



Application Note AN-P-088

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 metabolism. 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.

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 (5000xg) 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.



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 ($c(\text{Lactose})_S$ in mg/kg) and are calculated based on the sample weight to give the final lactose content ($c(\text{Lactose})_{\text{FIN}}$ in g/100 g):

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

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 $\leq 10\%$ and $\leq 7\%$, 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.



Figure 3. Lactose determination in baby food (NIST 2383a). Comparison of two sample preparation methods: Inline Dialysis ($c(\text{lactose}) = 51.1 \text{ mg/L}$, orange) and Carrez precipitation ($c(\text{lactose}) = 49.6 \text{ mg/L}$, 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 R_r 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 $520 \pm 27 \text{ mg/100 g}$ ($n = 7$), target $500 \pm 100 \text{ mg/100 g}$, recovery $106 \pm 6\%$)		
520 ± 27 (5.1)	528 ± 29 (5.5)	103 ± 2
NIST 1869 infant formula powder (average $569 \pm 33 \text{ mg/100 g}$ ($n = 7$), target $520 \pm 120 \text{ mg/100 g}$, target recovery $109 \pm 6\%$)		
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 $97 \pm 4\%$)		
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

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 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.

REFERENCES

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CONFIGURATION



940 Professional IC Vario ONE/Prep 1

Le 940 Professional IC Vario ONE/Prep 1 est l'appareil CI intelligent **sans suppression** combiné à une préparation des échantillons inline comme **l'ultrafiltration inline** ou **la dialyse inline**. L'appareil peut être utilisé avec n'importe quelles méthodes de séparation et de détection.

Domaines d'application typiques :

- Déterminations d'anions et de cations sans suppression après ultrafiltration inline ou dialyse inline
- Applications UV/VIS après ultrafiltration inline ou dialyse inline
- Applications avec détection ampérométrique après ultrafiltration inline ou dialyse inline



945 Professional Detector Vario – Amperometry

Détecteur intelligent et autonome équipé du détecteur IC Amperometric Detector. Il fait preuve d'une excellente sélectivité du fait de ses quatre modes de mesure : DC, PAD, flexIPAD et CV. L'excellent rapport signal/bruit et la disponibilité pour la mesure garantissent une très haute précision de la mesure. Pour utiliser avec les appareils CI intelligents ou en tant que détecteur autonome.



Metrosep Carb 2 - 250/4,0

La colonne CI Metrosep Carb 2 - 250/4,0 convient particulièrement à la détermination des hydrates de carbone en utilisant les éluants alcalins et la détection ampérométrique pulsée. La colonne d'échange d'anions de haute capacité est basée sur un copolymère styrène/divinylbenzène. Elle est stable dans la plage de pH = 0–14 et sépare les monosaccharides et les disaccharides. Elle convient en outre à l'analyse des alcools de sucre, des sucres anhydres, des osamines, etc. Le modèle 250 mm de la colonne de séparation Metrosep Carb 2 est optimisée pour les séparations complexes.



858 Professional Sample Processor – Pump

Le 858 Professional Sample Processor – Pump traite des échantillons de 500 μL à 500 mL. Le transfert des échantillons s'opère soit au moyen de la pompe péristaltique bidirectionnelle à deux voies intégrée soit par un 800 Dosino.



Équipement CI: dialyse inline Low Volume

Jeu d'accessoires pour la dialyse inline rapide. À utiliser avec le 858 Professional Sample Processor et une pompe péristaltique à 2 canaux supplémentaire.



Équipement CI : cellule Thin-Layer : Carb (Au, Pd)

Équipement constitué d'une cellule Thin-Layer et d'accessoires supplémentaires, d'électrodes destinées à une méthode analytique des hydrates de carbone, ainsi que d'une électrode de travail en or et d'une électrode de référence en Pd.