



Application Note AN-P-089

乳糖不耐症和对准确食品标签的依赖

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.

EXPERIMENTAL

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**.

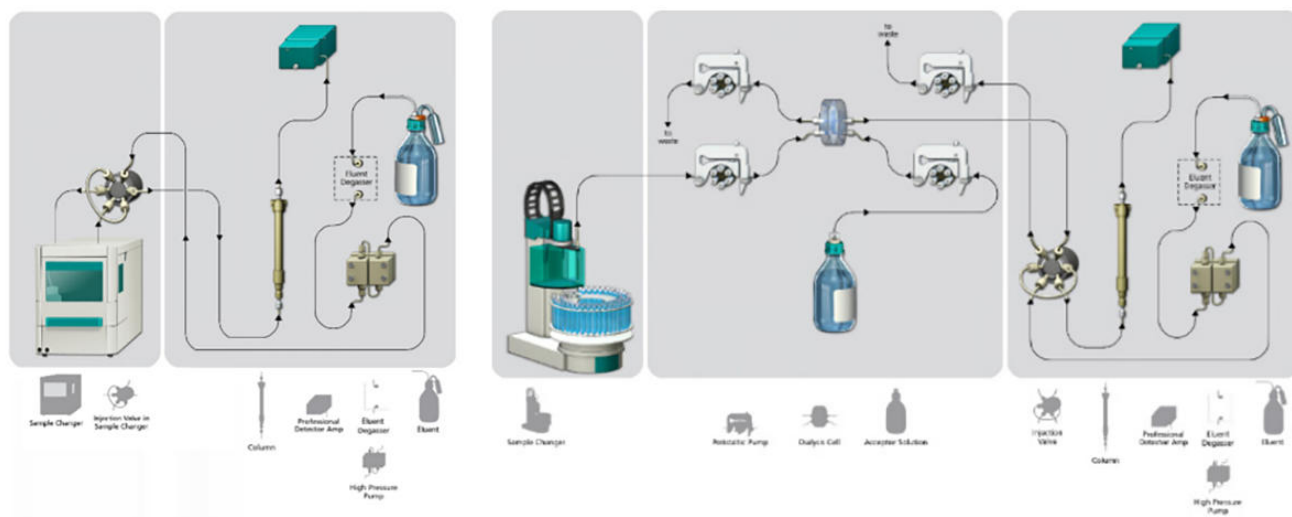


Figure 1. Example system configurations for direct lactose analysis using the Metrohm 889 Sample Center – cool (left). Sample preparation for direct analysis is mandatory as e.g., with Carrez precipitation to protect the analytical system. Inline Dialysis (right) can be added optionally to any existing 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

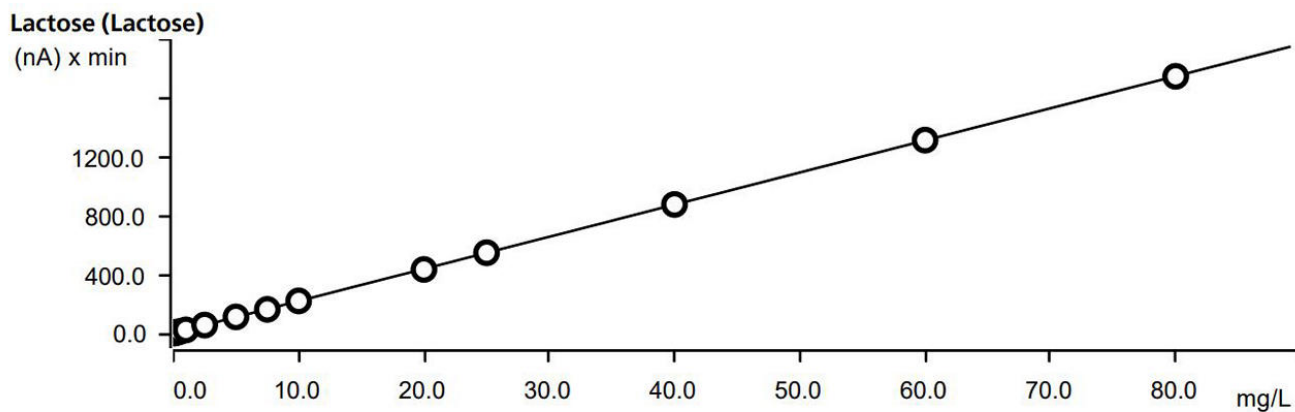
Lactose elutes in less than 30 minutes (**Figure 2–6**). The overall working range of the method is 0.05 to 80 mg/L for liquid lactose standards (**Figure 2 A**), with the ability to analyze samples in a range of 0.2 to 21,000 mg/ 100 g with respective dilution. In contrast to previously published chromatographic 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 ($c(\text{Lactose})_S$ in mg/kg) (**Figure 2 A**) and calculated to determine the final lactose content ($c(\text{Lactose})_{\text{FIN}}$ in g/ 100 g) based on the sample weight:

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

A



Function: $A = 0.0263117 + 2.18736 \times Q$

Relative standard deviation 0.418529 %

Correlation coefficient 0.999996

B

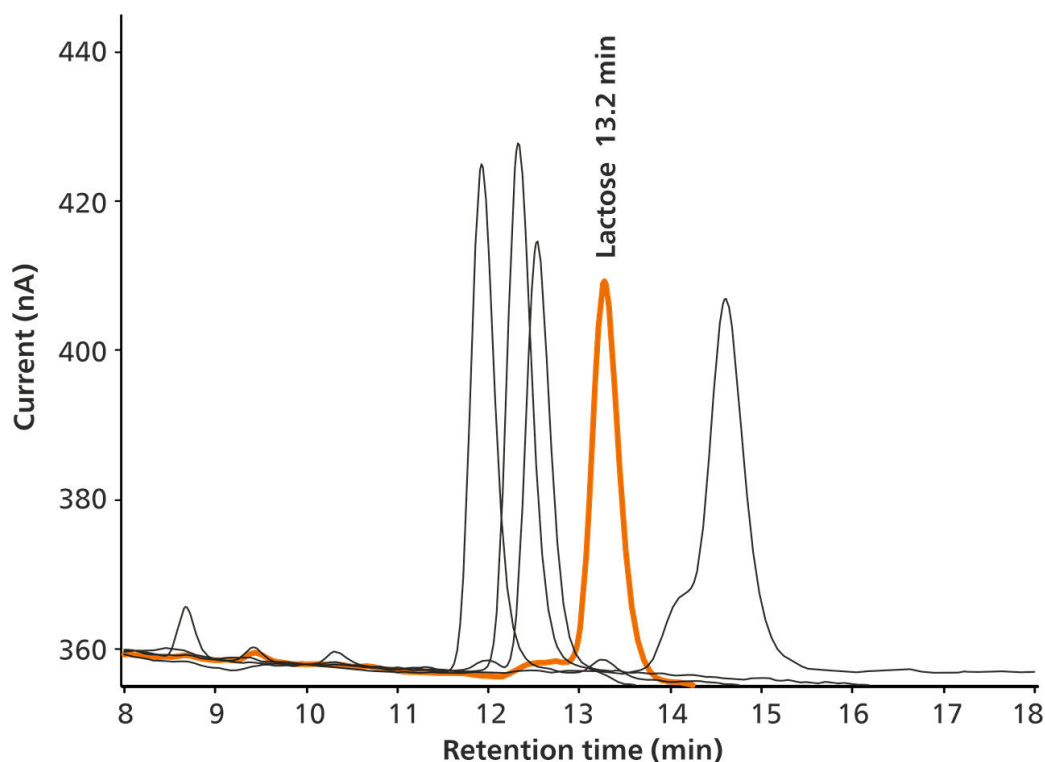


Figure 2. Calibration for lactose (A) showing strong linearity over the concentration range of 0.05 mg/L to 80 mg/L (validation requirement). A proper separation of lactose from interferences is mandatory. Beside other sugars, sugar alcohols, and inorganic ions, it is crucial to separate the structurally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

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 ($RSDs \leq 7\%$), 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 (*).

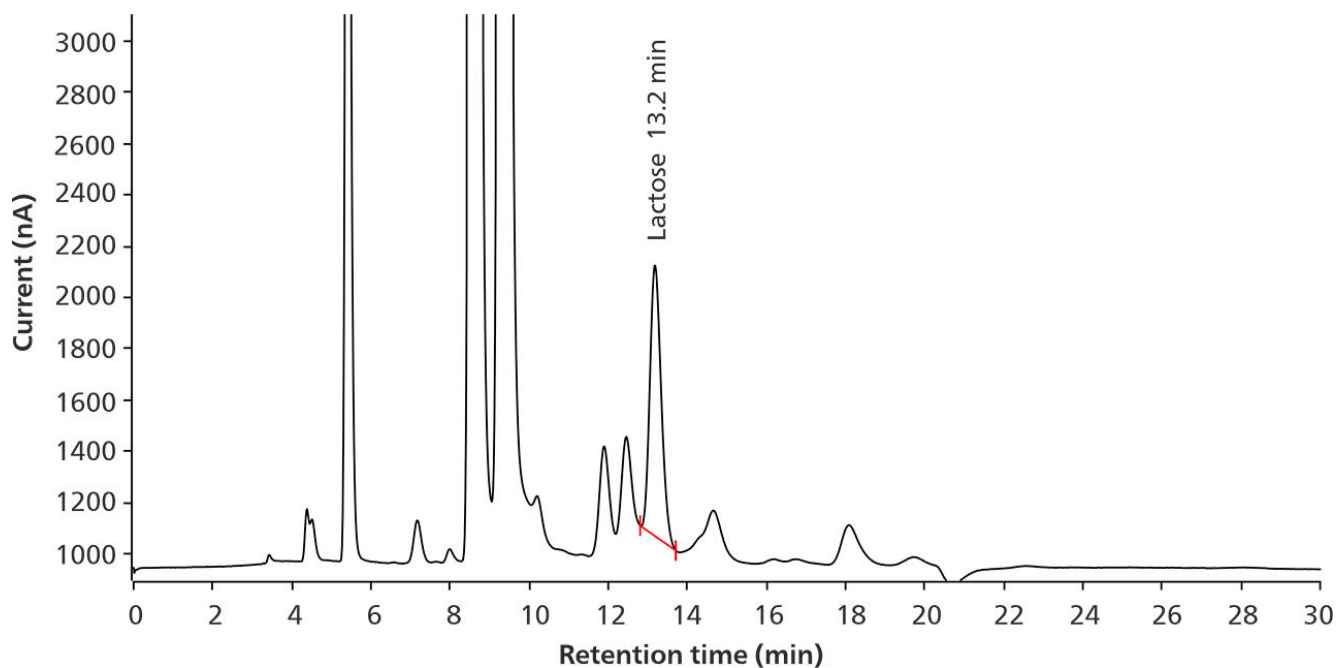


Figure 3. Lactose expressed as lactose monohydrate (conversion factor 1.05 for lactose into lactose monohydrate) in muva ML-2312 (target 217 ± 45 g/ 100 g).

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

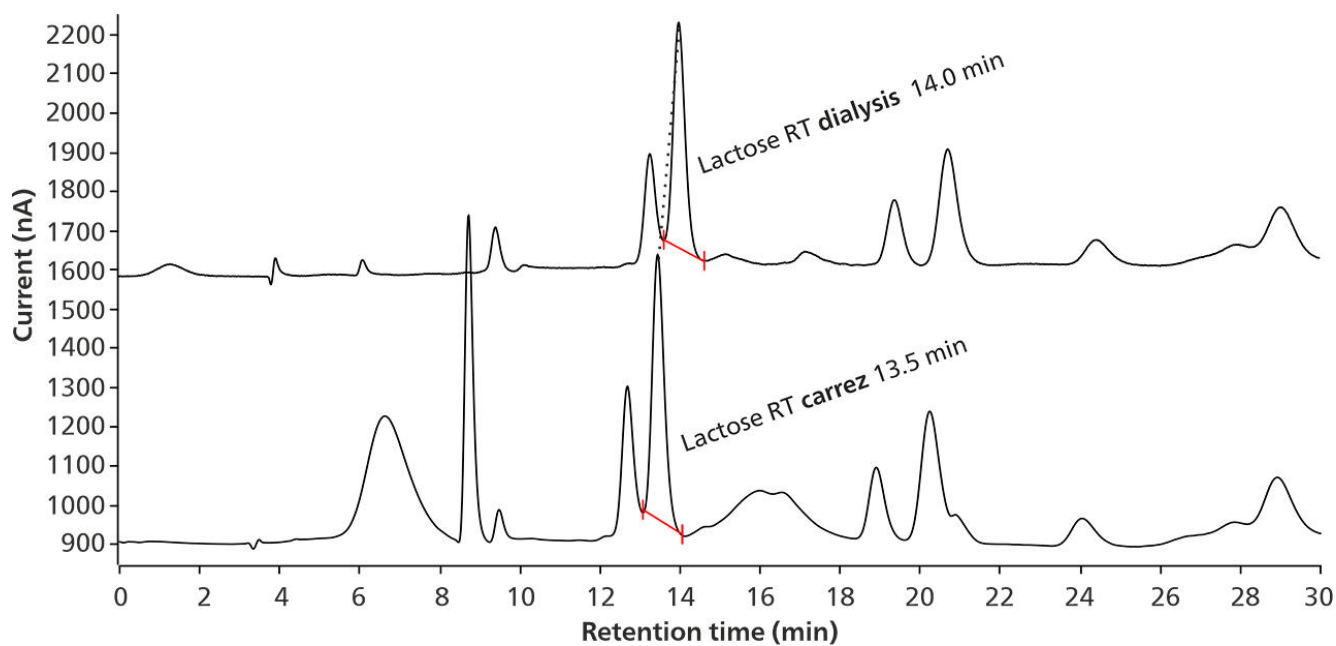


Figure 4. Lactose expressed as lactose monohydrate (conversion factor 1.05 for lactose into lactose monohydrate) in Bimuno daily (Targeted Digestive Nutrition).

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)*	$99 \pm 6^*$	1.9*
11795 ± 130 (1.1)	11807 ± 465 (3.9)	99 ± 5	1.6

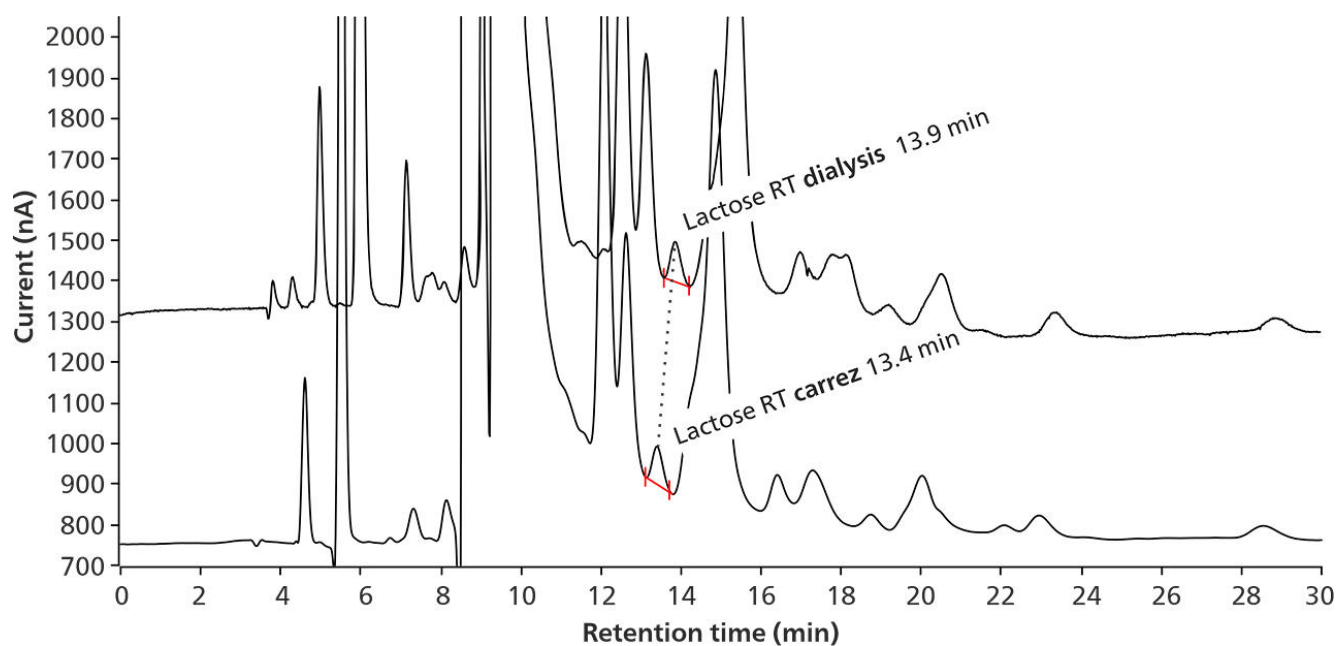


Figure 5. Lactose expressed as lactose monohydrate (conversion factor 1.05 for lactose into lactose monohydrate) in Yogurt, free from, Coop, lactose-free.

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)*	$94 \pm 3^*$	2.6*
4.60 ± 0.15 (3.3)	4.40 ± 0.05 (1.0)	96 ± 3	2.2

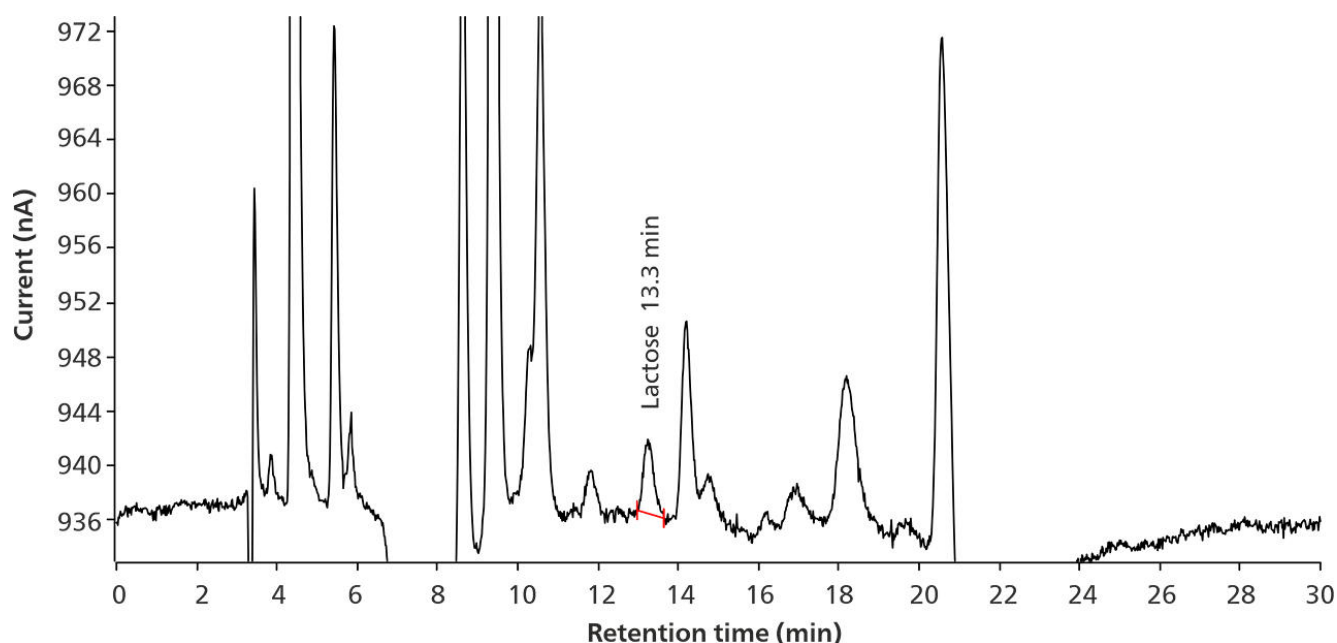


Figure 6. Lactose expressed as lactose monohydrate (conversion factor 1.05 for lactose into lactose monohydrate) in Butter, aha, Spar, lactose-free.

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

CONCLUSION

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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4. Facioni, M. S.; Raspini, B.; Pivari, F.; Dogliotti, E.; Cena, H. Nutritional Management of Lactose Intolerance: The Importance of Diet and Food Labelling. *Journal of Translational Medicine* **2020**, 18 (1), 260. <https://doi.org/10.1186/s12967-020-02429-2>.

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CONFIGURATION



940 Professional IC Vario ONE/Prep 1

940 Professional IC Vario ONE/Prep 1 是智能型 IC 仪器,无抑制,可与万通英蓝样品前处理以及英蓝超滤或英蓝渗析组合使用。该仪器可使用各种分离和检测方法。

典型的应用范围:

- 测定阴离子和阳离子,英蓝超滤或英蓝渗析之后无抑制
- 英蓝超滤或英蓝渗析之后的 UV/VIS 应用
- 英蓝超滤或英蓝渗析之后使用电流检测的应用



Metrosep Carb 2 - 250/4.0

Metrosep Carb 2 - 250/4.0 离子色谱柱特别适用于使用碱性淋洗液测定碳水化合物和脉冲安培检测。该大容量阴离子交换柱基于苯乙烯/二乙烯基苯聚合物。它在 pH = 0 - 14 的范围内较稳定,可分离单糖和双糖。此外还可用于分析糖醇、脱水糖和低聚糖等。250 mm 款型的 Metrosep Carb 2 分离柱极为适用于复杂的分离要求。



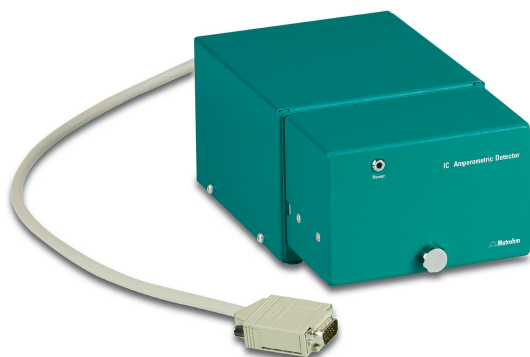
CarbAuPd

装备由喷壁式流通池组成,带有附件。用于碳水化合物分析,带一个金工作电极和一个铂参比电极。



“ ”

用于快速英蓝渗析的附件组。与 858 Professional Sample Processor 和一台附加的 2 通道蠕动泵一同使用。



IC Amperometric Detector

用于智能型离子色谱仪器的紧凑智能型安培检测器。多种可选的不同测量模式:DC、PAD、flexIPAD 和 CV,以及卓越的信号/干扰比例关系和极快进入测量准备就绪状态,这一切确保测量的高度精确性。



2 -

附件组,用于英蓝超滤 2 - 拉模式。用于搭配 858 Professional Sample Processor / 919 IC Autosampler plus 使用。



930 Compact IC Flex Oven/Deg

930 Compact IC Flex Oven/Deg 是智能型带柱加热炉、无抑制的 Compact IC 仪器,并且内置脱气装置。该仪器可使用各种分离和检测方法。

典型的应用范围:

- 阴离子和阳离子测定,无抑制的电导检测
- 使用 UV-VIS 或安培检测的简单应用