



## 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.

## 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 derivatives (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 lactosederivates (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  $217 \pm 45$  g/ 100 g).

| $R_r$ (mg/ 100 g) ( $RSD_r$ %) | $R_{Var}$ (mg/ 100 g) ( $RSD_{Var}$ %) | $R_S$ (%)   | R   |
|--------------------------------|----------------------------------------|-------------|-----|
| $226 \pm 7$ (3.2)              | $228 \pm 12$ (5.4)                     | $102 \pm 3$ | 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 <sub>r</sub> %) | $R_{Var}$ (mg/ 100 g) (RSD <sub>Var</sub> %) | $R_S$ (%)    | R    |
|----------------------------------------|----------------------------------------------|--------------|------|
| $13009 \pm 288$ (2.2)*                 | $13125 \pm 484$ (3.9)*                       | $99 \pm 6^*$ | 1.9* |
| $11795 \pm 130$ (1.1)                  | $11807 \pm 465$ (3.9)                        | $99 \pm 5$   | 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 <sub>r</sub> %) | $R_{Var}$ (mg/ 100 g) (RSD <sub>Var</sub> %) | $R_S$ (%)    | R    |
|----------------------------------------|----------------------------------------------|--------------|------|
| $4.69 \pm 0.18$ (3.9)*                 | $4.88 \pm 0.05$ (2.3)*                       | $94 \pm 3^*$ | 2.6* |
| $4.60 \pm 0.15$ (3.3)                  | $4.40 \pm 0.05$ (1.0)                        | $96 \pm 3$   | 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   |
|--------------------------------|----------------------------------------|-------------|-----|
| $0.40 \pm 0.02$ (5.9)          | $0.39 \pm 0.01$ (2.5)                  | $103 \pm 6$ | 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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## 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



#### 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.



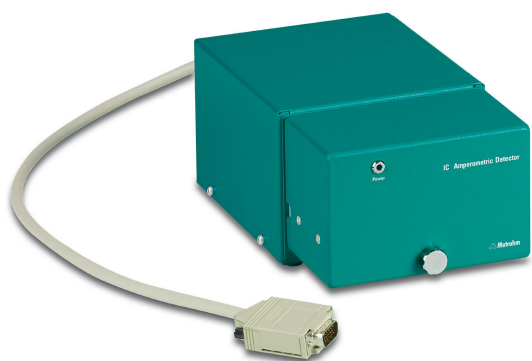
#### Équipement CI cellule Wall-Jet : Carb (Au, Pd)

Équipement comprenant une cellule Wall-Jet avec accessoires en supplément. Pour méthode analytique des hydrates de carbone avec une électrode de travail en or et une électrode de référence en Pd.



#### 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.



#### IC Amperometric Detector

Détecteur ampérométrique compact et intelligent destiné aux appareils CI intelligents. Ses quatre modes de mesure (DC, PAD, flexIPAD et CV) autorisent une excellente sélectivité ainsi qu'un excellent rapport signal/bruit et la grande rapidité de la disponibilité de mesure ; toutes ces caractéristiques garantissent l'extrême précision de la mesure.



### Équipement CI : ultrafiltration inline 2 - mode traction

Jeu d'accessoires pour l'ultrafiltration inline 2 - mode traction. À utiliser avec le 858 Professional Sample Processor / 919 IC Autosampler plus.



### 930 Compact IC Flex Oven/Deg

Le 930 Compact IC Flex Oven/Deg est un appareil CI compact intelligent avec un **four à colonne, sans suppression** et avec un **dégazeur** intégré. 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 avec détection de conductivité
- Applications simples avec UV/VIS ou détection ampérométrique