Application Note

Food, Beverage, Life Sciences

# Analysis of Lactose and isomers in 'Lactose-free' labelled products


- **Fast and sensitive HPAE-PAD analysis**
- **Lactose, lallolactose, epilactose and lactulose**
- **Milk, cheese, yoghurt, cookies and chocolate paste**
- **SenCell™ with Au working electrode**
- **'Green' method**

## Summary

Dairy products play a vital role in a healthy and balanced diet providing essential vitamins and minerals like calcium. Lactose-intolerance is a wide-spread condition, which prevents a large number of people of consuming dairy products as a part of their daily diet. It is estimated that an average of 65% of the global population is suffering from lactose-intolerance [1]. The global market for 'lactose-free' dairy products is rapidly growing and the criteria for 'lactose-free' labelled products are becoming stricter. In the EU for instance the threshold limit for lactose has been lowered to 10 mg per 100 gr product in the last years in a number of EU member states [2]. To check the lactose contents in these products there is a need for fast, sensitive and selective analytical methods.

In this application note a 'green' method is presented for fast and sensitive analysis of lactose, lactose isomers and lactulose using the DECADE Elite electrochemical detector and SenCell. The method is based on separation by High Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAE-PAD). The use of a narrow-bore HPAE column with 4µm particle size, allowed the separation of all compounds of interest in less than 8 min in combination with a four-fold reduction of mobile phase usage. A variety of dairy product were analyzed to demonstrate the versatility of the method. Besides the quantification of low concentrations of Lactose and derivatives also the major sugars in dairy products (Galactose, Glucose, Sucrose and Fructose) can be analyzed using the presented method.

The finest LC-EC Applications for Food & Beverage analysis

**Phenols**
Bisphenol A
Catechins
Flavonoids
Phenols
Antioxidants
Resveratrol
Epicatechin
Quercetin
Other polyphenols

**Carbohydrates**
Monosaccharides
Lactose
Mono- and Disaccharides
Other oligo- and Polysaccharides

**Vitamins, minerals etc.**
A, C, D, E, and K
Iodide
Q10, ubiquinols



## Introduction

Lactose is the main carbohydrate found in milk and dairy products (cow milk contains approximately 5% Lactose). It is a disaccharide composed of the monosaccharides D-glucose and D-galactose, joined in a ß-1,4-glycosidic linkage. In humans lactose is metabolized into glucose and galactose in the intestines by the enzyme lactase. Lactose intolerance is a condition caused by the inability to digest lactose due to a lactase deficiency. The most common symptoms of lactase deficiency are intestinal discomforts such as cramps, diarrhea, bloating, and gas. The reduction of lactase activity in humans starts already at infancy and might develop into a lactose intolerance during adulthood [3]. It is estimated that an average of more than 65% of the global population is suffering from lactose-intolerance. Rates of lactose intolerance vary between regions, from less than 10% in Northern Europe to as high as 95% in parts of Asia and Africa.

The global demand for 'lactose-free' dairy and other food products is rapidly growing and a large amount of commercial 'lactose-free' product are available nowadays. The majority of these products are produced by enzymatic hydrolysis using lactase containing yeast (*Kluyveromyces* strains), resulting in a reduction of lactose concentration up to < 0.01%. Currently there is no legislation in the US and EU with respect to the lactose concentration limits in 'lactose-free' labelled products, except for infant formulae [4]. However, in many EU member states a lactose threshold level of 10 mg per 100 g of product is adopted for 'lactose-free' labelled dairy [2]. To check if products meet these requirements there is a need for fast, sensitive and selective analytical methods to quantify lactose.

Besides Lactose, dairy products can also contain lactose isomers, such as allolactose, epilactose and Lactulose. These isomers can be formed enzymatically [5] or by heat treatment such as pasteurization [6]. Lactulose is a recognized laxative and food additive for digestive comfort. Furthermore, both epilactose and lactulose are considered prebiotic lactose isomers. The presence of these isomers, with their small structural differences, hampers the quantification of such low levels of lactose. Good chromatographic separation is necessary to avoid coelution and thus overestimation of the lactose contents in dairy samples. A wide range of different methods are available to measure lactose in food products based on different techniques (enzymatic assays, Mid Infrared, gravimetry, differential pH, polarimetry and HPLC), most of them are lacking sufficient selectivity and sensitivity [7].

High Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD) is the method of choice. It combines superior selectivity with sensitive detection. Due to the presence of hydroxyl groups which can be oxidized, carbohydrates can be detected without derivatization using pulsed amperometric detection with pico- and femtomol sensitivity [8-10]. Several HPAE-PAD methods for quantification of lactose are reported in literature based on conventional Anion-exchange columns with 6.5—10 µm particle sizes [11,12].

In this application note a 'green' method is presented based on a new type of HPAE column with 4µm particle size for the fast and sensitive analysis of lactose and its isomers.

## Method

The LC-EC conditions are listed in table 1. The analysis was performed using HPLC with a quaternary low-pressure gradient pump, autosampler and thermostatted column compartment. For detection a DECADE Elite electrochemical detector with SenCell flow cell (see figure 1) was used. The DECADE Elite was controlled via a PC using the Antec Dialogue Elite software.

Figure 1: Left: SenCell with Au working electrode and Pd/$H_2$ (HyREF) reference electrode. Right: DECADE Elite electrochemical detector.

**Table 1**

| Conditions | |
|---|---|
| LC system | Quaternary HPLC system |
| Detector | Antec DECADE Elite electrochemical detector |
| Columns | CarboPac PA210G-4µm column, 150 x 2.0 mm<br>CarboPac PA210G-4µm column, 30 x 2.0 mm ID<br>BorateTrap Inline Trap Column, 50 x 4.0 mm ID |
| Mobile phase (MP) | MP A: deionized (DI) water<br>(resistivity > 18 MOhm.cm and TOC<10ppb)<br>MP B: 200 mM KOH<br>Eluents blanketed with Helium 5.0 |
| Flow rate | 0.2 mL/min |
| Injection | 2.5 µL |
| Temperature | 30°C for separation, 35°C for detection |
| Flow cell | SenCell with Au WE, stainless steel AE and HyREF, AST 2 |
| Potential waveform (4-step) | E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V<br>ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s |
| I-cell | about 0.2— 0.4 µA |
| ADF | 0.5 Hz |
| Range | 500 nA/V or 5 µA/V |

**Separation**

Under alkaline conditions (pH > 12) carbohydrates can be separated by means of HPAE. Carbohydrates are weak acids with pKa values ranging between 12 and 14. At high pH they will be either completely or partially ionized depending on their pKa value. Due to the extreme alkaline conditions only polymeric anion-exchange columns are suitable for carbohydrate separation. The retention time of carbohydrates is inversely correlated with pKa value and increases significantly with molecular weight. The New CarboPac™ PA210-4µm column (150 x 2 mm ID) Anion-Exchange column with guard (30 x 2 mm ID) was chosen for the separation of lactose and isomers. This column with small particle size (4µm) and internal diameter (2 mm) combines high-resolution separation with low consumption of mobile phase. A BorateTrap™ inline trap column was installed in the solvent line between pump and autosampler to suppress tailing of the fructose and lactulose peaks due to complexation of these particular sugars with borate.

Table 2

| Step-gradient program | | | |
|---|---|---|---|
| *Time (min)* | *Mobile phase* | *B (%)* | *Description* |
| 0 - 10 | 20 mM KOH | 10 | Isocratic elution and detection |
| 10 - 15 | 100 mM KOH | 50 | Column clean-up and regeneration |
| 15 - 30 | 20 mM KOH | 10 | Equilibration, starting conditions |

The temperature for separation was set at 30°C . The analysis is based on a step-gradient, see Table 2. At a concentration of 20 mM KOH, carbonate ions ($CO3^{2-}$) present in the mobile phase will bind strongly to the active sites of the stationary phase resulting in a loss of retention and column efficiency. A column clean -up /regeneration step after isocratic elution with 200 mM KOH is therefore necessary to remove the bound carbonate ions and late eluting compounds like oligosaccharides present in dairy samples. This regeneration step assures reproducible retention behaviour for each run. The total cycle time for each run is 30 minutes.

For some samples it might be necessary to increase the time of the washing (and/or equilibration) step a bit to avoid late eluting interference in subsequent runs.

Note that all samples shown as example in this application note are analyzed with a wash step of 5 min (table 2). Under this conditions only the chocolate paste sample showed some late eluting interferences in the next run. Therefore, after analysis of this particular sample, a blank run was executed for clean-up.

To minimize the introduction of carbonate ions in the mobile phase the eluents were carefully prepared manually using a carbonate-free 50% w/w KOH solution (commercially available). The diluent was DI water (resistivity >18 MΩcm) which was sonicated and sparged with Helium 5.0 prior to use. The mobile phase should be prepared in plastic bottles instead of glass. NaOH is a strong etching agent and will react with the inner glass wall resulting in the release of silicates and borates. The appropriate amount of 50% w/w KOH solution was carefully pipetting into the diluent under gently stirring and Helium sparging to prepare the required the mobile phase solutions. The bottles with mobile phase and column clean-up solution were blanketed with Helium (0.5 bar overpressure) during the analysis to minimize the build-up of carbonate ions in the mobile phase and to assure a reproducible analysis.

**Detection**

For the pulsed amperometric detection of Lactose and isomers the Antec SenCell electrochemical flow cell is used. This novel flow cell [14] has a confined wall-jet design and consists of a Au working electrode (WE), HyREF (Pd/ $H_2$) reference electrode (RE) and stainless steel auxiliary electrode (AE). A 4-step potential waveform was applied as shown in figure 2. The temperature for detection was set to 35°C. The cell current was typical about 0.2—0.4 µA with these PAD settings under the specified conditions. This particular 4-step waveform with a pulse dura-

tion of 500 ms has been claimed to have as benefits: (1) a consistent long-term peak area response and (2) minimal electrode wear [15], resulting in less flow cell maintenance and system down time.

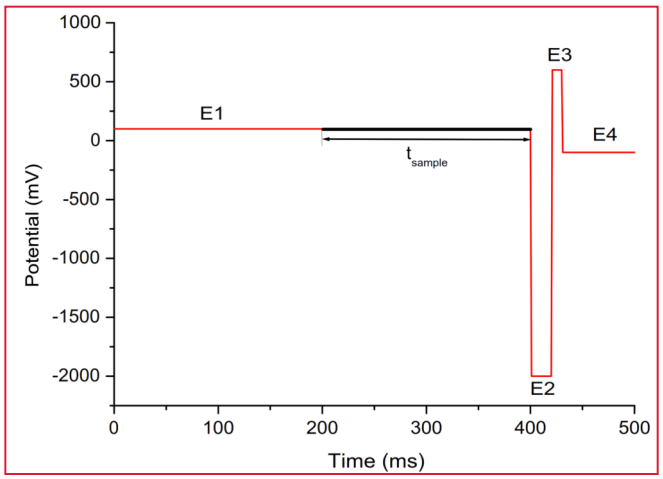


Figure 2: 4-step PAD potential waveform for the detection monosaccharides and other carbohydrates.

**Preparation of standards, reagents and samples**

Standards: 10 mM stock standards of the individual sugars were prepared in 95/5 (v/v%) water/acetonitrile. To prevent fast degradation 5% Acetonitrile was added to suppress bacterial and fungal growth. Stock standards under these conditions are stable for more than a month in the fridge at 4°C. Working standards in the concentration range of 100 nM—100 µM were prepared by dilution of the stock standards with DI water.

Carrez reagents: a Carrez clarification procedure is used for deproteination of the dairy samples. By removing all proteins also the enzymatic activity will be quenched, eliminating any unwanted conversion of the sugars inside the sample during the analysis process. The Carrez I solution was prepared by dissolving 15.0 g potassiumhexacyanoferrate(II) trihydrate in 100 mL of DI water in a volumetric flask. The Carrez II solution was prepared by dissolving 30.0 g zinc sulfate heptahydrate in 100 mL of water in a volumetric flask. Both Carrez solutions were filtered over a 0.2 µM syringe filter prior to use.

Sample preparation: The following lactose-free products were prepared and analyzed using the method described below:

- Semi-skimmed milk
- Cream cheese
- Low-fat yoghurt

Procedure:

1. 0.5 gram of dairy sample was weighted in a 50 mL volumetric flask and 10 mL DI water added.
2. Subsequently, 100 µL Carrez I and 100 µL Carrez II reagent was added (the solution was shortly vortexed after each addition of reagent). Followed by addition of DI water up to a total volume of 50 mL.
3. The solution was allowed to stand for 30 minutes and vortexed again to obtain a homogeneously turbid solution.
4. A few milliliters of the turbid sample solution was centrifuged in 2 mL Eppendorf vials at 6000 RPM for 15 minutes.
5. The supernatant was collected in a plastic 5 mL syringe and filtered over a 0.20 µm PE (Polyethersulfone) syringe filter.
6. 2.5 µL of the filtered supernatant was injected into the LC system and analyzed.

For the following dairy products a slightly modified procedure was used in step 1:

- Matured cheese
- Chocolate paste
- Chocolate-covered rice cake

1. 0.5 gram of (crushed) sample was weighted in a 50 mL disposable centrifuge tube (with cap) and 10 mL DI water added. The tubes with sample solutions were vortexed and transferred in a water bath of 65°C and heated for 20 minutes. During the heating process the tubes were taken out of the bath a few time and vortexed shortly to assure optimal dissolution and mixing.

Subsequently, step 2—6 from the procedure described for yoghurt, cheese and milk samples were followed.

## Results

In Figure 3 an overlay is shown of two chromatograms obtained with a 2.5 µL injection of a 10 µM standard mix containing the following sugars: fucose, arabinose, galactose, glucose, sucrose, fructose, allolactose, lactose, lactulose and epilactose. In the standard mix shown in the top chromatogram (red curve) also raffinose was present. Raffinose might be found in whole grain and cacao products. A concentration of 10 µM corresponds to 3.4 mg/L (ppm) of lactose and lactose isomers. All compounds of interest eluted within 8 minutes, and the total run cycle time is 30 minutes due to the wash and equili-

bration step. The analysis time is at least two times shorter than reported with conventional HPAE columns [11,12], resulting in a significant improvement in sample throughput. The retention time of Lactose was approximately 5.6 minutes. Not all sugars are completely baseline separated (resolution < 1.5), but under these conditions reliable quantification of lactose is still possible of the two raffinose containing samples shown as example in this application note. The elution order of Raffinose , between lactose and lactulose, deviates from the method reported in reference [13] in which a column was used with the same stationary phase.

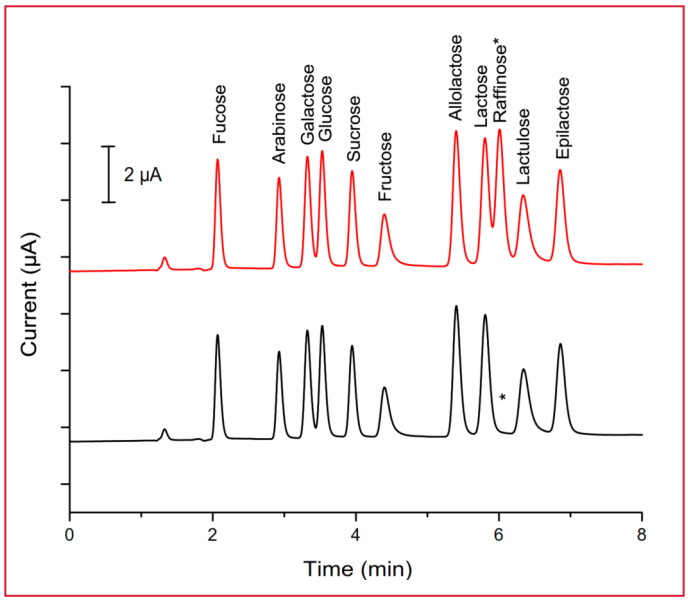


Figure 3: Two chromatogram of a 2.5 µL injection 10 µM sugar standard mix. (1) Fucose, (2) Arabinose, (3) Galactose, (4) Glucose, (5) Sucrose, (6) Fructose, (7) Allolactose, (8) Lactose, (9) Raffinose (10) Lactulose and (11) Epilactose. *) Raffinose only present in the mix of the top chromatogram (in red).

The peak efficiencies found for the sugars ranged from 20.000 to 85.000 theoretical plates/meter (fucose and epilactose, respectively). Fructose and lactulose showed slight tailing with a tailing factor between 1.7 - 1.8.

**Linearity**

The linearity was investigated in the concentration range of 100 nM - 100 µmol/L for all 11 sugars shown in figure 3. This corresponds with a concentration range of 34 µg/L - 34 mg/L for lactose and its isomers. In this concentration range the linearity is excellent and correlation coefficients for peak area were better than 0.999 for all sugars.

The calibration curves for lactose and the lactose isomers, used for the actual quantification of samples, are shown in figure 4

The linear correlation coefficient was 0.9999 or better for all 4 sugars. The calibration curve (0.25— 20 mg/L) lays within the expected concentration range of lactose in the samples. During sample preparation the dairy product is 100 x diluted. Therefore, a concentration of 10 mg lactose in 100 gram dairy product (upper limit for lactose in 'lactose-free' products) corresponds to a concentration of 1 mg/L (ppm) lactose in the samples actually injected into the LC system.

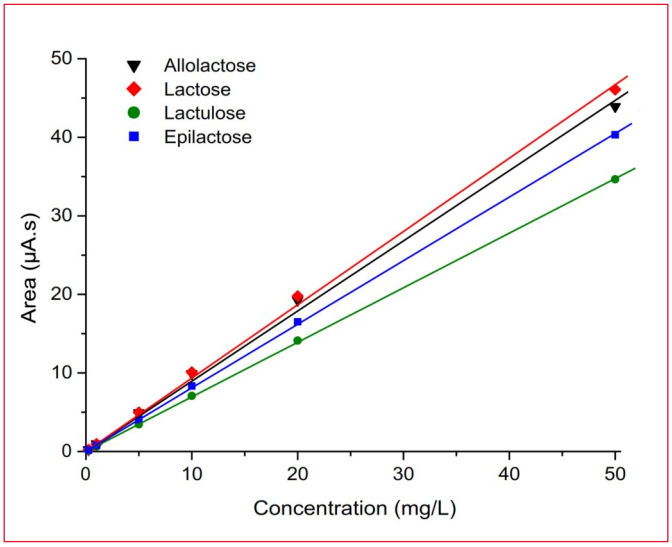


Figure 4: Calibration curve of Allolactose, Lactose, Lactulose and Epilactose in the concentration range of 0.25 - 20 mg/L.

**Repeatability**

The repeatability of the method and system was evaluated by repetitive injections with a 0.1, 1 and 10 µM sugar standard (mix of 10 sugars), corresponding with a concentration of 0.34, 3.4 and 34 mg/L for lactose and the isomers, respectively.

**Table 3**

Repeatability of 2.5 µL injections of a 10, 1 and 0.1 µM sugar standard mix in water (n=10)

| | *RSD's (%) 10 µmol/L* | | *RSD's (%) 1 µmol/L* | | *RSD's (%)* 0.1 µmol/L* | |
|---|---|---|---|---|---|---|
| *Compound* | $t_R$ | *Area* | $t_R$ | *Area* | $t_R$ | *Area* |
| Fucose | 0.04 | 0.59 | 0.07 | 1.27 | 0.08 | 1.70 |
| Arabinose | 0.06 | 0.32 | 0.08 | 1.53 | 0.09 | 1.70 |
| Galactose | 0.06 | 0.23 | 0.08 | 0.30 | 0.12 | 1.52 |
| Glucose | 0.07 | 0.21 | 0.10 | 0.37 | 0.12 | 1.63 |
| Sucrose | 0.10 | 0.22 | 0.10 | 0.57 | 0.14 | 0.86 |
| Fructose | 0.08 | 0.20 | 0.11 | 1.12 | 0.17 | 2.22 |
| Allolactose | 0.11 | 0.22 | 0.12 | 0.36 | 0.16 | 1.04 |
| Lactose | 0.11 | 0.21 | 0.12 | 0.53 | 0.15 | 1.60 |
| Lactulose | 0.11 | 0.22 | 0.13 | 0.84 | 0.12 | 1.67 |
| Epilactose | 0.12 | 0.19 | 0.15 | 0.68 | 0.17 | 1.69 |

*) n=5 for the repeatability test with the 0.1 µmol/L standard.



The relative standard deviations (RSD) for retention time and peak area for the different concentrations of sugar standards are listed in table 3. The good repeatability of the method is evident from the low RSD values obtained. RSD's for retention time were < 0.2%. For peak area the RSD's were < 1% for all sugars in the 10 µmol/L standard and < 2% for most of the sugars in the 100 nmol/L standard. These data demonstrate that with this method reproducible analysis of lactose and related sugars can be achieved.

**LOD and LOQ**

The Limit of Detection (LOD) for all sugars are shown in table 4 in mg/L (ppm) and molar concentrations. The LOD's were calculated as the analyte response corresponding to 3x the ASTM noise (average peak-to-peak baseline noise of 10 segments of 0.5 min). The noise was calculated based on a 5 minute section of the baseline close to the peaks of interest. The average responses of 5 replicate injections obtained with a 100 nmol/L standard mix in the 500 nA/V range were used to calculate the LOD's for all sugars.

**Table 4**

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

| | *Limit of detection (LOD)* | | *Limit of Quantification (LOQ)* |
|---|---|---|---|
| *Compound* | *mg/L (ppm)* | *nmol/L* | *mg/L (ppm)* |
| Fucose | 0.001 | 9 | 0.005 |
| Arabinose | 0.002 | 10 | 0.005 |
| Galactose | 0.001 | 8 | 0.005 |
| Glucose | 0.001 | 7 | 0.004 |
| Sucrose | 0.003 | 9 | 0.010 |
| Fructose | 0.003 | 19 | 0.012 |
| Allolactose | 0.002 | 7 | 0.008 |
| Lactose | 0.002 | 7 | 0.008 |
| Lactulose | 0.005 | 14 | 0.016 |
| Epilactose | 0.003 | 10 | 0.011 |

The excellent sensitivity of the method is evident from table 4. Detection limits around 10 nmol/L are attainable for most of the sugars. The calculated LOQ (10x S/N) for lactose is approximately 0.010 mg/L, which is a factor 100 below the upper limit of the lactose concentration expected in samples of 'lactose-free' labelled products.

**Sample analysis**

In total 6 commercially available 'Lactose-free' labelled products were purchased from supermarkets in the Netherlands and analyzed using the presented method, see table 5.

Table 5

'Lactose-free' labelled products

| *Product* | *Lactose content on product label* |
|---|---|
| Semi-skimmed milk UHT* | Lactose < 10 mg / 100 mL |
| Cream cheese | Lactose < 10 mg / 100 g |
| Low-fat yoghurt | Lactose < 10 mg / 100 g |
| Matured cheese | Lactose < 10 mg / 100 g |
| Chocolate paste | Lactose < 100 mg / 100 g |
| Chocolate-covered rice cake | Lactose < 100 mg / 100 g |

*) Ultra Hight Temperature sterilization.

The series of products include dairy, chocolate paste and chocolate-covered cookies. The lactose contents specified on the product labels ranged from < 10 mg to < 100 mg Lactose per 100 gr or mL of product. All samples were prepared following the sample preparation procedure described in the method section. The contents of lactose and lactose isomers in the samples was determined in two different ways, using:

- Calibration curve based on standards (0.25 - 20 mg/L)
- Standard addition method

The quantification with the standard addition method was based on a single point calibration by spiking the sample in the first dilution step during sample preparation with a known amount of a standard containing allolactose, lactose, lactulose and epilactose. The spike concentration was 3.3 µM (1.1 mg/L) for all 4 components in the final sample.

By using standard addition the method accuracy could be assessed, by calculating the sample recovery based on the responses of the analytes in the sample, spiked sample and 3.3 µM standard.

$$\text{Recovery (\%)} = \frac{\text{Area}_{\text{spiked sample}} - \text{Area}_{\text{sample}}}{\text{Area}_{\text{standard}}} * 100\%$$

The chromatograms of the samples are shown in figure 5 - 10. Every figure contains an overlay of chromatograms of the sample (red), spiked sample (black) and the 3.3 µM standard (grey).

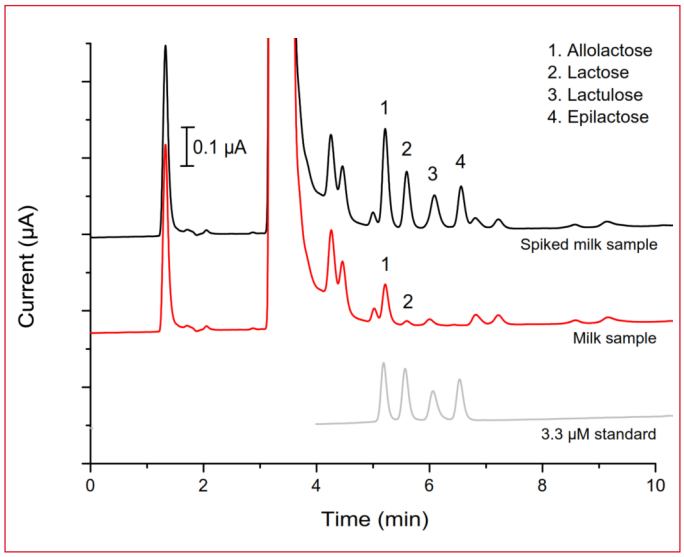


Figure 5: Chromatogram of the milk sample (red), spiked milk sample (black) and 3.3 µM standard of lactose and isomers (grey).

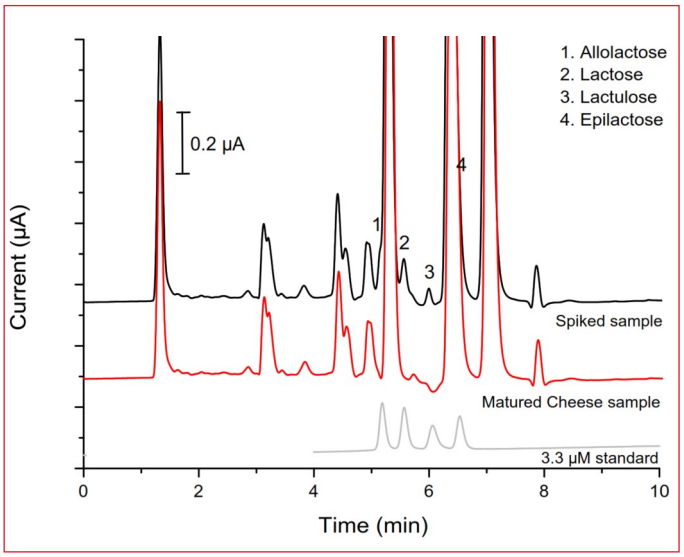


Figure 8: Chromatogram of the matured cheese sample (red), spiked matured cheese sample (black) and 3.3 µM standard (grey).

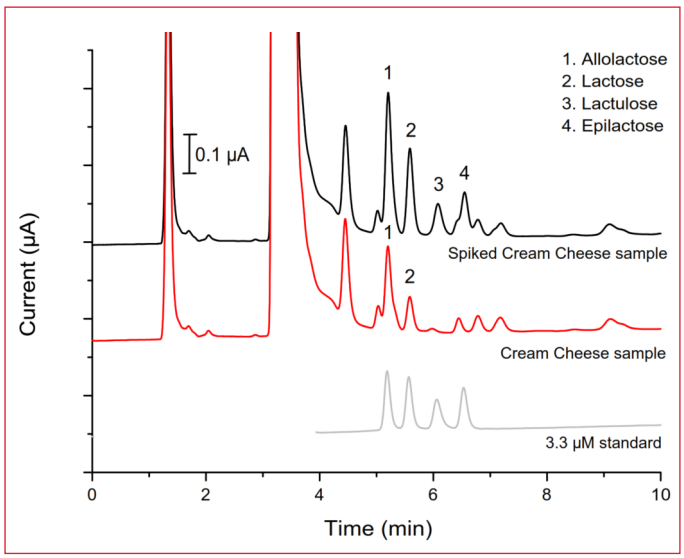


Figure 6: Chromatogram of the cream cheese sample (red), spiked cream cheese sample (black) and 3.3 µM standard (grey).

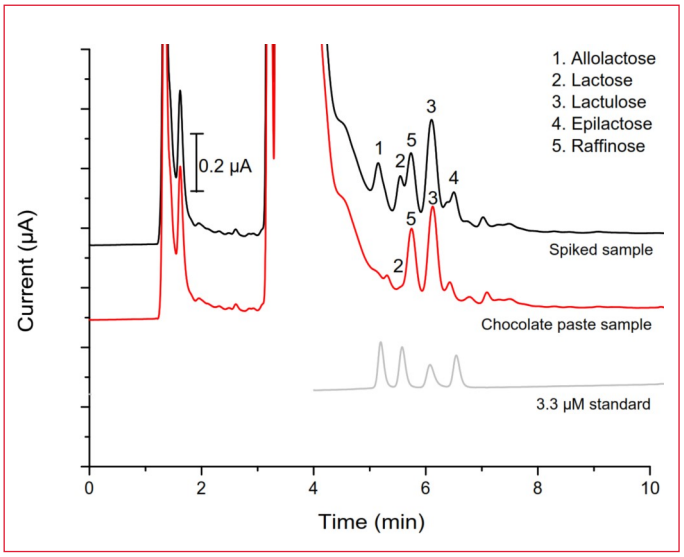


Figure 9: Chromatogram of the chocolate paste sample (red), spiked chocolate paste sample (black) and 3.3 µM standard (grey).

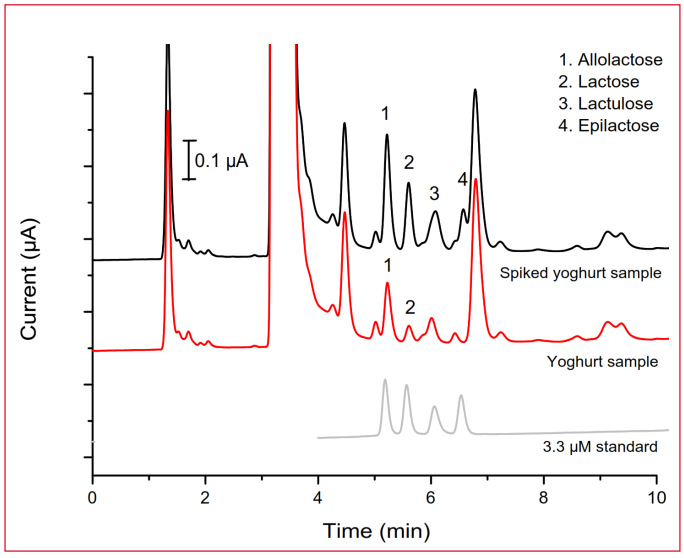


Figure 7: Chromatogram of the yoghurt sample (red), spiked yoghurt sample (black) and 3.3 µM standard (grey).

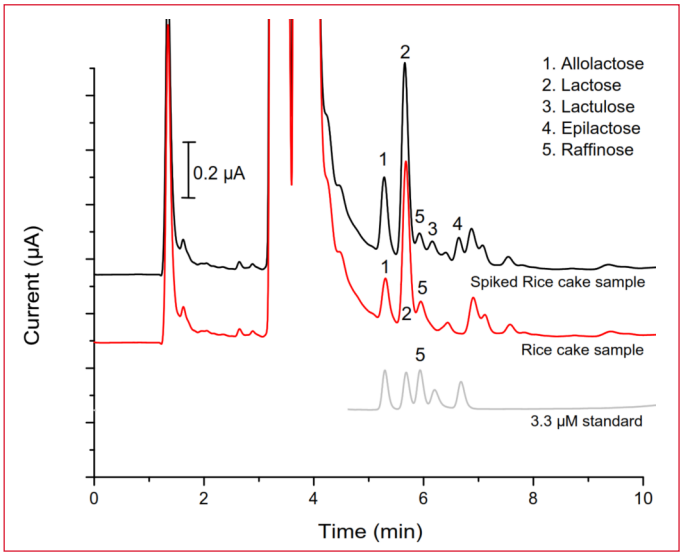


Figure 10: Chromatogram of the chocolate-covered rice cake sample (red), spiked rice cake sample (black) and 3.3 µM standard (grey).

The chromatograms in red, shown in figure 5-10, represent the unspiked samples. The lactose-related compounds present in the specific samples are marked with a number above the peaks, corresponding with the legend in the top right corner of the plot.

In none of the analyzed samples epilactose was found and lactulose was present in the chocolate paste sample only (figure 9). The concentration of lactulose in the sample was calculated based on standard addition and corresponds to 51 mg lactulose per 100 gram product (recovery 91.3%). The yoghurt sample shows an unidentified interference which coelutes closely to lactulose. The matured cheese (figure 8) did not contain a detectable amount of lactose or any of the other lactose isomers. Cheese is a fermented product and during fermentation lactose is converted into lactic acid.

In both the chocolate paste and chocolate-covered rice cake sample raffinose was present. Despite the presence of Raffinose it was possible to quantify the amount of lactose in the samples with sufficient accuracy. For lactose and allolactose , which are present in the majority of the 'lactose-free' products, the amounts found in the analyzed samples are listed in table 6.

**Table 6**

Lactose & allolactose, contents and sample recovery

| *Product* | *Lactose* | | *Allolactose* | |
|---|---|---|---|---|
| | *mg per 100 g product* | *Recovery (%)* | *mg per 100 g product* | *Recovery (%)* |
| Semi-skimmed milk UHT | 0.7 | 95.6 | 7.5 | 96.8 |
| Cream cheese | 6.7 | 90.5 | 17.6 | 94.4 |
| Low-fat yoghurt | 3.3 | 91.7 | 12.2 | 95.9 |
| Matured cheese | - | 94.6 | - | -* |
| Chocolate paste | 0.6 | 85.9 | - | 98.0 |
| Chocolate-covered rice cake | 60.5 | 87.1 | 13.9 | 95.2 |

*) recovery could not be calculated due to coeluting interference.

The concentrations shown in table 6 were calculated using the the standard addition method (single point calibration). There was a good correlation between the values in table 6 and the the concentrations calculated based on the calibration curve. The sample recovery (see table 6) found for lactose, ranged between 86% - 96%.

The lactose contents in all the 'lactose-free' products analyzed with the presented HPAE-EC method, fall within the specified limit of < 10 mg/100 g (dairy products) or < 100 mg/100 g (Chocolate paste, Chocolate-covered rice cake) listed on the product labels.

**Analysis of glucose, galactose and other sugars**

Although, the main focus in this application note is quantification of lactose and isomers, the method is also suitable to assess the contents of the other sugars present in dairy products (see figure 3). For accurate quantification of high abundant sugars it might be necessary to dilute the sample. As an example the milk sample shown in figure 5 was diluted 10x more to to get the response of Galactose en Glucose in range for quantification. The resulting chromatogram is shown in figure 11.

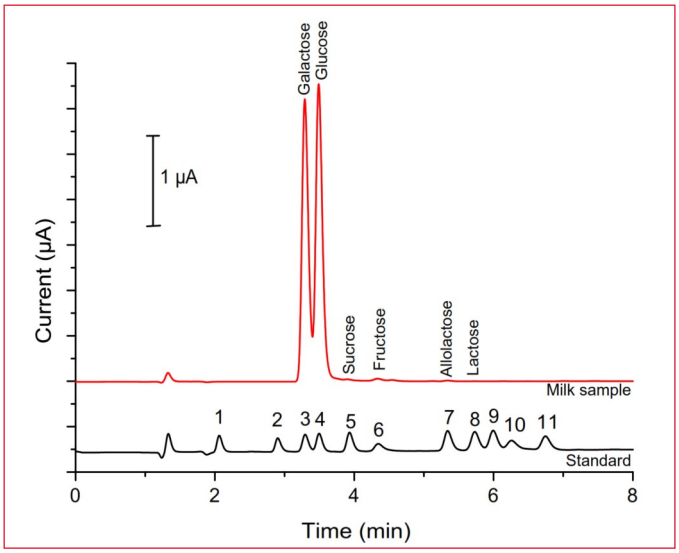


Figure 11: Chromatogram of a 10 x dilution of the worked-up milk shown in figure 5 (red) and a standard sugar mix for reference (black). See figure 3 for the legend of the sugars in the standard.

**Table 7**

Contents of sugars in milk sample

| *Sugar* | *Contents (mg / 100 g)* |
|---|---|
| Galactose | 1451 |
| Glucose | 1685 |
| Sucrose | 4.6 |
| Fructose | 22.9 |
| Allolactose | 7.5 |
| Lactose | 0.7 |
| *Total* | 3172 |

The sugars detected in the milk sample are tagged in the chromatogram. The calculated concentrations of the sugars in the sample are listed in table 7. It is evident that galactose and glucose, which are formed due to hydrolysis of lactose are the main sugar constituents in the 'lactose-free' milk product. The total amount of sugar found (3.2 g/100 g) is in agreement with the product label (an average sugar contents of 3.0 g/100 g).

## References


1. T.M Bayless, et al. , Lactase Non-persistence and Lactose Intolerance, Curr Gastroenterol Rep, 19 (2017) , 23.
2. ESFA panel, Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia, EFSA Journal , 8(9) (2010), 1777 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2010.177
3. National Institute of Health, Lactose-intolerance, https://ghr.nlm.nih.gov/condition/lactose-intolerance
4. EU directive 2006/141/EC on infant formulae, L401/1
5. E. Rentschler et al., Enzymatic production of lactulose and epilactose in milk, J Dairy Sci., 98(2015), 6767
6. A. Olano et al., Kinetics of lactulose, galactose and epilactose formation during heat-treatment of milk, Food Chemistry, 34, (1989), 239
7. L.M.L. Nollet, F.Toldra, Handbook of Dairy Foods Analysis, CRC press, Boca Raton, 2010, chapter 6 & page 397
8. D.C. Johnson, D. Dobberpuhl, R. Roberts, P. Vandeberg, Review: Pulsed amperometric detection of carbohydrates, amines and sulfur species in ion chromatography- the current state of research, J. Chromatogr., 640 (1993), 79 -96
9. D.C. Johnson, W.R. LaCourse, LC with pulsed ECD at gold and platinum, Anal. Chem., 62 (1990), 589A – 597
10. W.R. LaCourse, Pulsed Electrochemical Detection in High Performance Liquid Chromatography, John Wiley & Sons, New York, 1ed, 1997
11. W.B. van Scheppingen et al., Selective and sensitive determination of lactose in low-lactose dairy products with HPAEC-PAD, J. Chromatogr. B, 1060 (2017), 395
12. L. Monti et al., Lactose, galactose and glucose determination in naturally “lactose free” hard cheese: HPAEC-PAD method validation, Food Chemistry, 220 (2017), 18
13. M. Aggrawal, J. Rohrer, Fast and sensitive determination of Lactose in Lactose-free products using HPAE-PAD, Thermo Application note 72632 (2018)
14. H. Louw, H.J. Brouwer, N. Reinhoud, Electrochemical flowcell, (2016) US patent 9310330
15. R.D. Rocklin, A.P. Clarke, M. Weitzhandler, Improved long-term reproducibility for pulsed amperometric detection of carbohydrates via a new quadruple-potential waveform, Anal. Chem, 70 (1998), 1496 – 1501


## Conclusion

The DECADE Elite detector, in combination with the SenCell flow cell and a narrow-bore ‘fast-4µm’ HPAE column, offers a fast and sensitive analysis solution for the quantification of lactose and isomers in commercial Lactose-free products. The presented HPAEC-PAD method allows fast separation (within 8 min) of Lactose and isomers. Besides the quantification of low concentrations of lactose also the major sugars in dairy products (Galactose, Glucose, Sucrose and Fructose) can be analyzed using the presented method. A four-fold reduction of mobile phase usage was achieved by using a 2 mm ID column instead of a standard bore version (4 mm ID).



## Ordering information

Below all part numbers related to the DECADE Elite detector, and SenCell are listed.

| Ordering information | |
|---|---|
| 176.0035A | DECADE Elite SCC electrochemical detector |
| 116.4321 | SenCell 2 mm Au HyREF |
| | |
| 047078* | BorateTrap Inline Trap Column, 50 x 4.0 mm ID |
| 088954* | CarboPac PA210G-4µm analytical column, 150 x 2.0 mm ID |
| 088956* | CarboPac PA210G-4µm guard column, 30 x 2.0 mm ID |

*) The CarboPac and Borate trap columns used in this application are manufactured and sold by Thermo Scientific (https://www.thermofisher.com). CarboPac and BorateTrap are trademarks of Thermo Scientific.

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