Quality testing for infant formula

Fast and reliable low level lactose determination with IC-PAD

Lactose, a disaccharide made of glucose and galactose, is present in milk and milk products. As the major carbohydrate in human milk (55–70 g/L), it is also a main component of infant formulas with minimum recommendations of 4.5 g/100 kcal [1]. Analytically, such high concentrations can easily be determined by multi-component methods such as ISO 22184 or lower sensitivity methods like refractive index detection. In some cases, infants can suffer from lactose malabsorption. Here, the lactase enzymatic activity is either limited or absent from birth, impairing lactose metabolization. The lactose cannot be hydrolyzed into glucose and galactose in the small intestine, impeding the absorption of digestible carbohydrates as monosaccharides. Consequently, numerous gastrointestinal and extra-intestinal symptoms and complaints appear (e.g. diarrhea) [1–3]. Glucose-galactose malabsorption also affects a very small subset of the population. For all malabsorption types, it is critical to eliminate any dietary sources of lactose. Lactose-free nutritional sources are especially crucial for infants, and these foodstuffs must properly meet the regulation requirements (lactose <10 mg/100 kcal) [1,3–5]. To analyze such **low lactose contents** in complex matrices like infant formula, **ion chromatography with pulsed amperometric detection (IC-PAD)** offers a robust and sensitive solution.

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SAMPLES AND SAMPLE PREPARATION

Lactose determination was performed for a broad spectrum of low lactose sample matrices, comprised of infant formulas and follow-up baby food such as the reference materials NIST 2383a (baby food composite) and NIST 1869 (infant formula powder), an infant formula from HiPP (HiPP comfort, lactose reduced), and a commercially available lactose-free milk (1.5% fat, Spar Switzerland).

Powdered and liquid materials were homogenized and weighed directly into suitable containers (from 0.1–5 g, 50 mL polypropylene centrifuge tubes). The sample weight (W_S in g) was recorded to the nearest 0.001 g for later calculations. An aqueous extract was prepared by adding ultrapure water (UPW) to result in a total volume of 50 mL (W_{UPW} in kg). Afterwards, the vials were capped and mixed vigorously with a vortex mixer for approximately 20 seconds.

Carrez precipitation is a standard method to remove proteins and larger molecules from samples in order to protect the analytical system. Following this common practice, the reagents were added, and the final weight noted (W_{UPWc} in kg). After thorough mixing, the samples were centrifuged (5000×g) for 10 minutes and decanted. The covered vials were placed directly into the autosampler. Increased column protection can be secured by an additional ultrafiltration step.

As an alternative, automated sample preparation by Inline Dialysis with a Low Volume dialysis cell is recommended. For that, samples were prepared identically to the aqueous extracts, shaken well, and covered before placing on the autosampler rack. For dialysis, no Carrez precipitation is necessary prior to the analysis, saving time and chemical reagents. Using the Low Volume dialysis cell requires only 5 mL of sample.

EXPERIMENTAL

The quantity of lactose in aqueous sample extracts was determined by ion chromatography (IC) on a Metrosep Carb 2 - 250/4.0 column using an isocratic hydroxide eluent (400 mmol/L NaOH) and pulsed amperometric detection (PAD) with the sweep waveform (Figure 1).

Together with the Metrohm Thin-Layer amperometric cell (Au working and Pd reference electrode), a long electrode lifetime with minimal maintenance is obtainable. The sweep mode combined with less turbulent flow in the Thin-Layer cell results in a smooth baseline, a necessary precondition to analyze very low concentrations such as in low lactose products. Flow schemes for direct analysis and analysis after Metrohm Inline Dialysis are shown in Figure 2. Although the setup for the dialysis may look more complex, automation makes the effort worthwhile for the overall analytical process.
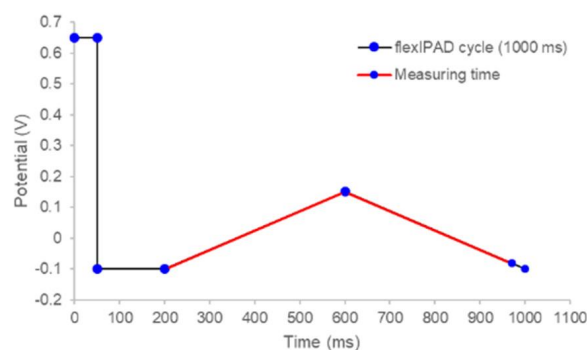
Figure 1. The sweep waveform for carbohydrate oxidation supports sensitive detection of carbohydrates with a low noise level.

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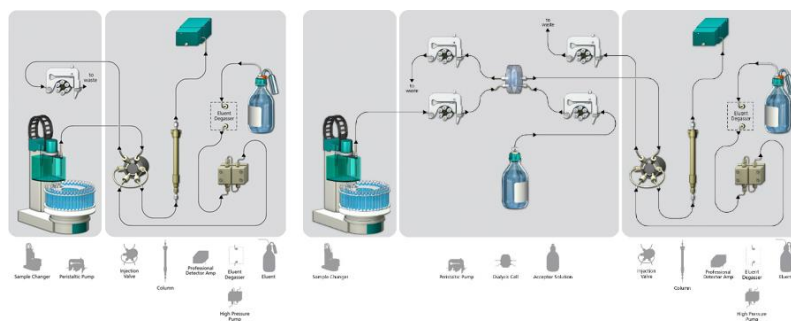
Figure 2. Example system configuration for direct lactose analysis (left, sample preparation mandatory as for example Carrez precipitation) and with optional dialysis (right, no additional sample preparation necessary). Inline Dialysis is optional and can be added to any existing instrumentation setup. For Carrez precipitation the sample path goes directly from the autosampler into the sample loop (PEEK, 10 µL). Sample transport occurs by peristaltic pump. From the sample loop, the sample is directly injected onto the column (Metrosep Carb 2 - 250/4.0), where it is eluted with a 400 mmol/L NaOH isocratic eluent before pulsed amperometric detection (PAD).

RESULTS AND DISCUSSION

Lactose elutes in under 20 minutes (**Figure 3**), independent of whether direct injection or Inline Dialysis is used. In contrast to previously published chromatographic methods, lactose derivatives (i.e., epilactose, lactulose, allolactose, and galactosyllactose), as from prebiotic additives, were successfully separated from lactose, increasing the selectivity and accuracy of the method. The overall working range of the method is 0.05 to 80 mg/L for liquid lactose standards, with the ability to analyze samples in a range of 0.2–21,000 mg/100g with respective dilution. The sample concentrations are determined from a linear calibration ($c(Lactose)_S$ in mg/kg) and are calculated based on the sample weight to give the final lactose content ($c(Lactose)_{FIN}$ in g/100 g):

$$c(Lactose)_{FIN} = \frac{100 \times c(Lactose)_S \times W_{UPW/UPWc}}{1000 \times W_S}$$

Validation results are shown for infant formula, baby food, and milk (**Table 1**). These display the compliance with the overall acceptance criteria of AOAC for a full single laboratory validation (RSD_r and variability ≤10% and ≤7%, spike recoveries 85–115% and 90–110% for an analytical range of 10–100 mg/100 g and >100 mg/100 g, respectively).

The results obtained for the analysis using Carrez precipitation prior to injection (**Table 1**) and using Inline Dialysis were comparable for selected test samples analyzed as shown in **Figure 3**. The data give an excellent verification for using Inline Dialysis as a time efficient alternative to Carrez precipitation.

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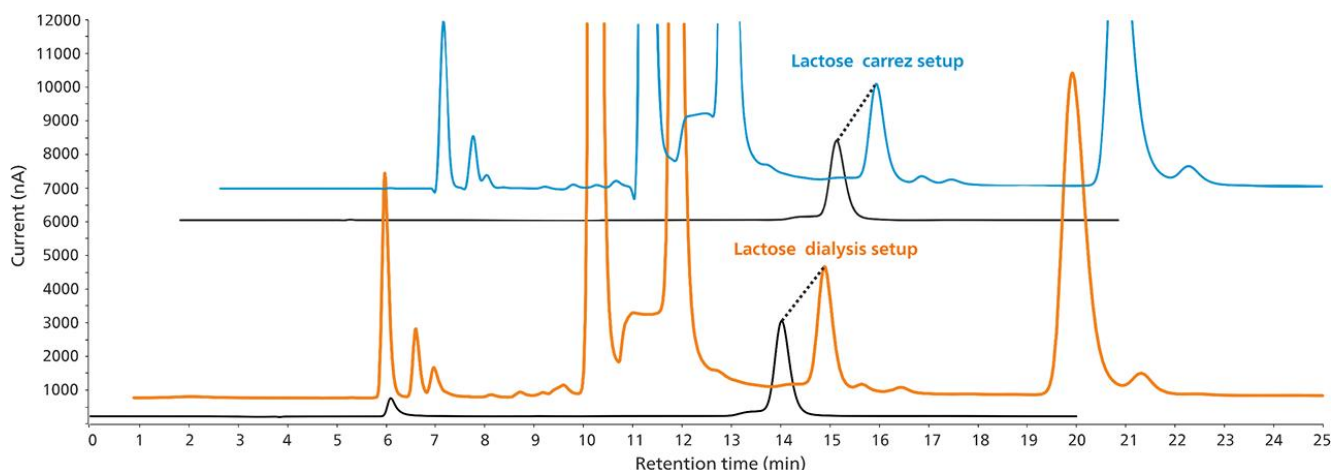
Figure 3. Lactose determination in baby food (NIST 2383a). Comparison of two sample preparation methods: Inline Dialysis (c(lactose) = 51.1 mg/L, orange) and Carrez precipitation (c(lactose) = 49.6 mg/L, blue). The relative standard deviation of the two samples is 2.1%. For comparison, a 40 mg/L lactose standard (black) is overlaid.

Table 1. Lactose expressed as lactose monohydrate (conversion factor 1.05) in lactose-free infant formula and milk samples determined after sample preparation by Carrez precipitation. The table shows the repeatability Rr as RSD from individually prepared samples measured within a short time period (n = 7), the day-to-day variability as RSD over individual prepared samples measured on different days (4–8 d), and spike recovery as the average of all spike experiments analyzed over several days.

Repeatability (mg/100 g) (RSD$_r$, %)	Day-to-day variability (mg/100g) (%)	Total spike recovery (%)
NIST 2383a infant food (average 520 ± 27 mg/100 g (n = 7), target 500 ± 100 mg/100 g, recovery 106±6%)		
520 ± 27 (5.1)	528 ± 29 (5.5)	103 ± 2
NIST 1869 infant formula powder (average 569 ± 33 mg/100 g (n = 7), target 520 ± 120 mg/100 g, target recovery 109 ± 6%)		
569 ± 33 (5.5)	523 ± 31 (5.9)	98 ± 3
HiPP Comfort infant formula (20.8 g/100 g (n = 7), target 22.1 g/100 g, target recovery 97 ± 4%)		
20751 ± 743 (3.6)	22163 ± 258 (1.2)	102 ± 3
Spar aha 1.5% fat, lactose-free milk		
2.35 ± 0.18 (7.9)	2.10 ± 0.05 (2.2)	111 ± 2

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CONCLUSION

Infant formula is a very complex and challenging matrix as it contains all kinds of nutrients (e.g., proteins, fats, carbohydrates, vitamins, and minerals). Dedicated sample preparation and analysis methods are needed to guarantee high accuracy, sensitivity, and selectivity for the determination of low lactose concentrations. IC-PAD overcomes such analytical challenges with underlined automated inline sample preparation options and results in excellent separation of lactose from other matrix components and lactose derivatives.

The estimated LOQ of 4 mg lactose/100 g in these sample matrices is lower than the requirements and known thresholds for lactose-free infant formula products. Thus, adequate sensitivity and robustness as shown by long-term analysis and spike experiments is given by this method. Additionally, the flexibility for user adapted applications, optional sample preparation, and/or automation make IC-PAD ideal for routine analytics and a highly valuable addition to the laboratory analytical portfolio.

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CONFIGURATION

940 Professional IC Vario ONE/Prep 1

The 940 Professional IC Vario ONE/Prep 1 is the intelligent IC instrument **without suppression** in combination with Metrohm Inline Sample Preparation, e.g., **Inline Ultrafiltration** or **Inline Dialysis**. The instrument can be used with any separation and detection methods.

Typical areas of application:

- Anion and cation determinations without suppression after Inline Ultrafiltration or Inline Dialysis
- UV/VIS applications after Inline Ultrafiltration or Inline Dialysis
- Applications with amperometric detection after Inline Ultrafiltration or Inline Dialysis

945 Professional Detector Vario – Amperometry

Intelligent stand-alone detector equipped with the IC Amperometric Detector. Outstanding selectivity due to the four measuring modes: DC, PAD, flexIPAD, CV. The excellent signal/noise ratio and the very fast start-up guarantee the highest in measurement precision. For use with intelligent IC instruments or as independent detector.

Metrosep Carb 2 - 250/4.0

The Metrosep Carb 2 - 250/4.0 IC column is particularly suitable for the determination of carbohydrates using alkaline eluents and pulsed amperometric detection. The high-capacity anion exchanger column is based on a styrene-divinylbenzene copolymer. It is stable in the range of pH = 0 - 14 and separates monosaccharides and disaccharides. It is also suitable for the analysis of sugar alcohols, anhydrous sugars, amino sugars, etc. The 250 mm version of the Metrosep Carb 2 separation column is optimized for complex separations.

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858 Professional Sample Processor – Pump

The 858 Professional Sample Processor – Pump processes samples from 500 µL to 500 mL. The sample transfer takes place either with the installed bidirectional two-channel peristaltic pump or with an 800 Dosino.

IC equipment: Low Volume Inline Dialysis

Accessory set for rapid Inline Dialysis. For use with the 858 Professional Sample Processor and an additional two-channel peristaltic pump.

IC equipment: Thin-Layer cell: Carb (Au,Pd)

Equipment comprising Thin-Layer cell with additional accessories, with electrodes for carbohydrate analysis with a gold working electrode and a Pd reference electrode.

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Professional PC program for controlling all intelligent Professional IC systems, Compact IC systems and their peripherals, all detectors and various Auto samplers, 800 Dosino, 771 Compact Interface, etc. The software permits checks, data acquisition, data evaluation and data monitoring as well as report generation of ion chromatographic analyses.

Graphical user interface for routine operations, extensive database programs, method development, configuration and manual system control; very flexible user management, powerful database operations, extensive data export functions, individually configurable report generator, control and monitoring of all system components and the chromatography results.

MagIC Net Professional complies fully with FDA Regulation 21 CFR Part 11 as well as GLP.

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The installation and documents are supplied on a USB flash drive.

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