Application Note AN-P-089

Lactose intolerance and reliance on accurate food labels

Fast and robust low level lactose analysis with IC-PAD

Worldwide, milk and dairy products are vital sources of nutrition for humans [1]. Beside nutrients, minerals, proteins, and fat, lactose is a major component of dairy products, serving as an energy source. To efficiently metabolize lactose, the enzyme lactase is indispensable [2]. However, globally nearly 70% of the population is lactose intolerant, i.e., they have difficulty digesting lactose [3, 4]. Lactose malabsorption leads to numerous gastrointestinal and extra-intestinal symptoms and other complaints with varying extents. While some lactose intolerant people are sensitive to any amounts, others can consume a small quantity without ill effects. A specific cutoff value and regulations for lactose-free product labels and production are still lacking— therefore it is necessary to accurately analyze and label foods using sensitive standard techniques. **Ion chromatography with pulsed amperometric detection** (IC-PAD) enables the determination of very low lactose contents. Validation according to AOAC requirements shows the high sensitivity and reliability of this method as a routine analysis.

SAMPLE AND SAMPLE PREPARATION

Lactose determination was performed for a broad selection of sample matrices comprising infant formulas and follow-up baby food (AN-P-088), certified references (e.g., the low lactose milk reference muva ML-2312), as well as 15 commercially available low lactose and lactose-free products including yogurt, butter, cream, cottage cheese, milk drinks, milk chocolate, and supplements.

Solids (e.g., cheese and milk chocolate) were chopped, while the powders and liquid materials were homogenized. Following this pretreatment, the samples were weighed directly into suitable containers (0.1–5 g, 50 mL polypropylene 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 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. To improve the solubility of certain samples (e.g., cream cheese or chocolate), vials were heated up in a 70 °C tempered water bath for 10 minutes.

Carrez precipitation is a standard method to remove proteins and larger molecules 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 achieved by an additional ultrafiltration step.

Alternatively, automated sample preparation using Inline Dialysis with the Low Volume dialysis cell is recommended. For this, samples were prepared as described previously regarding aqueous extracts, shaken well, and covered before placing them directly on the autosampler rack. When using dialysis, no Carrez precipitation is necessary prior to analysis, saving time and chemicals. Using the Low Volume dialysis cell requires only 5 mL of sample.

The quantity of lactose in the aqueous sample extracts was determined by ion chromatography (IC) with a Metrosep Carb 2 - 250/4.0 separation column using an isocratic hydroxide eluent (400 mmol/L NaOH) and pulsed amperometric detection (PAD) with the sweep waveform (AN-P-088, WP-077). A long electrode lifetime with minimal maintenance requirements is possible by using the Metrohm Thin-Layer amperometric cell (Au working and Pd reference electrode). The sweep mode combined with the less turbulent flow in the Thin-Layer cell results in a smooth baseline with low noise – a necessary precondition to analyze very low concentrations, such as in low lactose products.

Flow schemes for the direct analysis of lactose with mandatory sample preparation such as Carrez precipitation are shown in Figure 1.

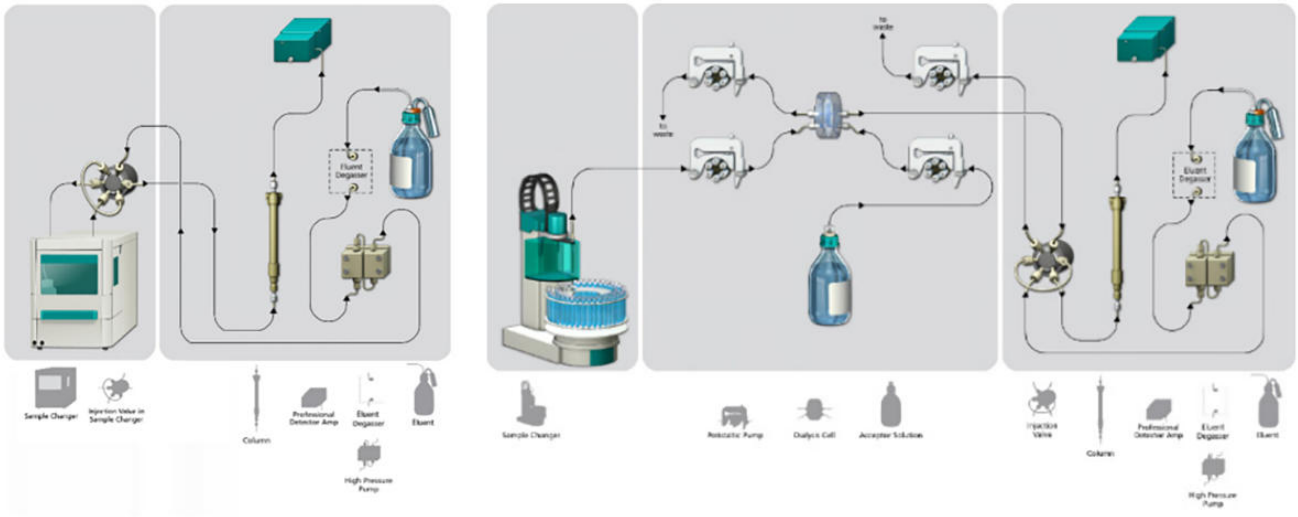


Figure 1. Example system configurations for direct lactose analysis using the Metrohm 889 Sample Center – cool (left). Sample preparation for direct analysis is mandatory as e.g., with Carrez precipitation to protect the analytical system. Inline Dialysis (right) can be added optionally to any existing instrumentation, which enables an automated alternative to the conventional sample preparation. Sample transport and liquid handling can be either performed with a peristaltic pump, Dosino, or directly using the 889 autosampler. In both examples isocratic elution is performed with a sodium hydroxide eluent prior to detection by PAD.

Sample stability is improved using the 889 IC Sample Center – cool. Automated sample preparation by Inline Dialysis can be added to any existing configuration setup. More details are available in Metrohm literature for Metrohm Inline Sample Preparation as well as in AN-P-088.

Any liquid handling for sample transport or sample path cleaning can also be automated using Metrohm's most flexible tool for liquid handling – the Dosino.

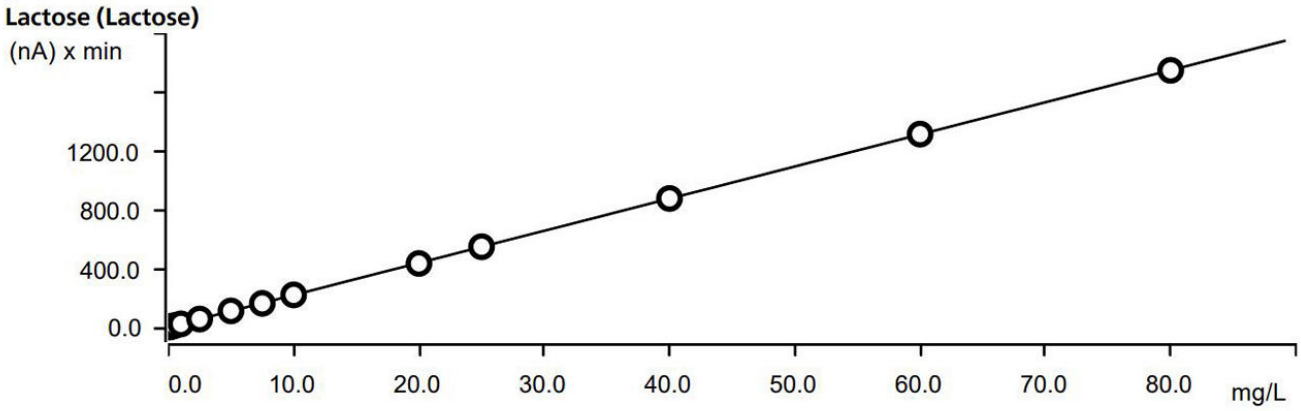
RESULTS AND DISCUSSION

Lactose elutes in less than 30 minutes (Figure 2–6). The overall working range of the method is 0.05 to 80 mg/L for liquid lactose standards (Figure 2 A), with the ability to analyze samples in a range of 0.2 to 21,000 mg/ 100 g with respective dilution. In contrast to previously published chromatographic methods, lactose derivates (epilactose, lactulose, allolactose, and galactosyllactose), such as those from prebiotic additives, were successfully separated from lactose, increasing the selectivity and accuracy of the method (Figure 2 B).

The sample concentrations are determined from the linear calibration ($c(Lactose)_S$ in mg/kg) (Figure 2 A) and calculated to determine the final lactose content ($c(Lactose)_{FIN}$ in g/ 100 g) based on the sample weight:

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

A



Function: . . . . . *A = 0.0263117 + 2.18736× Q*

Relative standard deviation . . . . . *0.418529 %*

Correlation coefficient *0.999996*

B

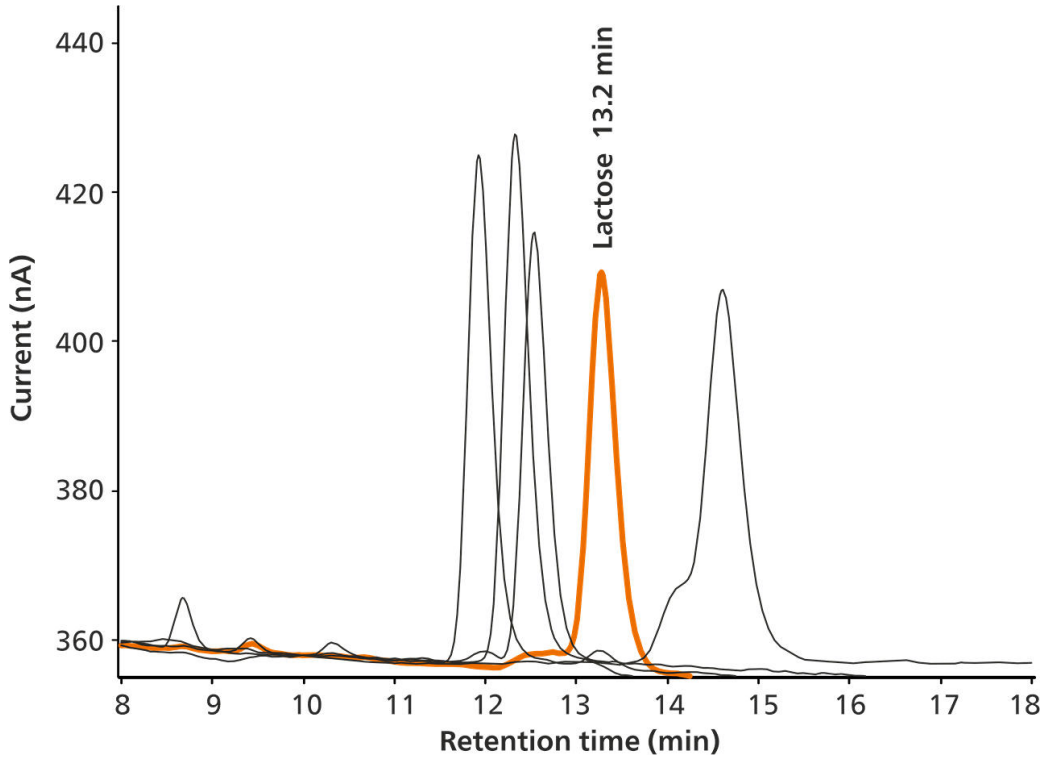


Figure 2. Calibration for lactose (A) showing strong linearity over the concentration range of 0.05 mg/L to 80 mg/L (validation requirement). A proper separation of lactose from interferences is mandatory. Beside other sugars, sugar alcohols, and inorganic ions, it is crucial to separate the structurally similar lactosederivates (B): epilactose, lactulose, allolactose, and galactosyllactose (peaks from left to the right), which is possible with the described elution conditions.

RESULTS AND DISCUSSION

Examples of validation results are shown for selected samples in Figure 3–6 (low lactose milk reference material (muva), a supplement containing lactose beside galacto-oligosaccharides (Bimuno), a lactose-free yogurt, and a lactose-free butter). Concentrations of lactose ranged from <0.5 mg/ 100 g (low lactose butter) to almost 13 g/ 100 g (prebiotic supplement). The data display the compliance with AOAC acceptance criteria for repeatability and day-to-day variability (RSDs ≤7%), spike recoveries (90–110%), and a resolution (>1.5, i.e., baseline separation) ascertained within the single laboratory validation.

The results obtained for the analysis using Carrez precipitation prior to injection and using Inline Dialysis showed comparable results for selected test samples (Figure 3–6, AN-P-088). The results for a set of six different matrices comprising short-term replicates and spike tests (as described in Figure 3–6) differ with RSDs of less than 7% (average 3.2%).

Figure 3–6 show the average concentrations and the repeatability R_r as RSD from individually prepared samples measured within a short time (n = 7) or as concentration determined over individual prepared samples measured at different days (4–8 d) (day-to-day-variability (R_{Var}) and their RSDs), and the total spike recovery R_S as average over all spike experiments analyzed over several days. The resolution of lactose to the subsequent peaks is expressed as R. Results from Inline Dialysis for selected samples are marked with a star (*).

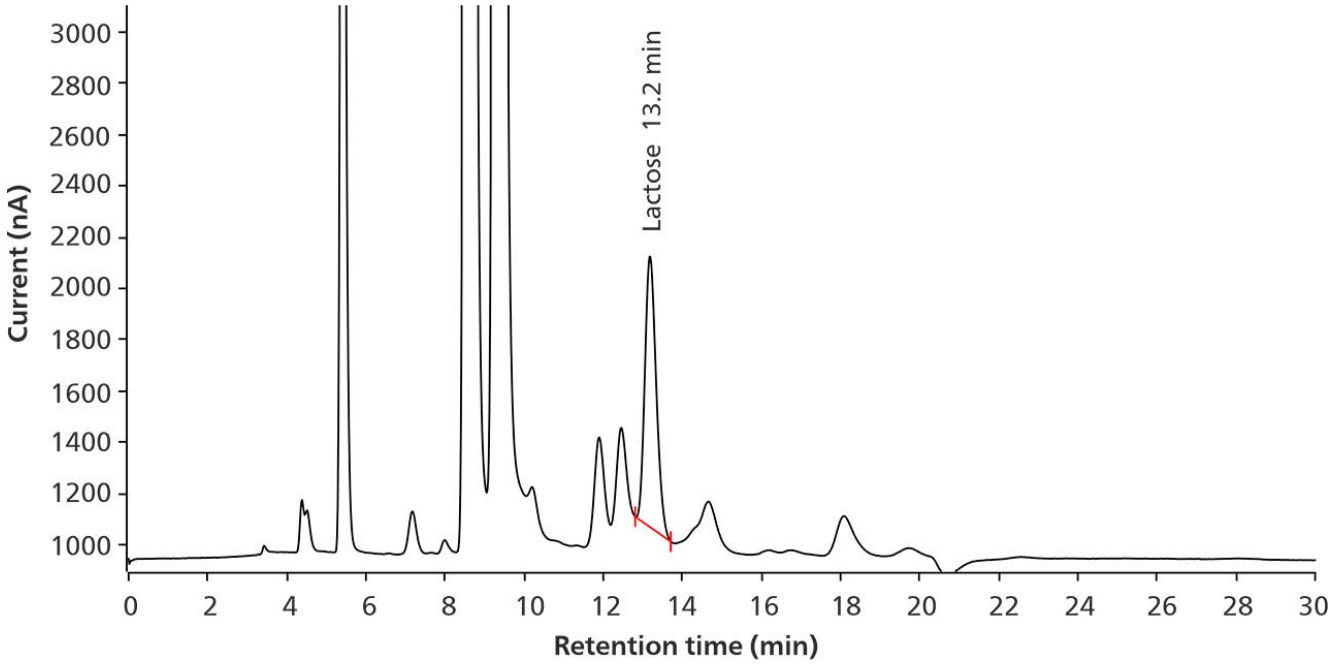


Figure 3. Lactose expressed as lactose monohydrate (conversion factor 1.05 for lactose into lactose monohydrate) in muva ML-2312 (target 217 ± 45 g/ 100 g).

R_r (mg/ 100 g) (RSD_r %)	R_{Var} (mg/ 100 g) (RSD_{Var} %)	R_S (%)	R
226 ± 7 (3.2)	228 ± 12 (5.4)	102 ± 3	2.3

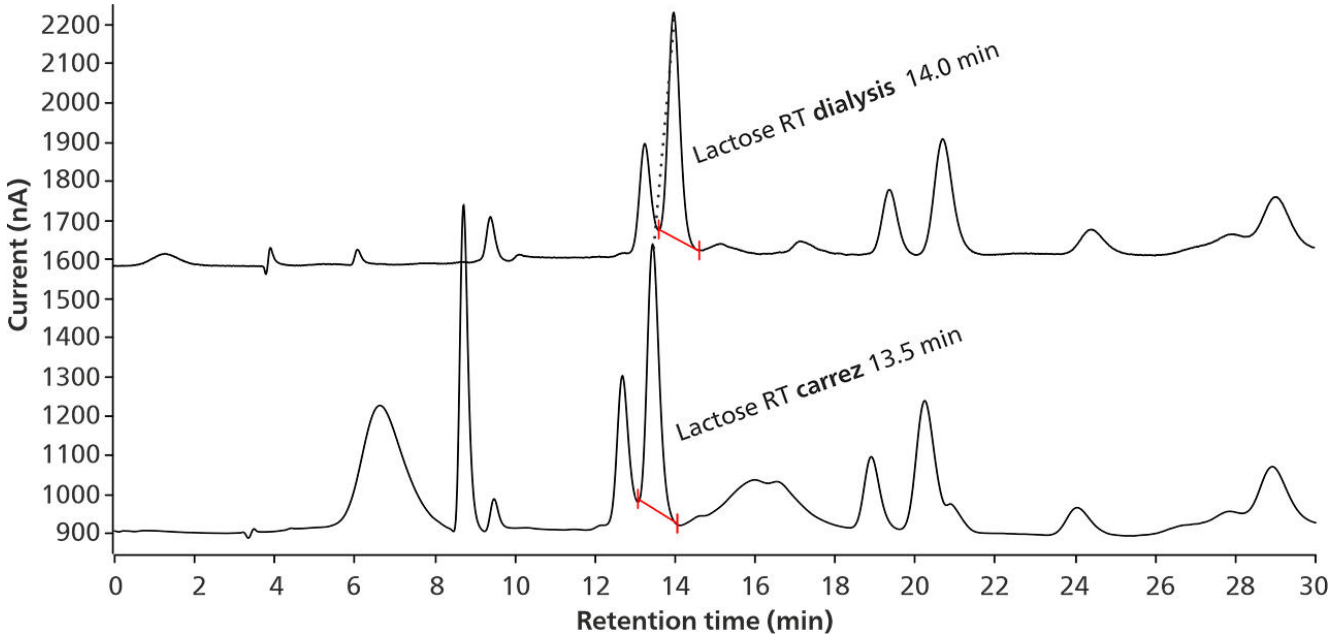


Figure 4. Lactose expressed as lactose monohydrate (conversion factor 1.05 for lactose into lactose monohydrate) in Bimuno daily (Targeted Digestive Nutrition).

R_r (mg/ 100 g) (RSD_r %)	R_{Var} (mg/ 100 g) (RSD_{Var} %)	R_S (%)	R
13009 ± 288 (2.2)*	13125 ± 484 (3.9)*	99 ± 6*	1.9*
11795 ± 130 (1.1)	11807 ± 465 (3.9)	99 ± 5	1.6

RESULTS AND DISCUSSION

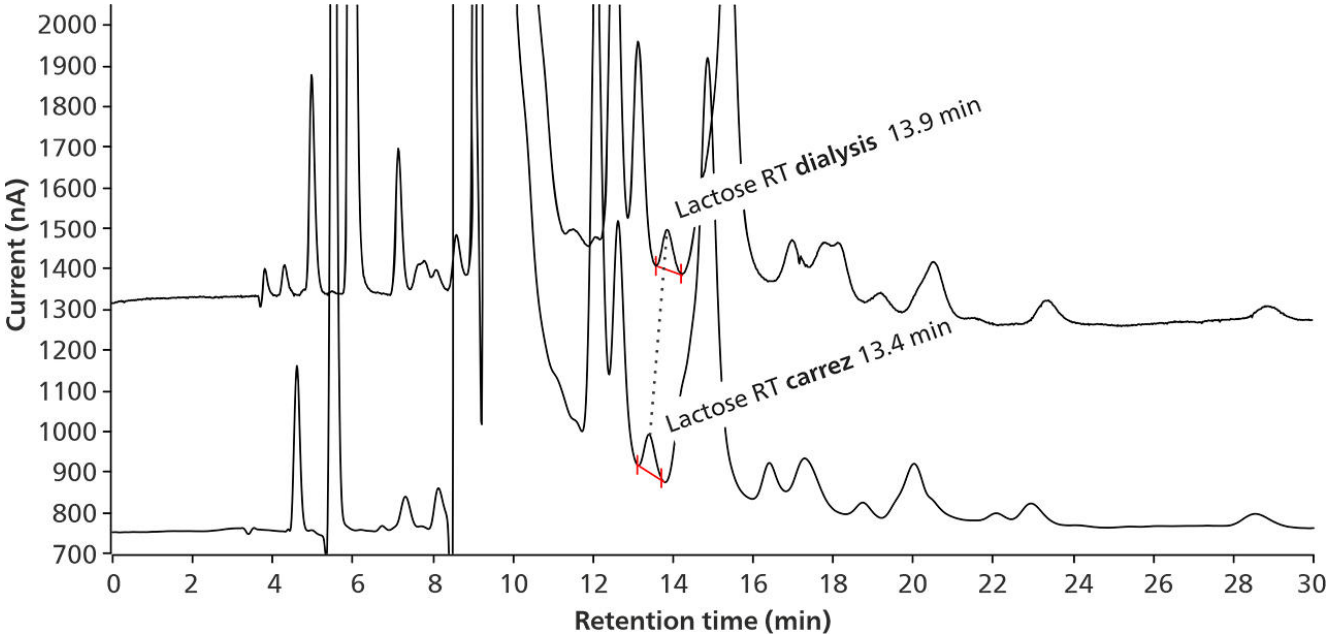


Figure 5. Lactose expressed as lactose monohydrate (conversion factor 1.05 for lactose into lactose monohydrate) in Yogurt, free from, Coop, lactose-free.

R_r (mg/ 100 g) (RSD_r %)	R_{Var} (mg/ 100 g) (RSD_{Var} %)	R_S (%)	R
4.69 ± 0.18 (3.9)*	4.88 ± 0.05 (2.3)*	94 ± 3*	2.6*
4.60 ± 0.15 (3.3)	4.40 ± 0.05 (1.0)	96 ± 3	2.2

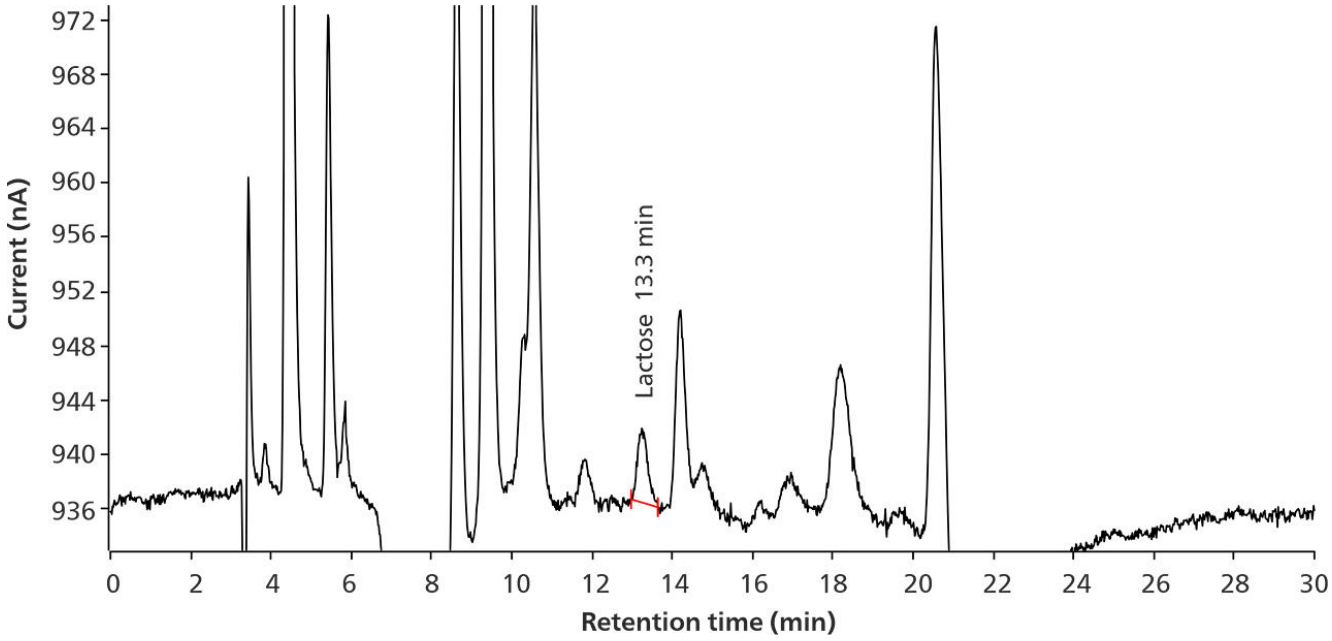


Figure 6. Lactose expressed as lactose monohydrate (conversion factor 1.05 for lactose into lactose monohydrate) in Butter, aha, Spar, lactose-free.

R_r (mg/ 100 g) (RSD_r %)	R_{Var} (mg/ 100 g) (RSD_{Var} %)	R_S (%)	R
0.40 ± 0.02 (5.9)	0.39 ± 0.01 (2.5)	103 ± 6	2.3

Validity according to the criteria for an AOAC single laboratory test shows the appropriate reliability, sensitivity, and robustness of the described IC-PAD method for the determination of lactose in low lactose and lactose-free dairy products. Comparative analysis for sample preparation with Carrez precipitation and Metrohm Inline Dialysis showed excellent conformity. Metrohm Inline Dialysis is recommended as a time-efficient alternative to Carrez precipitation. Metrohm IC systems are characterized by a high degree of flexibility—the systems can be upgraded e.g., to additionally integrate dilution, also allowing for inline calibration and intelligent dilution. Any automation makes the method even more straightforward and suitable for high-throughput and routine analysis.

REFERENCES

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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

Metrosep Carb 2 - 250/4.0

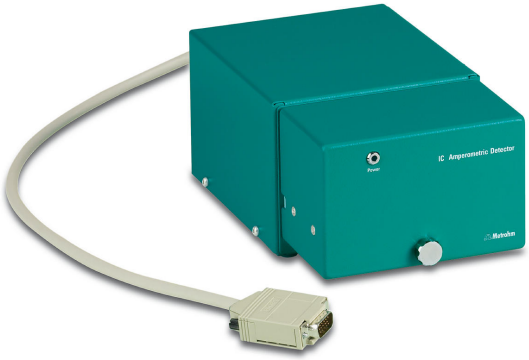
The Metrosep Carb 2 - 250/4.0 IC column is particularly suitable for the determination of carbohydrates using alkaline eluents and pulsed amperometric detection. The high-capacity anion exchanger column is based on a styrene-divinylbenzene copolymer. It is stable in the range of pH = 0 - 14 and separates 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.

IC equipment Wall-Jet cell: Carb (Au, Pd)

Equipment comprised of Wall-Jet cell with additional accessories. For carbohydrate analysis with a gold working electrode and a Pd reference electrode.

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 Amperometric Detector

Compact and intelligent amperometric detector for intelligent IC instruments. Outstanding selectivity due to the four measuring modes: DC, PAD, flexIPAD and CV, as well as the excellent signal/noise ratio and the very fast stabilization of the measuring signal guarantee the highest in measurement precision.

IC equipment: Inline Ultrafiltration 2 - pull mode

Accessory set for Inline Ultrafiltration 2 - pull mode. For use with the 858 Professional Sample Processor/ 919 IC Autosampler plus.

930 Compact IC Flex Oven/Deg

The 930 Compact IC Flex Oven/Deg is the intelligent Compact IC instrument with **column oven**, **without suppression** and with built-in **degasser**. The instrument can be used with any separation and detection methods.

Typical areas of application:

- Anion and cation determinations without suppression with conductivity detection
- Simple applications with UV/VIS or amperometric detection