Metrohm

Application Work AW IC ES6-0004-052016_G

Fast HPIC-PAD determination of several carbohydrates on food products with Metrosep Carb 2

Branch

Food, stimulants, beverages, flavors

Keywords

IC PAD 930 919 inositol xylitol sorbitol glucose fructose lactose sucrose maltose, Drinks, Food, Vegetables, Meat, Alcohols, Pulsed Amperometric Detection, PAD-IC, Metrosep Carb 2-150/4.0, branch 7

Summary

HPIC-PAD (Pulsed Amperometric Detection) technique for Carbohydrates determination in several matrices was studied. This AW describes the use of Metrosep Carb 2 150/4.0 column in combination with a high concentrated NaOH/ CH_3COONa eluent. The use of this eluent eliminates the problem of retention time shifting because of carbonation.

Samples

Food aqueous extracts of several matrices (chocolate, cookies, chewing gum)

Instruments

930 Compact IC pro	2.930.2460
919 Professional Sample Processor	2.919.0020
IC Amperometric Detector	2.850.9110
IC equipment Wall-Jet cell: Carb (Au, Pd)	6.5337.010
Metrosep Carb 2 150/4.0	6.1090.420
Metrosep Carb 2 Guard	6.1090.500
MagIC Net™ 3.1 Professional	6.6059.242
IC equipment for ultrafiltration	6.5330.110
Adsorbing tube, large and bent	6.1609.000

Reagents

- Sodium hydroxide, puriss. 50 – 52%;
  CAS 1310-73-2, Fluka 72064
- Sodium Acetate anhydrous >99,0 % CAS 127-09-3; Sigma-Aldrich S2889
- Carbohydrates reagent standards Sigma Aldrich Analytical grade
- Soda lime p.a., with indicator, pellets; CAS 8006-28-8; Sigma-Aldrich 72073
- Ultrapure water (UPW) resistivity >18.2 MΩ·cm (25 °C), type I grade (ASTM D1193) from Flex 5 ELGA water system

Solutions

Eluent	Sodium Hydroxide 300.0 $mmol \cdot L^{-1}$ Sodium Acetate 1.00 $mmol \cdot L^{-1}$ *CO_2 free ultrapure water*
Rinsing Solutions	UPW : Methanol (1:1)

Standard solutions

Sorbitol and Xylitol from 0.5 to 10 $mg \cdot L^{-1}$

Mono and di-saccharides from 0.5 to 50 $mg \cdot L^{-1}$

Sample preparation

An aqueous extraction of every matrix was performed. For example 1 to 5 g of sample was diluted with 50 to 200 mL of UPW (depending on sample matrix). Heating slightly (<50ºC)

and stirred for 30 min. Extracting solution was centrifuged to separate solids. Depending of sample concentration an additional dilution was done before transferring to sample vials.

Analysis

Every solution was filtrated with 0.2 um inline ultrafiltration previously to injection.

Eluent Flow	0.5 mL·min^{-1}
Detector Mode	PAD (3 pulse steps)
E1	0.05 V
E2	0.55 V
E3	-0.10 V
T1	300 ms
T2	50 ms
T3	200 ms
Measurement duration	100 ms
Range	200 µA
Channel measurement	Current (A)
Col. Oven temperature	30 °C
Detector temperature	35 °C
Sample Loop	20 uL

Calculation

Automatic integration using peak areas evaluated with MagIC Net™ 3.1

Results

See appendix with chromatograms below

Comments

In this study was found the combination of high concentration NaOH for eluent with the Metrosep Carb 2 column showed high stability of retention times of carbohydrates almost for 4-5 days working with the same eluent bottle.

It was shown the degassing of water previously to eluent preparation improve the stability of the eluent more days in comparison with a non-degassed one. A degassing of 10-15 minutes for a liter of water was assayed with successful result.

It was found the using of plastic bottle (HDPE) for eluent is preferred to a glass made one due to strong alkaline conditions and certain peak shapes.

Using inline ultrafiltration for sample preparation showed 24-48 hours of continuous operability with one 0.2 um filtration membrane (RC) for several matrices such meat and vegetable extracts, honey, candies, juices, pills, and another foods.

Sample line cleaning between samples was performed with UPW: Methanol (50:50 %vol.) and UPW.

Metrosep Carb 2 showed a high capacity, stability and robustness working with the high concentrate eluent. Nevertheless, is advisable to rinse high pressure pump with UPW to avoid pump problems (almost once a week when working continuously)

In Appendix are showed some chromatograms of several determinations and a calibration example of poli-alcohols and carbohydrates.

References

[1] Determination of Lactose in Lactose-free Yogurt, with Pulsed Amperometric Detection Ion Chromatography (PAD-IC). AW IC ES6-0003-092013_G Gomensoro IC team SPAIN

[2] Application Work AW IC CH6-1105-082012, Sugar analysis in lactose free products using the amperometric detector and Metrosep Carb 1 - 150/4.0 Metrohm AG, Herisau

[3] AN-P-046 Lactose in lactose-free dairy products applying pulsed amperometric detection after Inline Dialysis, Metrohm AG

[4] Application Work AW IC CH6-1278-042016 Determination of 5 sugars with a highly concentrated eluent on a Metrosep Carb 2 - 250/4.0 Metrohm AG

[5] Application Work AW IC CH6-1241-052015

Sugar analysis using amperometric detection and Metrosep Carb 2 150/4.0 Metrohm AG

Date

18th May 2016

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Appendix

Example Chromatograms

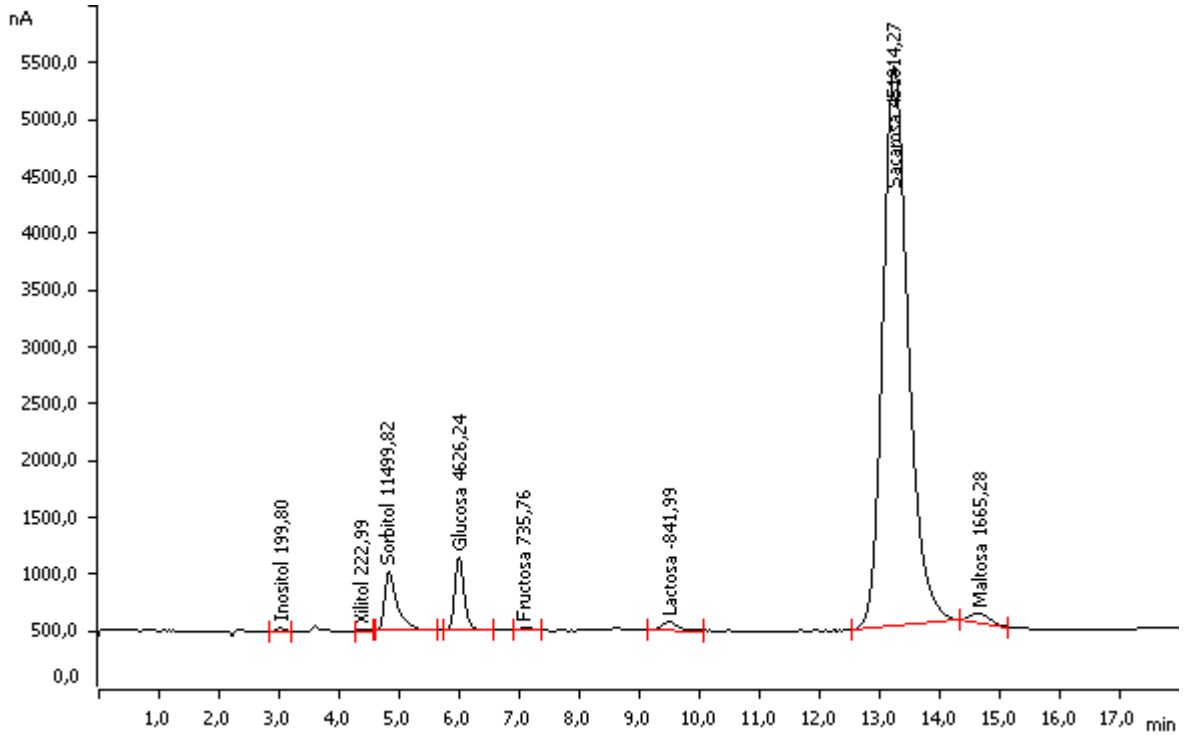


Fig 1. *10 uL injection of Milk chocolate (diluted 1:4000)*

Peak number	Component name	Retention time min	Area (nA) x min	Sample g/100g
1	Inositol	3,033	4,3304	0,020
2	Xilitol	4,390	2,5689	0,022
3	Sorbitol	4,829	127,6198	1,150
4	Glucosa	6,002	117,8079	0,463
5	Fructosa	7,102	6,5974	0,074
6	Lactosa	9,503	23,5793	n/a
7	Sacarosa	13,242	2416,3244	45,181
8	Maltosa	14,653	31,3096	0,167

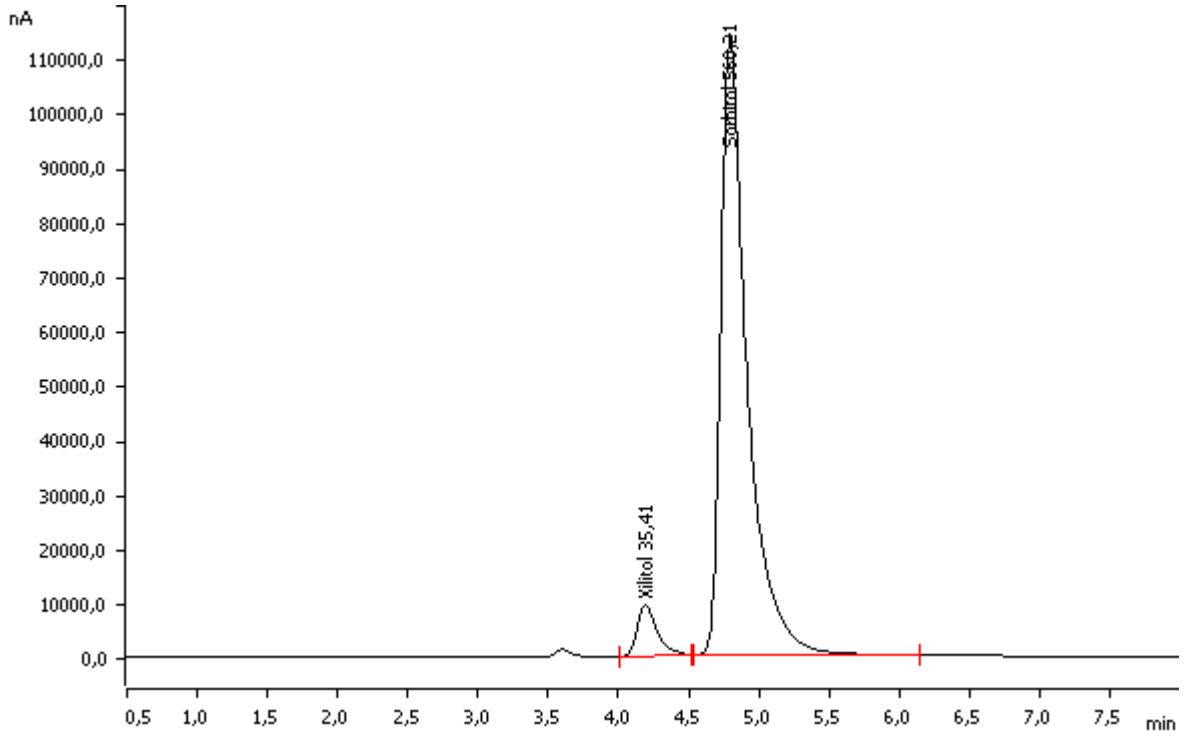
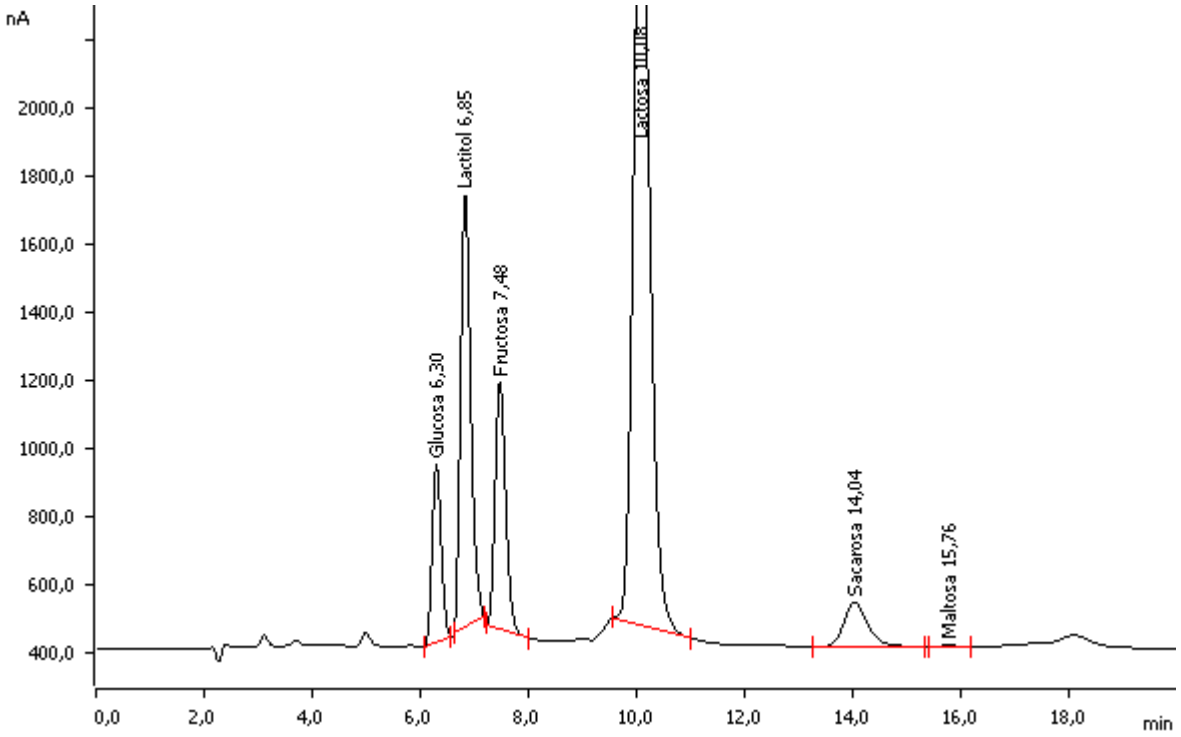


Fig 2. *10 uL injection of Chewing gum (diluted 1:1000)*

Peak number	Component name	Retention time min	Area (nA) x min	Sample g/100g
1	Xilitol	4,197	1534,2795	0,071
2	Sorbitol	4,802	24850,8251	1,120



Fi 3. 20 uL injection of Yogurt (diluted 1:2000)

Component name	Retention time min	Area (nA) x min	Concentración total %
Glucosa	6,305	100,8777	0,255
Fructosa	7,478	166,2853	0,561
Lactosa	10,080	822,3908	3,646
Sacarosa	14,039	66,4474	0,313
Maltosa	15,762	2,0346	0,013

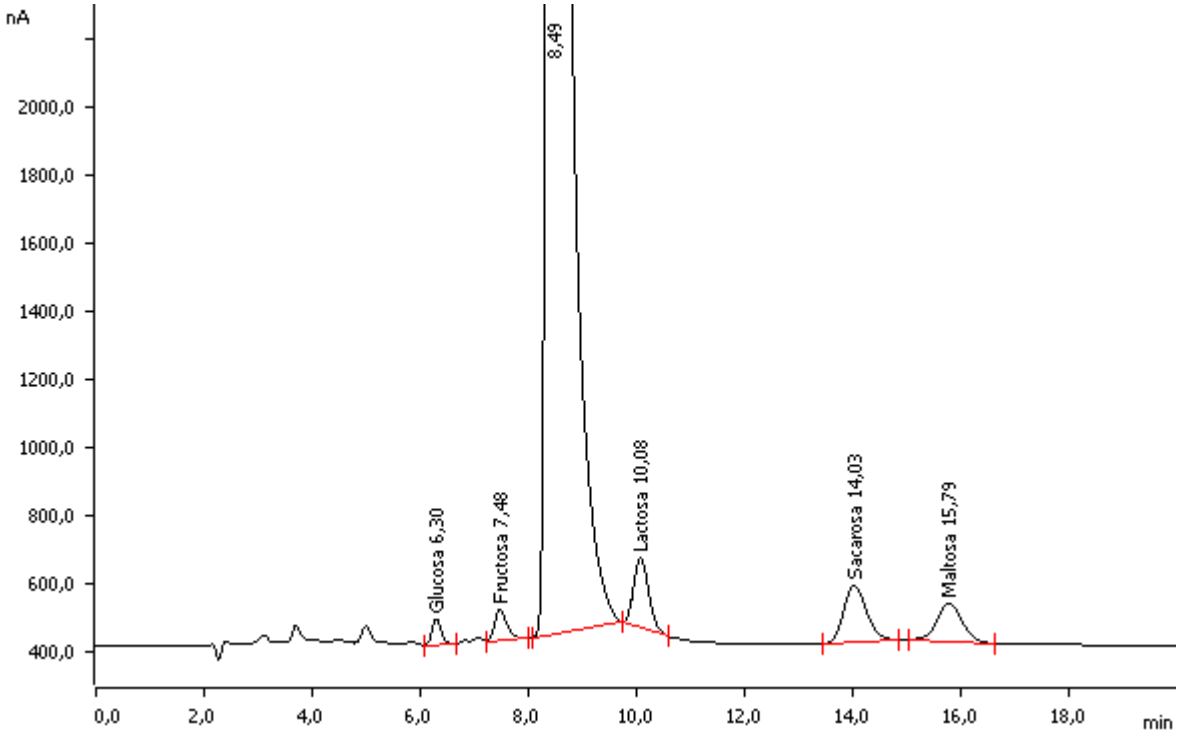


Fig 4. 20 uL injection of Cookies (diluted 1:2000)

Component name	Retention time min	Area (nA) x min	Concentración total %
Glucosa	6,305	14,6767	0,037
Fructosa	7,478	22,9278	0,071
Lactosa	10,080	65,7438	0,286
Sacarosa	14,030	82,3066	0,388
Maltosa	15,790	60,3789	0,412

Example of Repeatability

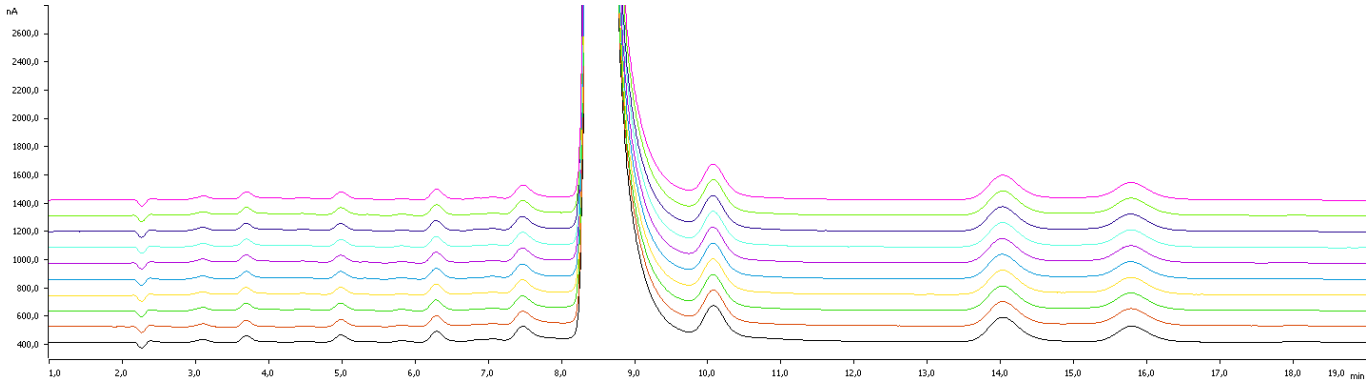


Fig 5. *Overlay of 10 chromatograms of cookies sample*

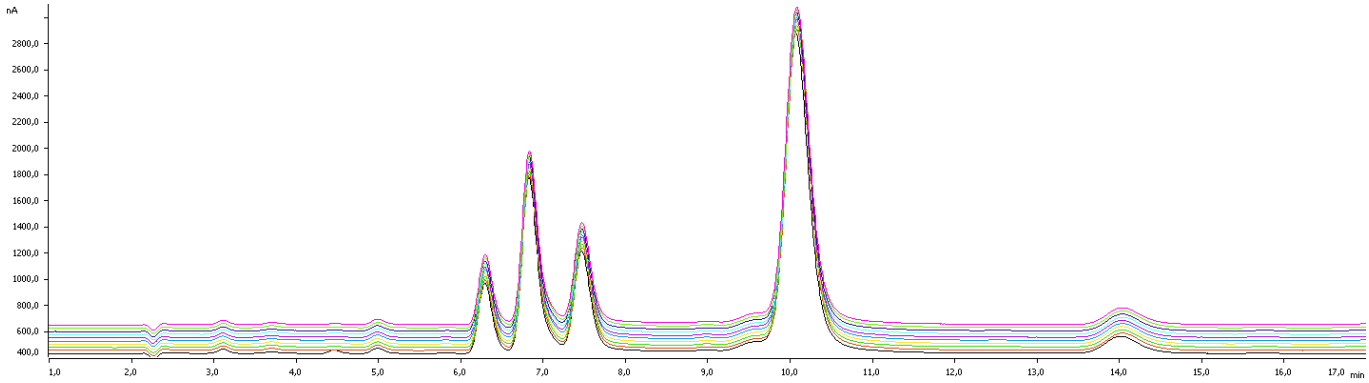


Fig 6. *Overlay of 10 chromatograms of yogurt sample*

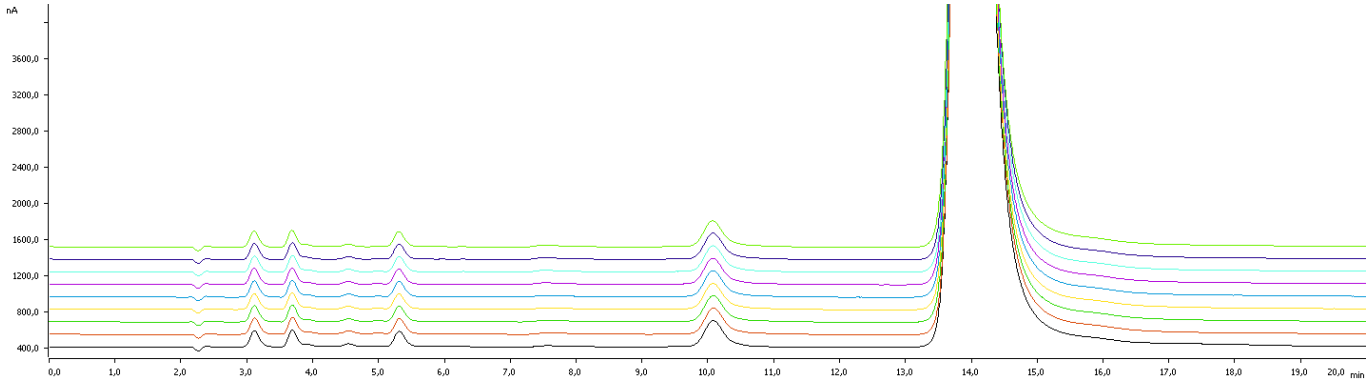


Fig 7. Overlay of 10 chromatograms of chocolate sample

Example of Standard Chromatograms

20 uL of injection

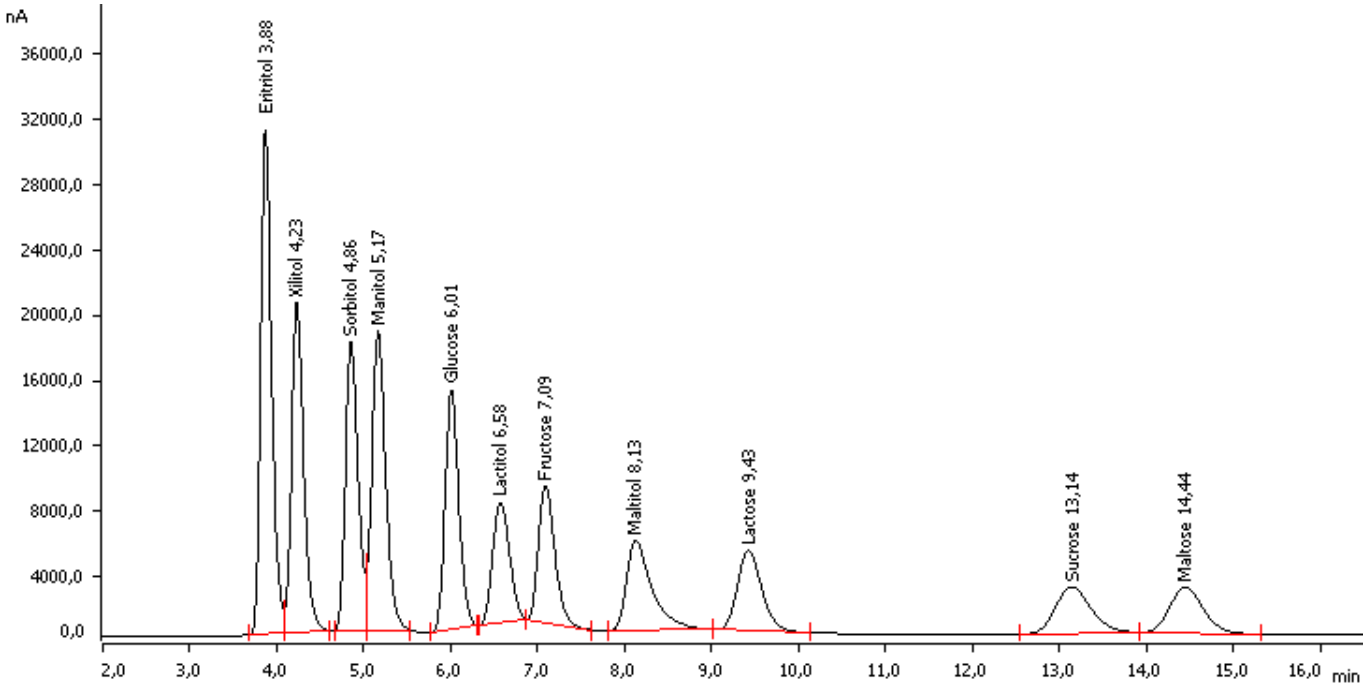


Fig 8. Standard containing 11 carbohydrates (Poly-alcohols and mono-di saccharides) 10 mg·L^{-1}

Eritritol, Xylitol, Sorbitol, Mannitol, Glucose, Lactitol, Fructose, Maltitol, Lactose, Sucrose, Maltose

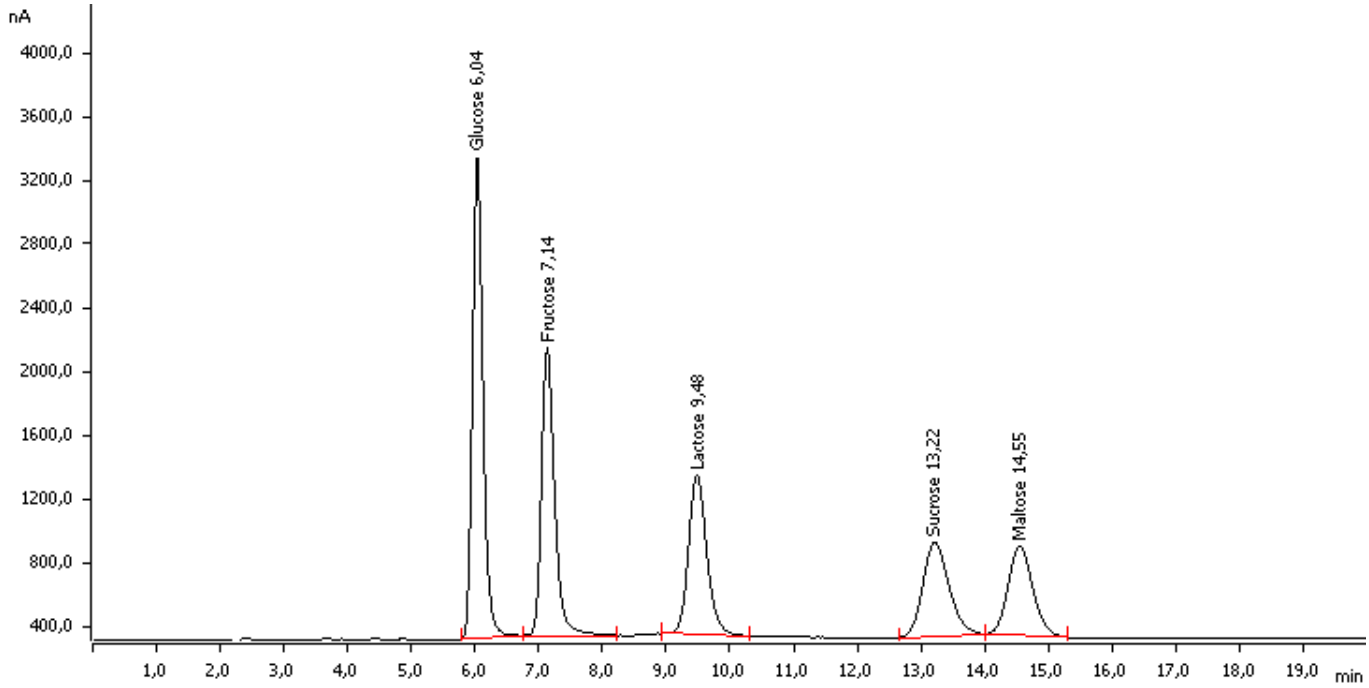


Fig 9. Standard containing 5 carbohydrates (Mono-di saccharides) 10 mg·L^{-1}

Glucose, Fructose, Lactose, Sucrose, Maltose

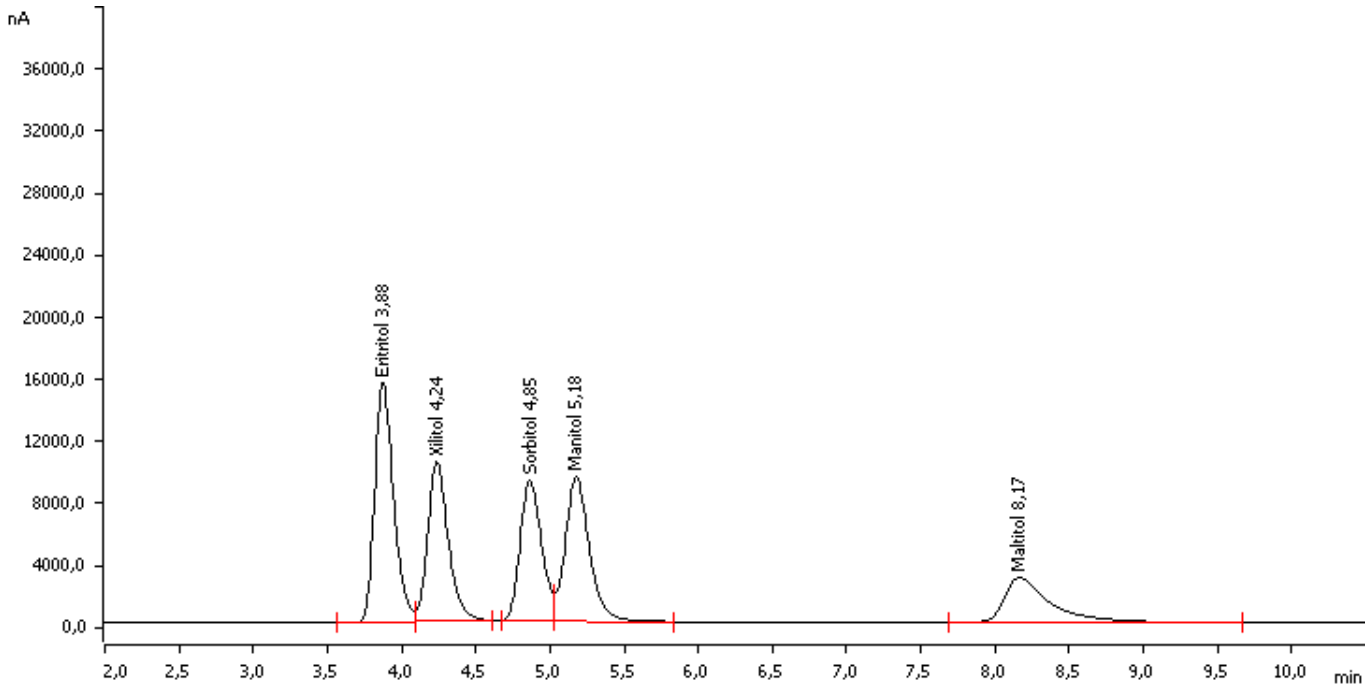
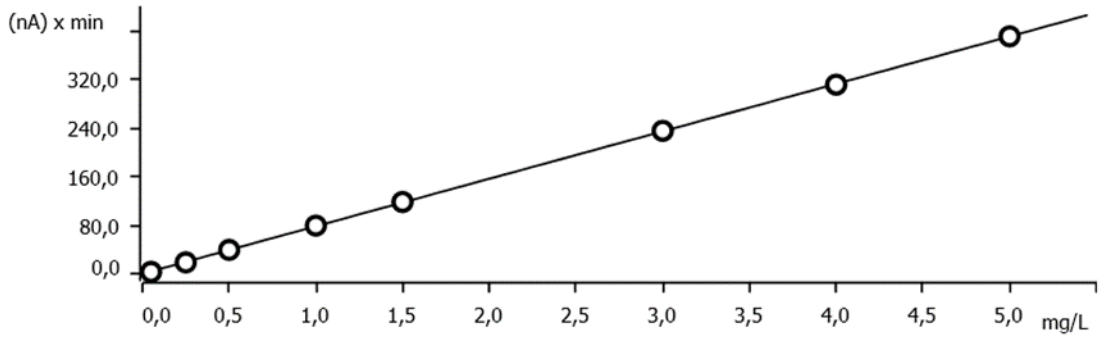


Fig 10. Standard containing carbohydrates (5 Poly-alcohols) 10 mg·L^{-1}

Eritritol, Xylitol, Sorbitol, Mannitol, Lactitol, Maltitol

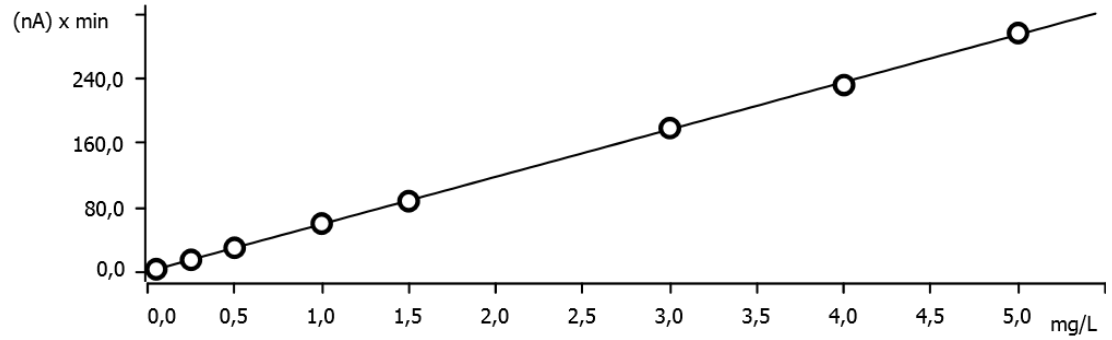
Calibration example

Glucosa (Azucares)



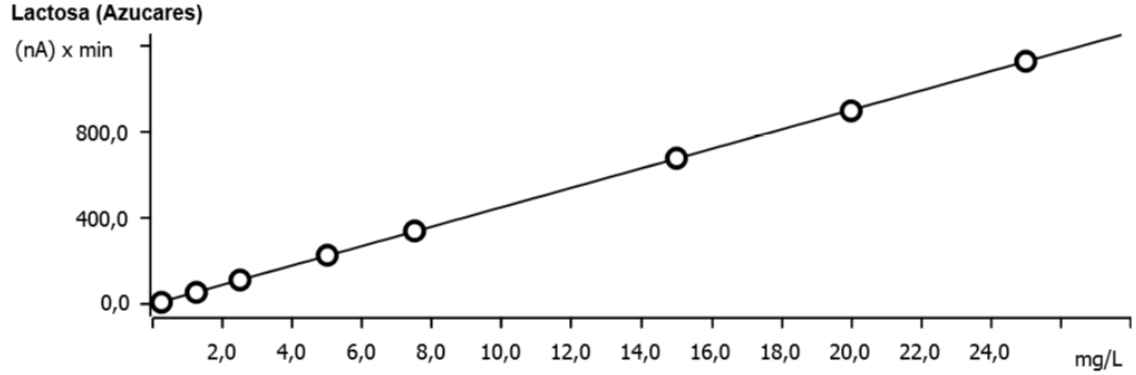
Function: . . . . . *A = 0,964816 + 3,89963× Q*
Relative standard deviation . . . . . *0,639005 %*
Correlation coefficient *0,999981*

Fructosa (Azucares)



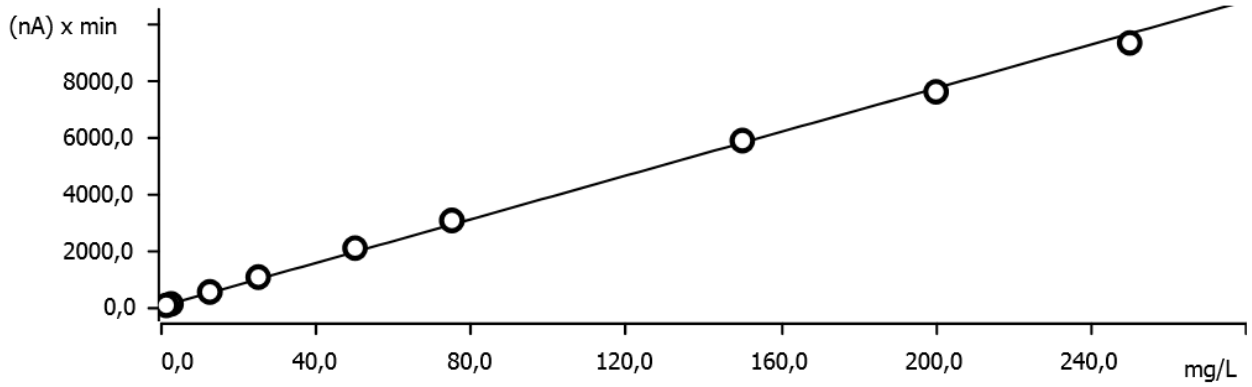
Function: . . . . . *A = 1,92504 + 2,92222× Q*
Relative standard deviation . . . . . *1,672611 %*
Correlation coefficient *0,999869*

Lactosa (Azucares)



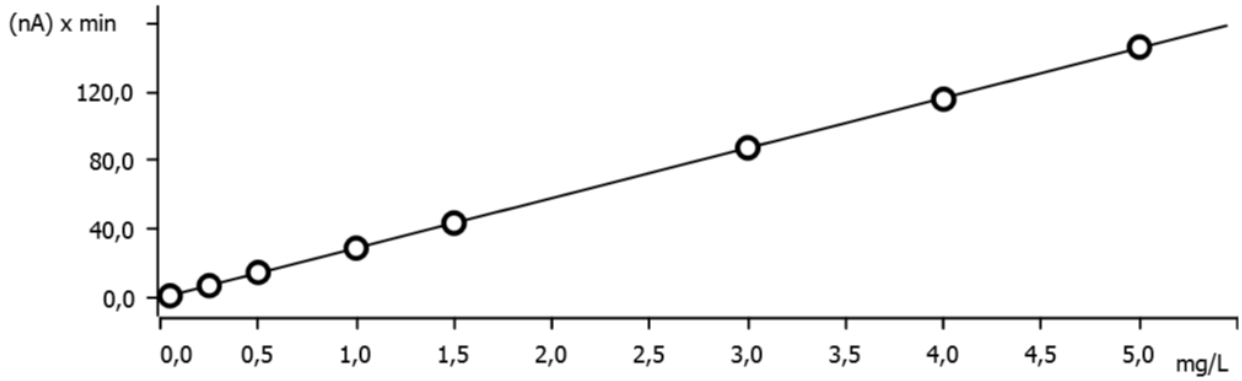
Function: . . . . . *A = 2,30776 + 2,24321× Q*
Relative standard deviation . . . . . *0,556416 %*
Correlation coefficient *0,999986*

Sacarosa (Azucares)



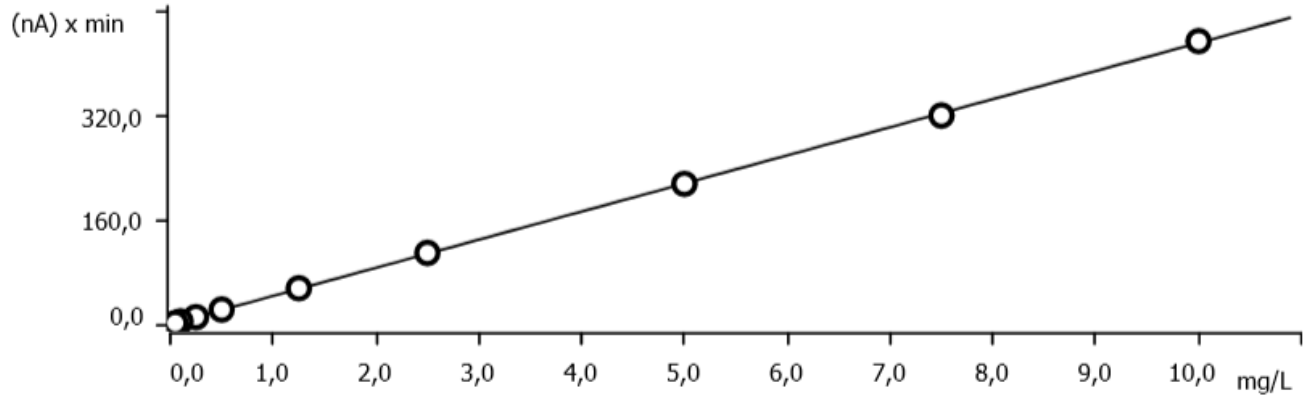
Function: . . . . . *A = 13,7402 + 1,92309× Q*
Relative standard deviation . . . . . *4,816282 %*
Correlation coefficient *0,999085*

Maltosa (Azucares)



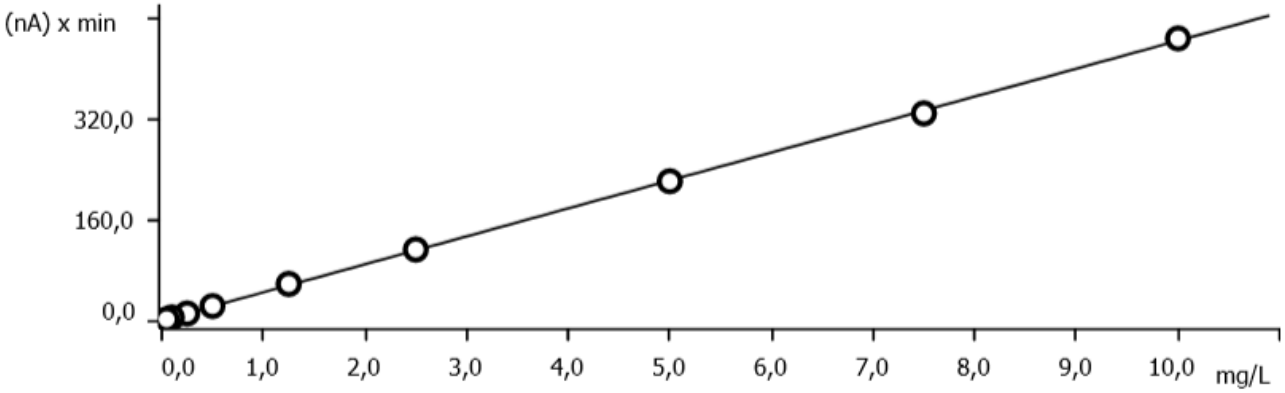
Function: . . . . . *A = 0,151435 + 1,45365× Q*
Relative standard deviation . . . . . *0,720794 %*
Correlation coefficient *0,999976*

Xilitol (Carbohidratos)



Function: . . . . . *A = 0,153353 + 4,33303× Q*
Relative standard deviation . . . . . *1,428467 %*
Correlation coefficient *0,999939*

Sorbitol (Carbohidratos)



Function: . . . . . *A = 0,0866613 + 4,43601× Q*
Relative standard deviation . . . . . *1,839478 %*
Correlation coefficient *0,999900*