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 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.

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.

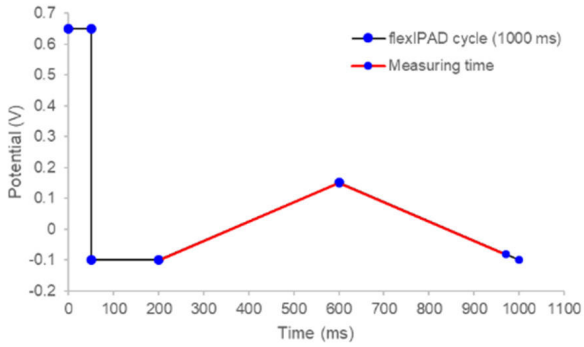


Figure 1. The sweep waveform for carbohydrate oxidation supports sensitive detection of carbohydrates with a low noise level.

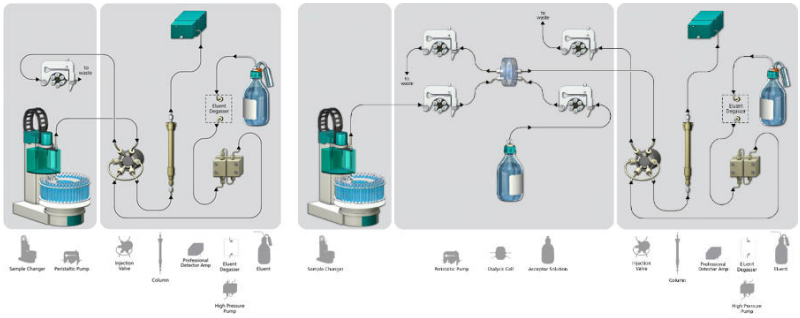


Figure 2. Example system configuration for direct lactose analysis (left, sample preparation mandatory as for example Carrez precipitation) and with optional dialysis (right, no additional sample preparation necessary). Inline Dialysis is optional and can be added to any existing instrumentation setup. 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).

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):

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

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.

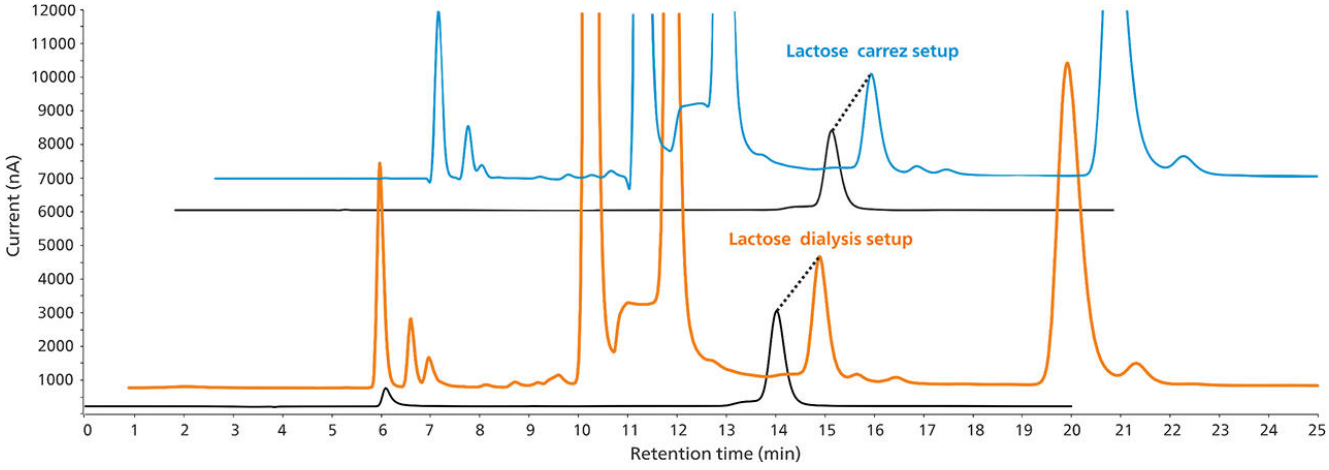


Figure 3. Lactose determination in baby food (NIST 2383a). Comparison of two sample preparation methods: Inline Dialysis (c(lactose) = 51.1 mg/L, orange) and Carrez precipitation (c(lactose) = 49.6 mg/L, blue). The relative standard deviation of the two samples is 2.1%. For comparison, a 40 mg/L lactose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

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

1. EFSA. Scientific Opinion: Essential Composition of Infant and Follow-up Formulae. *Eur. Food Saf. Auth. EFSA J.* **2014**, *12* (7), 3760.
2. Facioni, M. S.; Raspini, B.; Pivari, F.; Dogliotti, E.; Cena, H. Nutritional Management of Lactose Intolerance: The Importance of Diet and Food Labelling. *J. Transl. Med.* **2020**, *18* (1), 260.
3. EFSA. Scientific Opinion on Lactose Thresholds in Lactose Intolerance and Galactosaemia. *Eur. Food Saf. Auth. EFSA J.* **2010**, *8* (9), 1777.
4. European Commission. Comission Delegated Regulation (EU) 2016/127 of 25 September 2015 Supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as Regards the Specific Compositional and Information Requirements for Infant Formula and Follow-on Formula and as Regards Requirements on Information Relating to Infant and Young Child Feeding. *Off. J. Eur. Union 251* **2016**, 29.
5. FAO/WHO. CODEX Alimentarius - General Standard for the Labelling of Prepacked Foods CXS 1-1985. *Int. Food Stand.*

Internal reference: AW IC CH6-1435-022021

CONTACT

Metrohm AG
Ionenstrasse
9100 Herisau

info@metrohm.com

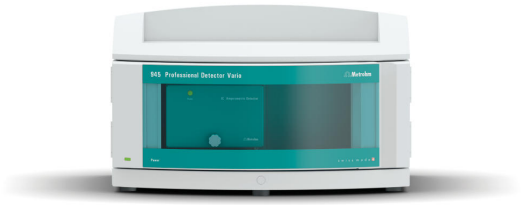
CONFIGURATION

940 Professional IC Vario ONE/Prep 1

940 Professional IC Vario ONE/Prep 1 是智能型 IC 器,**无抑制**,可与万通英品前理以及**英超**或**英析**合使用。 器可使用各分和方法。

典型的用范:

- 定子和子,英超或英析之后无抑制
- 英超或英析之后的 UV/VIS 用
- 英超或英析之后使用流的用

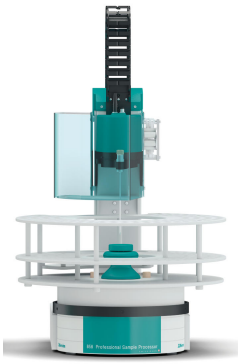


945 Professional Detector Vario – Amperometry

智能型机器配一台 IC Amperometric Detector。 具有四可的不同量模式:DC、PAD、flexIPAD 和 CV。卓越的信号/干比例和迅速的量准,能保最高的量精度。 与智能型子色合使用,或作独立的器使用。

Metrosep Carb 2 - 250/4.0

Metrosep Carb 2 - 250/4.0 / pH = 0 - 14 ,250 mm
Metrosep Carb 2



858 Professional Sample Processor – Pump

858 Professional Sample Processor – Pump 500 L
500 mL , 800 Dosino

""

用于快速英析的附件。与 858 Professional Sample Processor 和一台附加的 2 通道蠕一同使用。

CarbAuPd

装包含了有外附件的薄式流通池,配用于水化合物分析的,一个金工作和一个参比。