Determination of Lactose Content using a HILIC Amide Column on a Single Quad LCMS

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Introduction

Acid whey is a low value by-product of Greek yogurt production which contains significant amounts of lactose. The accurate determination of lactose content in acid whey samples is important for further processing, quality assurance, and high-value food product applications. However, it is difficult to analyze lactose due to its acidic nature, presence of isomers, and other reducing sugars. Liquid Chromatography (LC) with refractive index detector (RID), amperometry, and other methods are normally used, but may not be suitable for the analysis of sugars in whey due to their matrix effect. The aim of this study was to use LCMS for analysis of milk sugars.

Methods

LC-MS (Shimadzu LC-40D and LCMS-2020) with a single quad detector and amide column (Waters) was used to analyze lactose standards. Lactose standards were prepared in LC-MS grade water. Mobile phase consisted of LC-MS grade acetonitrile:water:methanol (90:6:4). Samples and mobile phases were filtered with 0.2 µm nylon filters before injection (Fig. 1). Column oven temperature was 90°C, isocratic flow was 0.6 mL/min, and injection volume was 1 µL. For the MS, the desolvation line temperature was 250 °C, and the heat block temperature was 400 °C.

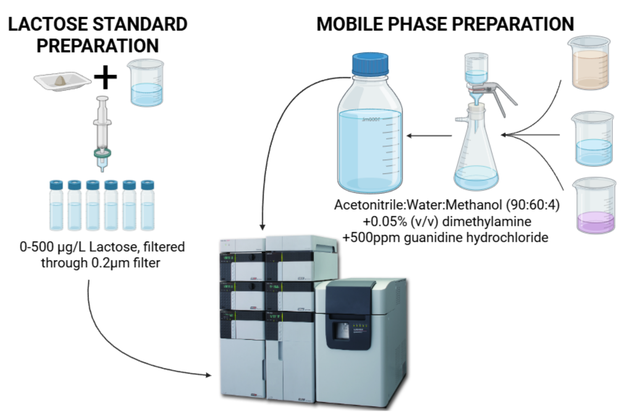

Figure 1: Lactose standard preparation/ mobile phase preparation

Nebulizing gas (N_2) flow was 1.5 L/min, and drying gas (N_2) flow was 15 L/min. Selective Ion Monitoring (SIM) was used in negative ion mode with *m/z* 377 and *m/z* 379 monitored for lactose. All samples were analyzed in triplicate.

Results

The raw chromatogram showed the peak intensity vs retention time (Fig. 2). Retention time decreased from 19.5 min to 7.5 minutes with an increase in mobile phase flow rate from 0.2 to 0.8 mL/min (Fig. 3).

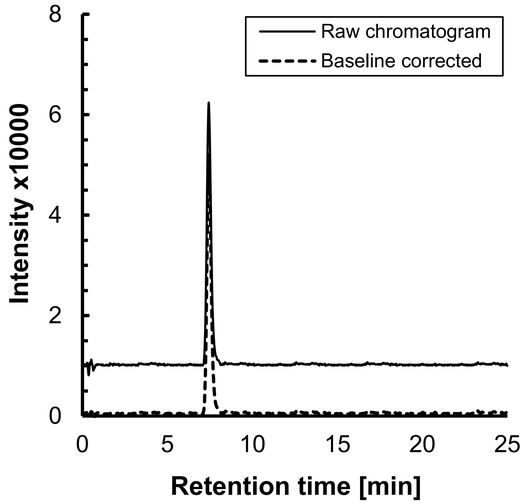

Figure 2: Raw and corrected chromatogram of lactose.

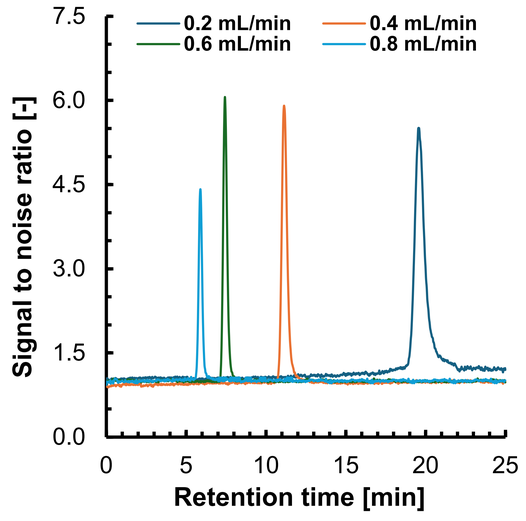

Figure 3: Effect of flow rate on peak intensity and RT.

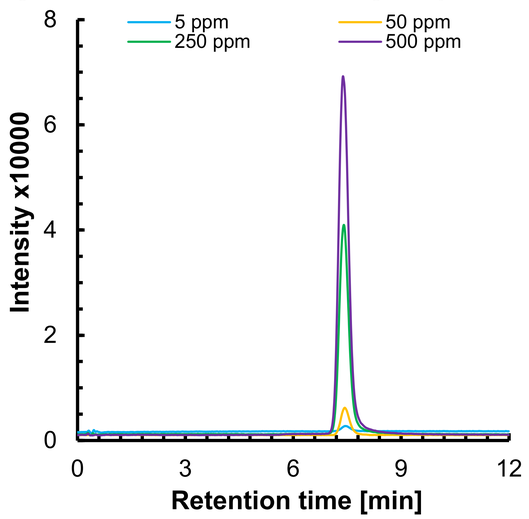

Figure 4: Effect of lactose concentrations on peak area.

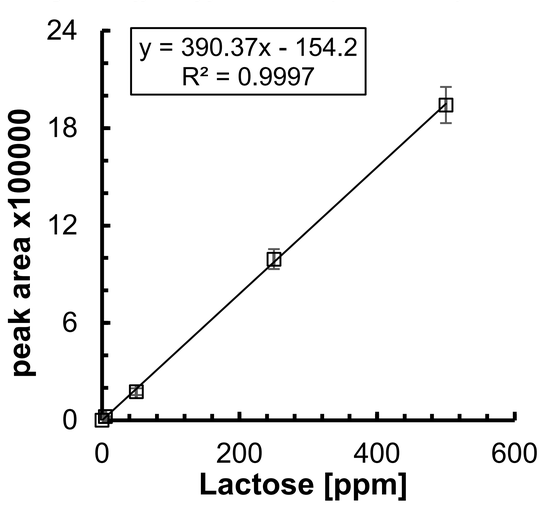

Figure 5: Lactose standard calibration curve.

Table 1: Signal-to-Noise (S/N) ratio and RT at different flow rates

Flow rate	S/N	RT
0.2 ml	5.51	19.56
0.4 mi	5.91	11.13
0.6 ml	6.06	7.43
0.8 ml	4.42	5.9

The impact of LC flow rate to maximize the peak efficiency (peak resolution and signal-to-noise ratio) was optimized (Fig. 3) and the best peak was observed at 0.6 mL/min compared with other flow rates (Table 1). The calibration curve was constructed with peak area vs concentration of lactose standard using 0.6 mL/min (Fig. 5). The maximum peak was linearly dependent on lactose concentration (Fig. 4-5). The method showed good linearity, sensitivity with a limit of detection of 12.0 ppm, and repeatability (RSD < 6%).

Conclusion

A method was successfully developed to quantify lactose sugar using LCMS system. The flow rate of 0.6 mL/min was optimal for signal-to-noise ratio, and a concentration of 500 ppm had the highest peak intensity when compared with other, lower concentrations. This method showed good linearity, sensitivity, precision, the R^2 value, and the limit of detection. This method could be beneficial for the analysis of sugars found in acid whey and other dairy products that can be difficult to analyze with other methods.

Acknowledgements

This research was funded by the BUILD DAIRY Program of the Western Dairy Center (Utah State University, Logan) with financial support from Dairy West (Meridian, ID) and regional dairy processing companies and by the Dairy NutriSols through NSF convergence accelerator funding program.

References

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