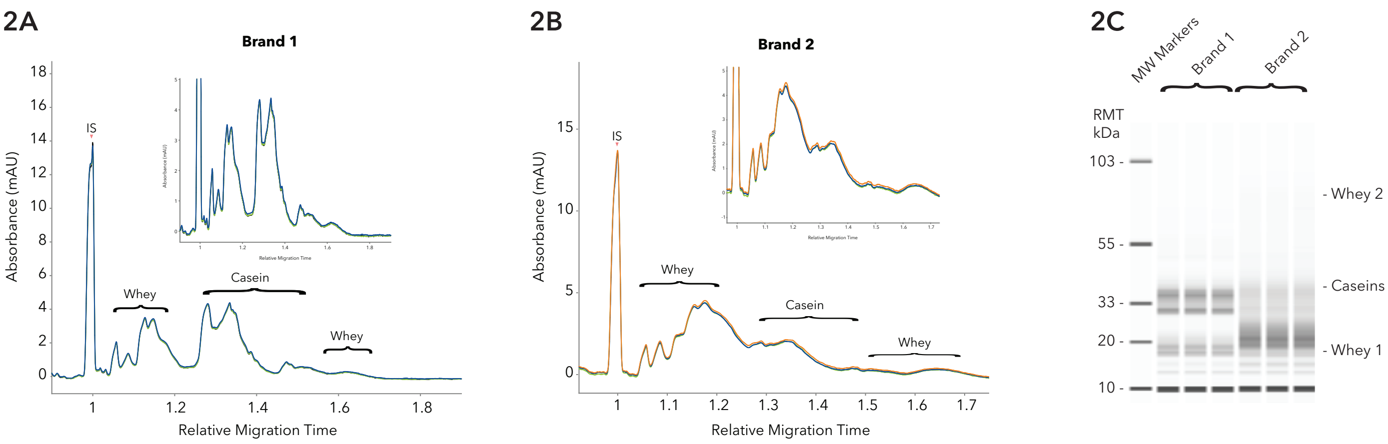
Percent Peak Area (n=2)			
Milk Protein Standard	Average	Standard Deviation	%RSD
Alpha Lactalbumin	93.95	0.07	0.08
Beta Lactoglobulin	99.30	0.00	0.00
Beta Casein	91.25	0.21	0.23
Alpha S Casein	97.90	0.14	0.14
Kappa Casein*	NA	NA	NA
BSA	97.30	0.00	0.00
IgG Light Chain	35.60	0.14	0.40
IgG Heavy Chain	64.30	0.14	0.22

The average percent peak area (n =2) was calculated for each protein detected, along with the standard deviation and relative standard deviation (%RSD).
*There were too many fragments for κ-casein, preventing the assessment of percent peak area.

Infant Formula

Infant formulas are crafted to mimic the nutritional contents of human breast milk, particularly with respect to whey and casein ratio. In this study, baby formulas from two well-known commercial brands were analyzed with the Maurice CE-SDS PLUS method, and the ratio of whey and casein was determined. **Figures 2A** and **2B** illustrate the data for each brand respectively.

FIGURE 2. Maurice CE-SDS PLUS analysis of proteins in commercial infant formulas



2A. Analysis results for Brand 1 and **2B.** Brand 2. Notable differences in protein composition can be observed between Brand 1 and Brand 2.
2C. A gel-like representation of the CE-SDS results, visualized using the "Lane View" feature on the Compass for iCE software.

The ratio of whey and casein was calculated based on the percent peak area, as shown in **Table 3**. Analysis of Brand 1 yielded a ratio of **~45:55**, while Brand 2 resulted in **~70:30**. These results are close to the expected ratio for baby formulas (60:40)[3].

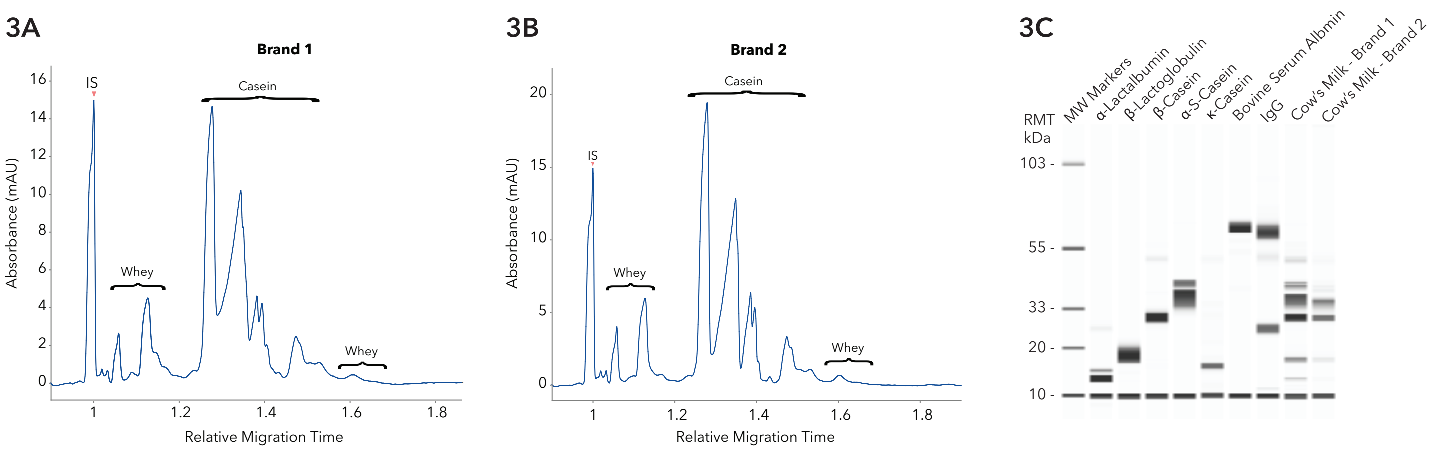
TABLE 3. Analysis of the average percent peak area standard deviation, and %RSD for whey and casein proteins for two different brands of infant formulas

Percent Peak Area (n=3)						
	Brand 1			Brand 2		
Protein	Average	Standard Deviation	%RSD	Average	Standard Deviation	%RSD
Whey	44.85	0.37	0.83	70.10	0.62	0.89
Casein	55.15	0.37	0.68	29.90	0.62	2.08

Cow's Milk

Being the most popular type of milk consumed across the globe, cow's milk is known to have a whey to casein ratio of approximately 20:80[3]. In this study, two popular commercial brands from the United States were analyzed with Maurice CE-SDS PLUS, as shown in **Figures 3A** and **3B**. Additionally, **Figure 3C** shows a gel-like view of the CE-SDS results. Both brands resulted in similar profiles as well as expected protein ratios, as summarized in **Table 4** and graphically depicted in **Figure 4**.

FIGURE 3. CE-SDS PLUS analysis of cow's milk from two commercial brands



Distribution of whey and casein proteins in **3A.** Brand 1 and **3B.** Brand 2. **3C.** A gel-like view of proteins from both brands alongside milk protein standards.

FIGURE 4. A graphical representation of the whey:casein ratio found in commercial cow's milk, analyzed using CE-SDS PLUS

Both brands were found to contain a similar ratio (~20:80), and the results align with those published in literature.

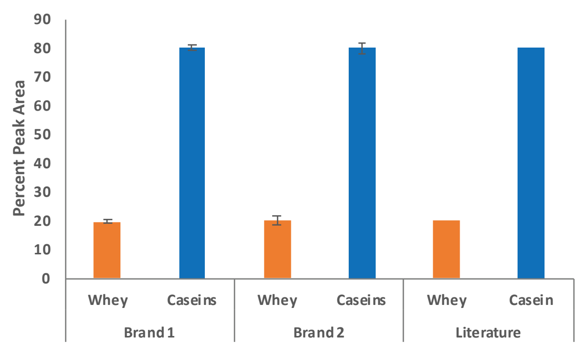


TABLE 4. Quantification of protein content in two commercial brands of cow's milk, resulting in a whey to casein ratio of ~20:80

Percent Peak Area (n=4)						
	Brand 1			Brand 2		
Protein	Average	Standard Deviation	%RSD	Average	Standard Deviation	%RSD
Whey	19.85	0.80	4.04	20.13	1.74	8.63
Casein	80.15	0.80	1.00	79.88	1.74	2.17

Goat's Milk

Although similar in whey to casein ratio to cow's milk[3], goat's milk is higher in fat, calories, vitamins, and minerals. Maurice CE-SDS PLUS analysis of a top goat's milk brand is showcased in **Figure 5**, with the main protein ratio (~20:80) detailed in **Table 5**. The profile of goat's milk proteins appears very similar to that of cow's milk.

FIGURE 5. CE-SDS PLUS analysis of goat's milk with distinct peaks of whey and casein proteins

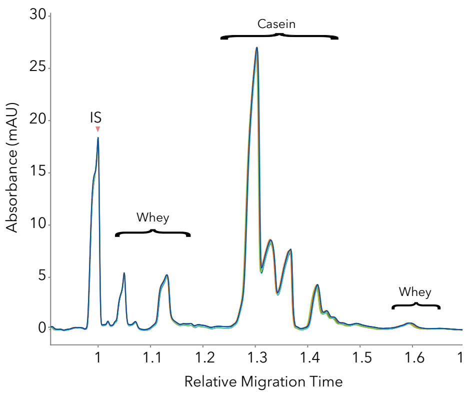


TABLE 5. Quantification of protein content in commercial goat's milk, resulting in an expected whey:casein ratio of ~21:79

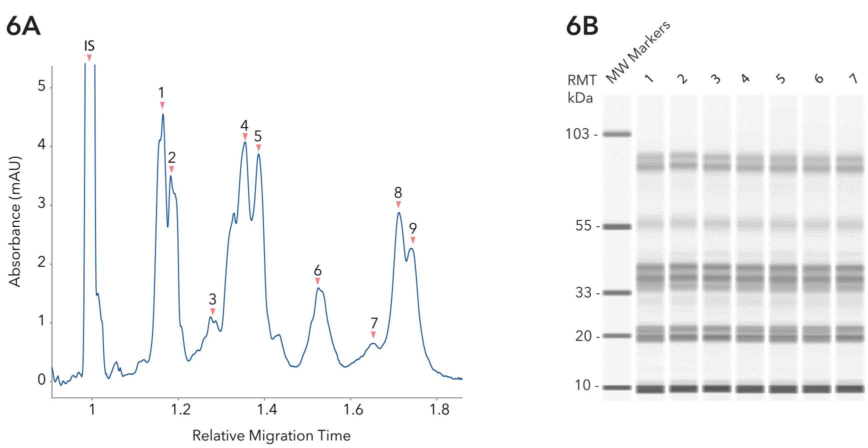
Percent Peak Area (n=5)			
Protein	**Average**	**Standard Deviation**	**%RSD**
Whey	20.78	0.76	3.64
Casein	79.22	0.76	0.95

Non-Dairy Sources of Milk

Although widely known to have far less protein compared to animal-based milk, non-dairy beverages such as soy milk are almond milk are increasingly being consumed due to the popularization of plant-based diets. Produced from whole soybeans or soy protein isolate, the protein profile of soy milk is distinct from animal-derived milk, as seen in **Figure 6A**.

While further experiments are needed to accurately identify all the peaks, β-conglycinin and glycinin are known to account for 70% of proteins in soy milk[4] and hence most likely correspond to peaks with the highest percent peak area. **Figure 6B** shows results from the Lane View, while the details are highlighted in **Table 6**.

FIGURE 6. CE-SDS PLUS analysis of soy milk proteins from a commercial brand



6A. Electropherogram of soy milk proteins. The Maurice system detected several peaks, many of which could be enzymes and minor storage proteins that are often found in soy milk. **6B.** Results of the same run from Lane View, showing gel-like data for seven consecutive injections.

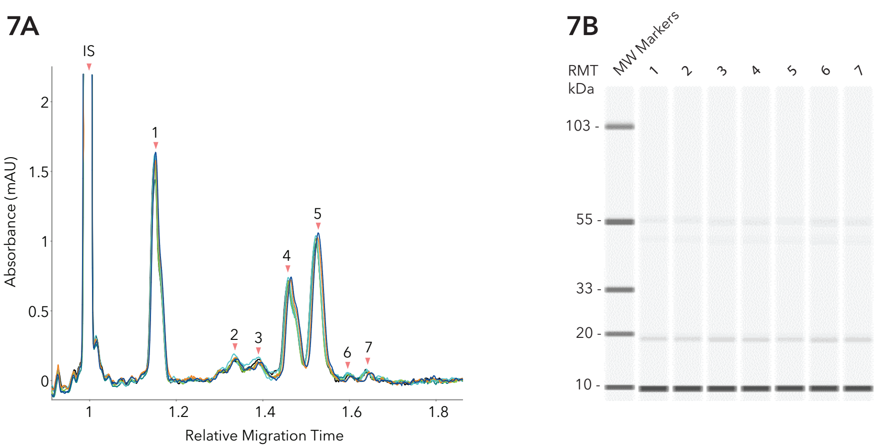
Non-Dairy Sources of Milk (continued)

TABLE 6. Quantification of soy milk proteins based on percent peak area

Percent Peak Area (n=7)									
Injection	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 6**	**Peak 7**	**Peak 8**	**Peak 9**
Average	16.86	11.91	6.50	21.69	15.37	8.70	3.21	8.93	6.84
Standard Deviation	0.21	0.17	0.30	0.46	0.28	0.30	0.17	0.20	0.29
%RSD	1.23	1.41	4.62	2.10	1.83	3.45	5.21	2.21	4.29

Almond milk is derived from a mixture of ground almonds and water, typically containing around 1g of protein per serving. This relatively low protein content is evident in the CE-SDS protein profile, where fewer dominant peaks are apparent (**Figure 7A**). The consistency of these major peaks was confirmed across multiple injections, as detailed in **Table 7**. The presence of several minor peaks suggests the addition of other components, possibly additives common in commercial almond milk. A more in-depth analysis is required to precisely identify these peaks. The results from the Lane View are depicted in **Figure 7B**.

FIGURE 7. CE-SDS PLUS analysis of almond milk proteins from a commercial brand



7A. Electropherogram of almond milk proteins, where three peaks are the most abundant **7B.** Results of the same run using Lane View, showing data for seven consecutive injections.

TABLE 7. Quantification of almond milk proteins based on percent peak area

Percent Peak Area (n=7)							
Injection	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 6**	**Peak 7**
Average	40.69	6.34	5.31	20.19	25.67	0.71	1.06
Standard Deviation	1.36	0.71	0.98	0.57	0.63	0.30	0.29
%RSD	3.35	11.18	18.42	2.82	2.46	42.33	27.23

Conclusion

Analyzing milk proteins is critical for ensuring the quality and safety standards of milk products consumed globally. Traditional methods have their limitations in accurately measuring true protein content and can be error prone. This application note emphasized the capabilities and advantages of the Maurice™ system's CE-SDS method for rapid and precise milk protein analysis. In particular, the study showed how the method can be used to determine protein quantity in various milk products. The findings underscore the method's reliability, accuracy, and speed, making it a suitable analytical tool for the analysis of food products, especially in quality control and nutritional labeling applications. To learn more about the Maurice system and its capabilities, visit our website.

References

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