KNAUER

Application Note

► Sensitive analysis of the lactose content of lactose-free labelled products using HPAEC-PAD

Category	Food analysis
Matrix	Extracts from various food products
Method	HPAEC-PAD
Keywords	Lactose free
Analytes	Lactose
ID	**VFD0137N**, 10/15

Summary

The market for lactose-free products is growing rapidly and constantly and Europe is a worldwide leader in the lactose-free market. Between 2012 and 2016 the sales of lactose-free products are expected to increase by 75 %.[1,2] Studies additionally state that customers who are lactose intolerant or believe they are, will pay a big premium for the right product.[1] From these statements it becomes obvious that a huge demand for lactose-free products is existing in the industry.
An HPLC method that easily reaches the required LOD by using High-Performance Anion-Exchange Chromatography coupled to pulsed amperometric detection (HPAEC-PAD) on a KNAUER AZURA® Analytical HPLC system coupled to the DECADE II electrochemical detector is presented. Besides lactose, also sucrose and glucose were analyzed to prove the method´s ability to differentiate several sugars.

Introduction

Generally, lactose intolerance is the inability to digest lactose caused by the deficiency in the enzyme lactase which hydrolyzes lactose into glucose and galactose. More than 65 % of the world´s population loses the ability to completely digest lactose after infancy what is called primary or late onset lactose intolerance.[3] Reduction in lactase activity is rarely total but decreases to 10 – 30 % of the initial level between the ages 5 and 20.[4] Additionally, secondary and developmental lactose intolerance occur and it can be stated that lactose intolerance is an important subject worldwide.
Although in European countries like Sweden and Finland for example, lactose tolerance levels of 74 % and 82 % are widespread, the market for lactose-free products is growing and the regulations are getting harder.[5]
In many European countries, the limit of lactose in lactose-free labeled products was decreased from former 100 to now 10 mg/100g product in the last years.[6] This makes an HPLC method with low detection limits inevitable for the quality control of these products. Special methods and systems are needed because classical determination of sugars in food products is far too insensitive in this special case. The lactose content of food products can generally be determined in several ways. Validated methods do exist for enzymatic essays, polarimetry, gravimetry, differential pH and HPLC.

Today, HPLC is the method of choice when sugar contents in dairy products have to be analyzed because it is a highly specific method with the ability to differentiate other sugars. The typically used and validated method is HPLC on an ion exclusion column coupled to RI detection. But in the special case of lactose analysis in lactose-free products, this method is far too insensitive. Therefore, special methods have to be applied to reach the wanted low detection limit of 10 mg/100 g sample

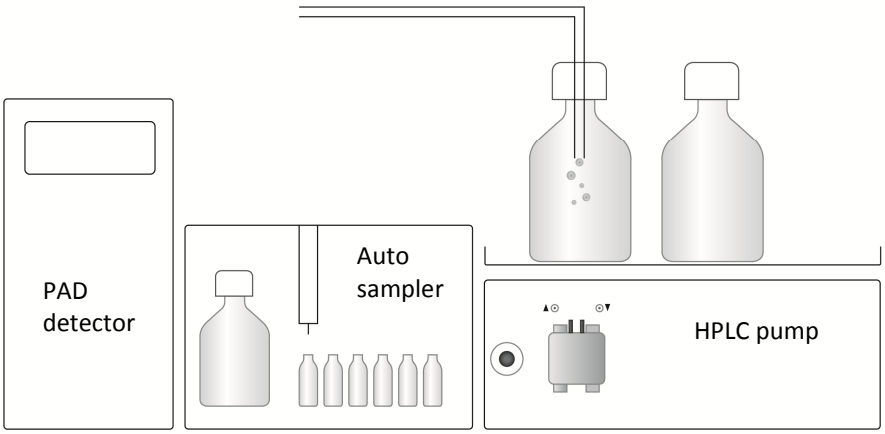


Fig. 1

System for the sensitive analysis of lactose

Experimental sample preparation

Samples from different nondairy food products were extracted using various extraction protocols, filtered and injected to the HPLC system.

Experimental preparation of standard solution

Standards of lactose, sucrose and glucose were weighed in and dissolved in deionized water. They were afterwards diluted in deionized water to reach low concentrations down to less than 100 µg/L lactose. The allowed content of lactose in lactose-free products from 10 mg/100 g food sample has to be reached, taking into account that the sample preparation involves an additional sample dilution and no 100 % lactose recovery depending on the food product. Therewith, the target LOD was set to < 10 mg/L.

Method parameters

The HPLC analysis was performed using a KNAUER AZURA Analytical HPLC system consisting of an isocratic high pressure pump P 6.1L in the metal free ceramic edition, an autosampler 3950 and the DECADE II electrochemical detector. The mobile phase was continuously sparged with helium to keep it inert. A schematic drawing of the system can be seen in Fig. 1. The applied anion exchange column is stored in the tempered section of the DECADE II detector.
The system was flushed and allowed to equilibrate overnight because the applied method is really sensitive to any changes. A sufficient equilibration time is recommended especially when the system is running this method for the first time.

Method parameters

Column	RCX-10, 7 µm, 250 x 4.6 mm, PEEK hardware
Column Order No.	25EE158HML
Eluent A	30 mM NaOH continuously sparged with helium
Gradient	Isocratic
Flow rate	2 ml/min
Injection volume	50 µL
Temperature	30 °C (column and flow cell)
System pressure	approx. 120 bar
Detection	ECD (electrochemical detection) E cell: E1, E2, E3: 0.05, 0.75, -0.80 Volts ts, t1, t2, t3: 0.06, 0.5, 0.13, 0.12 seconds I-cell: 300 – 500 nA

Results

Figure 2 shows an overlay of four chromatograms measured with the described method. It is obvious that a separation of the three applied sugars is easily possible. Additionally, really low lactose concentrations could still be detected.

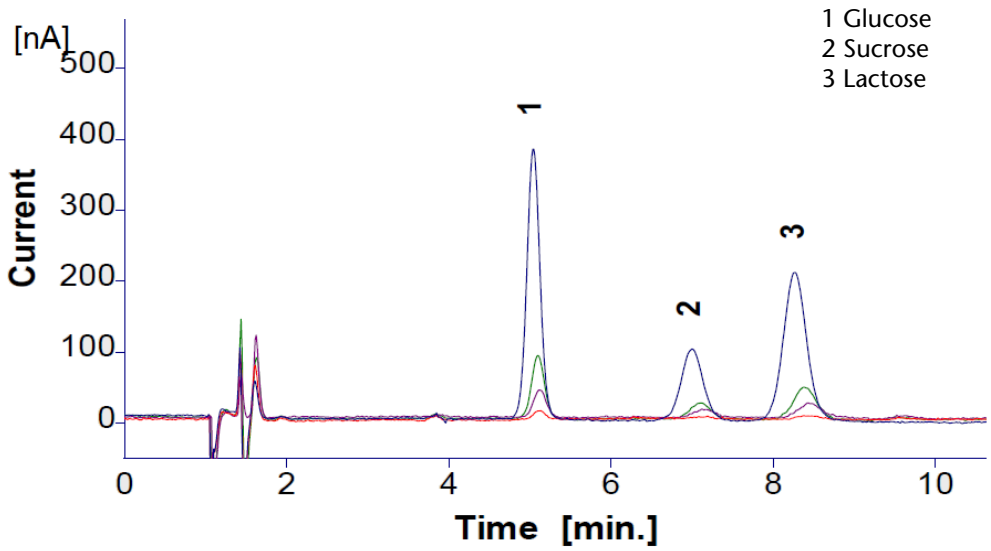
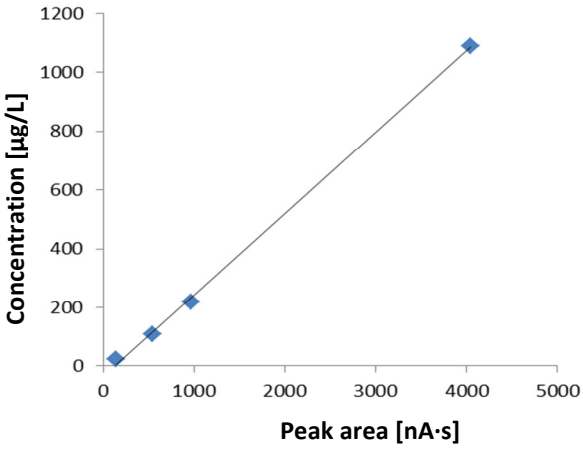


Fig. 2

Chromatograms of a standard solution at different concentrations: Blue: 1090.0 µg/L, green: 218.0 µg/L, violet: 109.0 µg/L, red: 21.8 µg/L

To figure out the method´s limit of detection and the limit of quantification, standard dilutions from around 1000 down to 21 µg/L were injected. Using the resulting peak heights, the parameters could be calculated. Figure 3 shows the calibration curve and the parameters calculated for the method´s performance.



Method performance

Correlation factor	> 0.999
Limit of detection (LOD)	15 µg/L
Limit of quantification (LOQ)	59 µg/L

Fig. 3
Calibration curve for lactose concentrations in the range of 21.8 µg/L to 1090.0 µg/L and results for method performance

The following analysis of four samples from typical German food proved that most of them were lactose-free and therewith allowed to use this label, even with the new lactose limits given by the EU. Figure 4 shows the chromatograms of the sugar standard (blue) overlayed with the samples (red) from *Hähnchenspieß* (chicken skewers), *Leberkäse* (meatloaf), *Paprikalyoner* (sausage) *and Nürnberger* (sausage). Only in one case a significantly high lactose peak could be found. Therewith, using the presented method, 3 out of 4 samples could be declared as lactose-free and one sample is not allowed to be called a lactose-free product.

Additionally the chromatograms show that not a lot of disturbing peaks were detected. This is caused by the specialized detection method which is sensitive to sugar analysis and does not show many of the samples impurities.

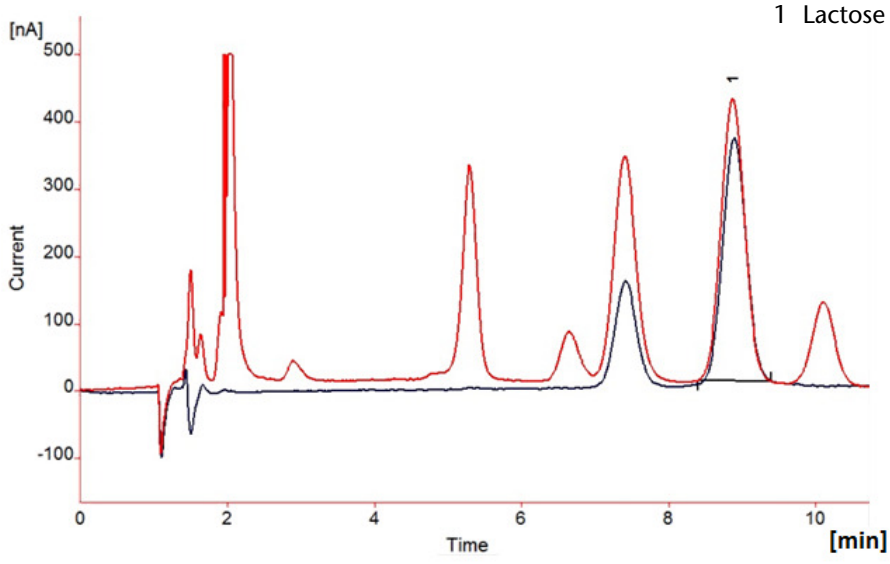


Fig. 4
Chromatogram of "Hähnchenspieß" chicken skewers (red) with an overlay of the sugar standard (blue)

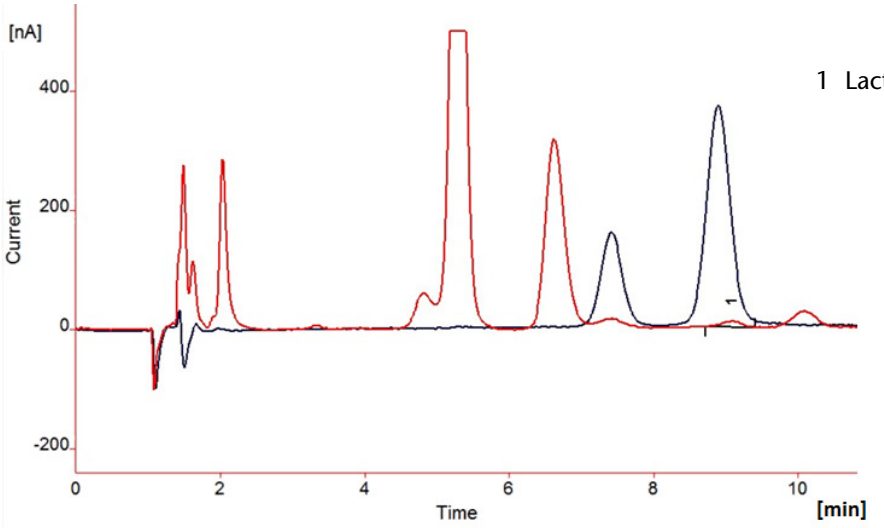


Fig. 5
Chromatogram of "Paprikalyoner" sausage (red) with an overlay of the sugar standard (blue)

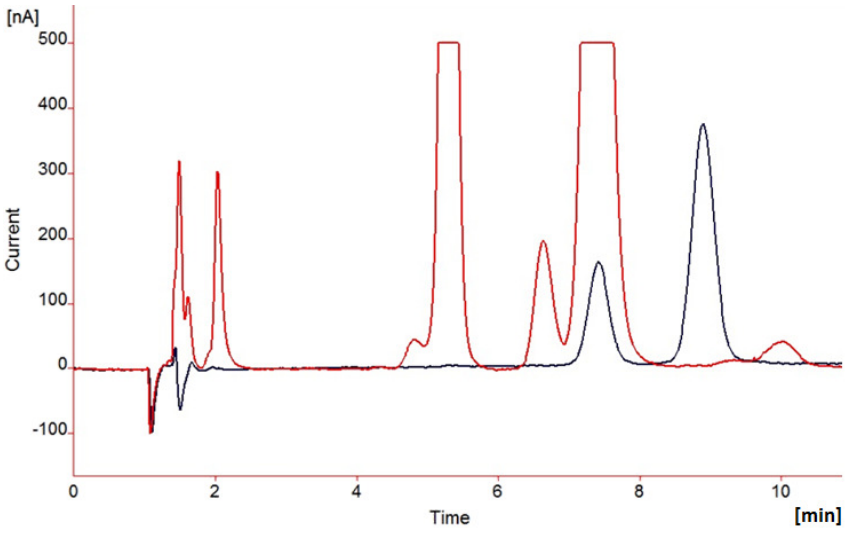


Fig. 6
Chromatogram of "Leberkäse" meatloaf (red) with an overlay of the sugar standard (blue)

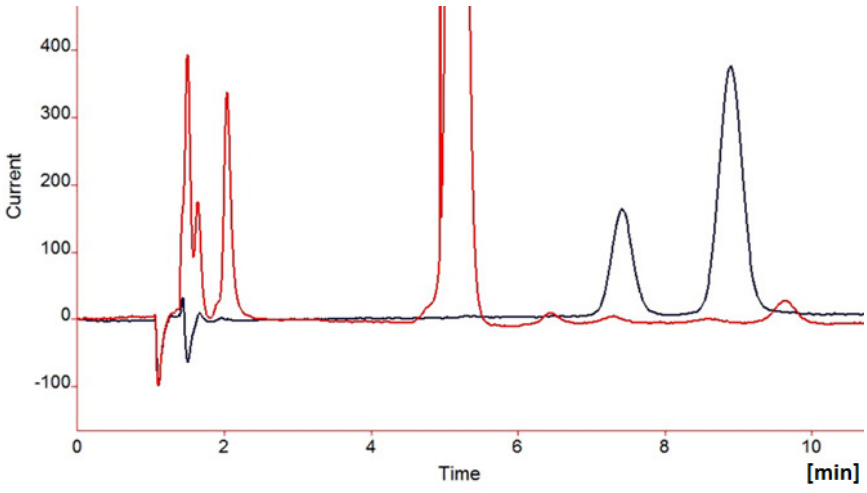


Fig. 7
Chromatogram of "Nürnberger" sausage (red) with an overlay of the sugar standard (blue)

Using the calibration, the lactose contents in the samples were determined. Table 1 shows the results.

Table 1
Determination of lactose contents in the real samples

Sample	Peak area	Calculated concentration* [µg/L]	Lactose content in 100 g product [mg]	Result**
Paprikalyoner	209	23	9	Lactose-free
Leberkäse	NF	< 15	< 6	Lactose-free
Hähnchenspieße	9342	25544	10217	Not lactose-free
Nürnberger	NF	< 15	< 6	Lactose-free

* dilution of the sample already included
**lactose-free means that the sample contains less than 10 mg lactose per 100 g product

Three out of four samples can be declared as lactose-free according to the definition.
If there is a need in the future to detect even lower concentrations, this method allows for optimization by less dilution of the samples. This becomes possible by the very specific detection method where nearly no interfering matrix peaks are seen.

Conclusion

The presented method of HPAEC-PAD on a KNAUER HPLC system proved to be well-suited to determine low limits of sugars in food products. The detection principle was quite specific for sugars and only showed very little interference by matrix peaks. Using the AZURA analytical system combined with the DECADE II electrochemical detector, the analysis of lactose in lactose-free labelled products can be carried out in a robust and reproducible manner. This system reaches the detection limits defined by the EU and can therefore ideally be used in food control.

References

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6 Leo M.L. Nollet et al: Handbook of Dairy Foods Analysis, (2010) Taylor and Francis Group LLC, USA, ISBN 9781420046311, S. 397

Physical properties of recommended column

RCX-10 carbohydrate analysis columns are designed for the isocratic or gradient separation of carbohydrates. The exchange capacity of the RCX-10/RCX-30 is greater than that of the PRP-X100, leading to characteristics better suited for the separation of carbohydrates.

Stationary phase	RCX-10, 7 µm
USP code	L47
Pore size	100 Å
Particle size	7 µm
Support material	PSDVB with Tri-methyl ammonium Exchanger
Dimensions	250 x 4.6 mm, PEEK hardware
Order number	25EE158HML

Recommended instrumentation

Detector: DECADE II SCC

HPLC system: AZURA

Description	Order No.
AZURA P 6.1L Isocratic analytical HPLC pump, metal-free 10 ml pump head	APH60EB
Electrochemical Detector DECADE II SCC	A07543
Autosampler 3950 analytical HPLC autosampler with biocompatible flow path	A50052-1
AZURA Eluent tray E 2.1L Eluent tray for up to 6 x 1000 ml bottles (delivery without bottles) or 4 x 2.5 l bottles or 2 x 5 l bottles or 1 x 10 l bottle	AZC00
ClarityChrom® 5.0.5 single instrument license one time base	A1670-8

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