Detection of sugars and uronic acids by PAD-HPAEC

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Application field

Third Party, Others

Abstract

In this paper, 16 kinds of sugars and uronic acids were determined using the Shine CIC-D200E coupled with a PA20 (3.0 × 150mm) column. The results showed that under the chromatographic conditions of this experiment, the resolution of N-acetylglucosamine and N-acetylgalactosamine was poor, while xylose and mannose were basically separated. Besides these, the other sugars were well separated.

Key words

CIC-D200E, sugars, uronic acids

Instrument

Name of the equipment	Model	Brand
PAD-HPAEC	CIC-D200E	Qingdao Shine Chromatograph Technology Co., LTD
Automatic sampler	SHA-18i	

Reagents and consumables

Reagents and standard solutions	Brand
16 kinds of sugars and uronic acids standard stock solution	Provided by the customer
50% sodium hydroxide solution	Sigma

Parameters of ion chromatograph instruments

Chromatographic column	SH-PA20 (3.0×150mm)； SH-PA20 Guard(3.0×30mm)
Eluent	A: ultrapure water B: 200 mM NaOH C: 200 mM NaOH+500 mM NaAc D: 20 mM NaOH

	Time/min	A%	B%	C%	D%
	0-10	90	0	0	10
	12	86	4	0	10
	15-25	76	14	0	10
	25.1-45	56	14	20	10
	45.1-55	0	90	0	10
	55.1-70	90	0	0	10
Flow velocity	0.4 mL/min				
Column temperature	30℃				
Cell temperature	30℃				
Injection volume	10 μL				
Detection mode	Pulsed amperometric mode				
Potential waveform diagram	E1-E4: 0.10V/-2.00V/0.60V/-0.10V t1-t4: 0.4s/0.02s/0.01s/0.07s ts: 200ms				
Working Electrode	Au working electrode				

Result

No.	Name	Concentration mg/L	Time (min)	Area (nA*min)	Height (nA)	Tailing factor	R
1	Fuc	7	4.508	318.4083	2231.3600	1.205	11.457
2	GalN	20	9.250	1262.0167	4088.1453	1.311	1.479
3	Rha	10	9.942	131.3225	548.7765	1.218	1.551
4	Ara	19	10.583	518.3333	1969.2133	1.243	2.768
5	GlcN	10	12.117	775.5625	1893.7000	1.020	2.524
6	Gal	23	13.708	482.7817	1379.3782	1.189	3.709
7	Glu	12.5	15.742	458.6558	1529.2840	1.094	2.257
8	GlcNAc	10	16.892	376.8303	1395.2170	0.915	1.073
9	GalNAc	10	17.175	211.3072	867.1830	1.684	2.743
10	Xyl	10	17.658	395.5463	1923.2772	1.093	1.090
11	Man	12.5	18.033	451.5337	2126.3327	1.166	6.183
12	Rib	10	20.650	330.8925	1091.9333	1.467	38.049
13	GacA	2.5	39.042	78.0408	275.6460	0.998	3.761
14	GulA	2.5	40.858	63.2067	218.5577	1.101	2.959
15	GlcA	2.5	42.333	134.9317	455.7231	1.054	2.855
16	ManA	2.5	43.692	79.4158	289.0065	1.245	0.000

Note:

(1) The resolution of GlcNAc and GalNAc was poor, Xyl and Man were basically separated, and the remaining sugars could achieve baseline separation.

(2) When the eluent was adjusted to a 30 mM NaOH solution, the resolution of Glu, GlcNAc, GalNAc, Xyl, and Man was improved; however, galactose (Gal) overlapped with GalNAc, making it

difficult to achieve satisfactory separation for all these components simultaneously.

Conclusion

In this paper, 16 kinds of sugars and uronic acids were determined using the Shine CIC-D200E coupled with a PA20 (3.0 × 150mm) column. The results showed that under the chromatographic conditions of this experiment, the resolution of N-acetylglucosamine and N-acetylgalactosamine was poor, while xylose and mannose were basically separated. Besides these, the other sugars were well separated.

Date

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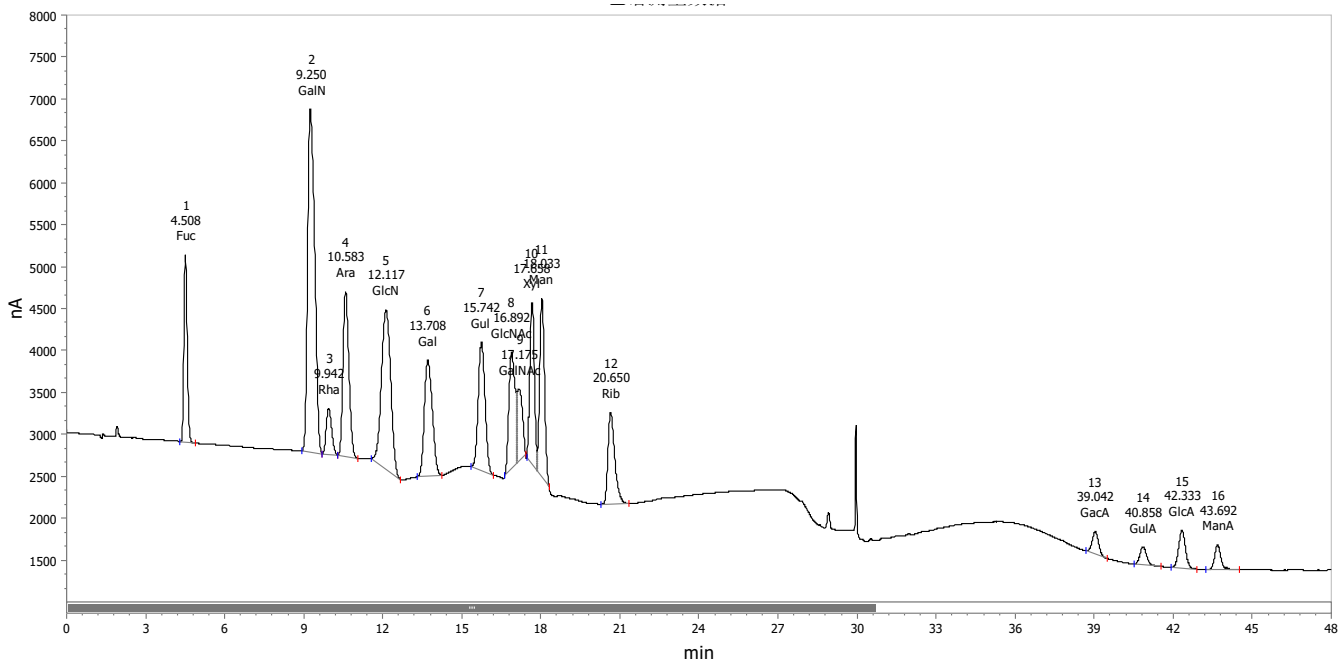
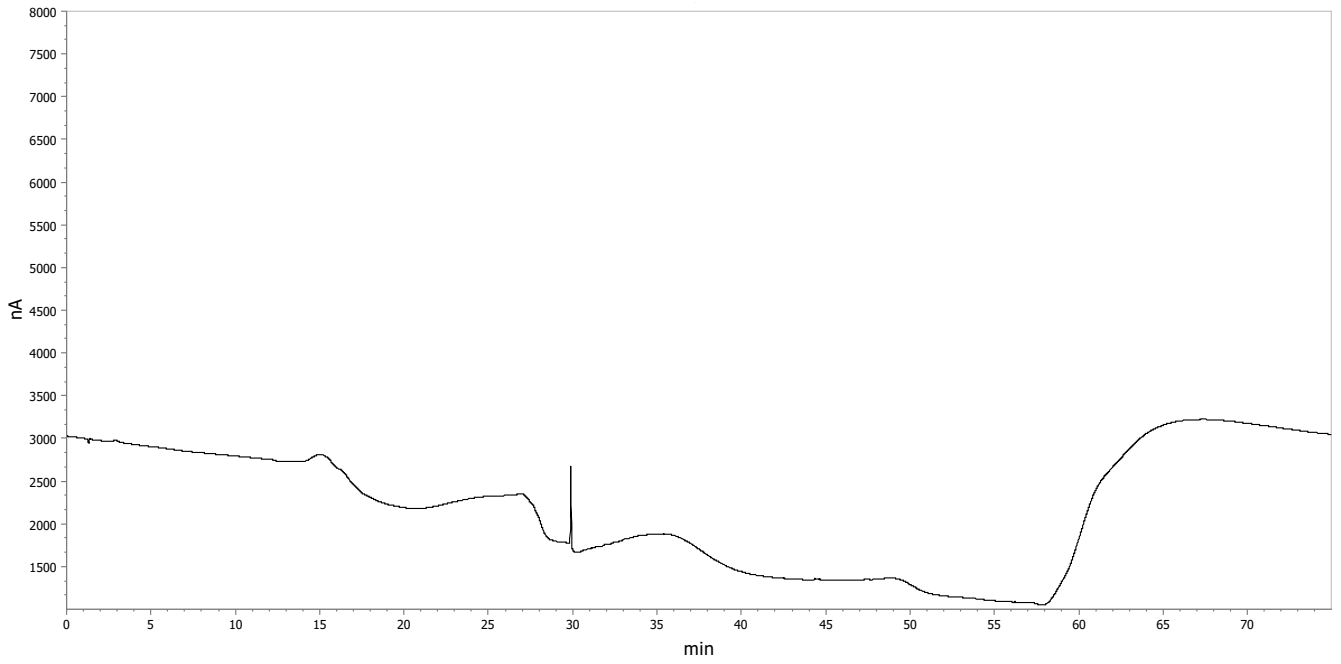
Appendix

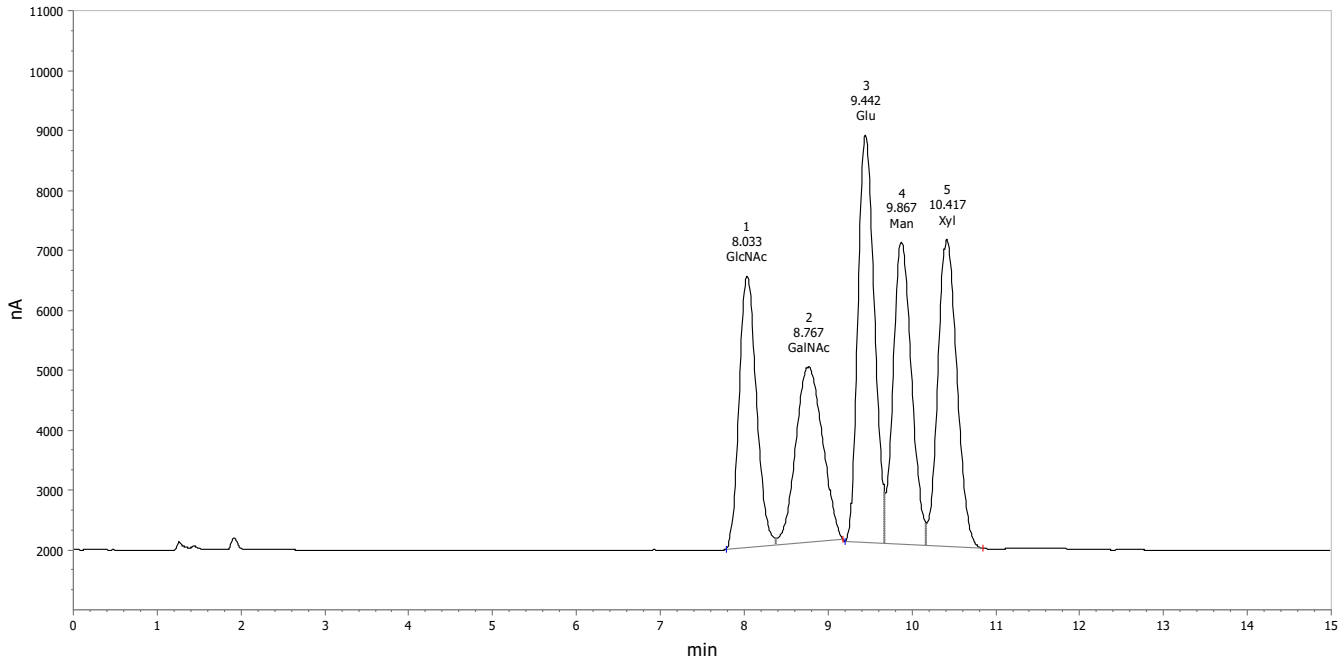
Typical chromatograms



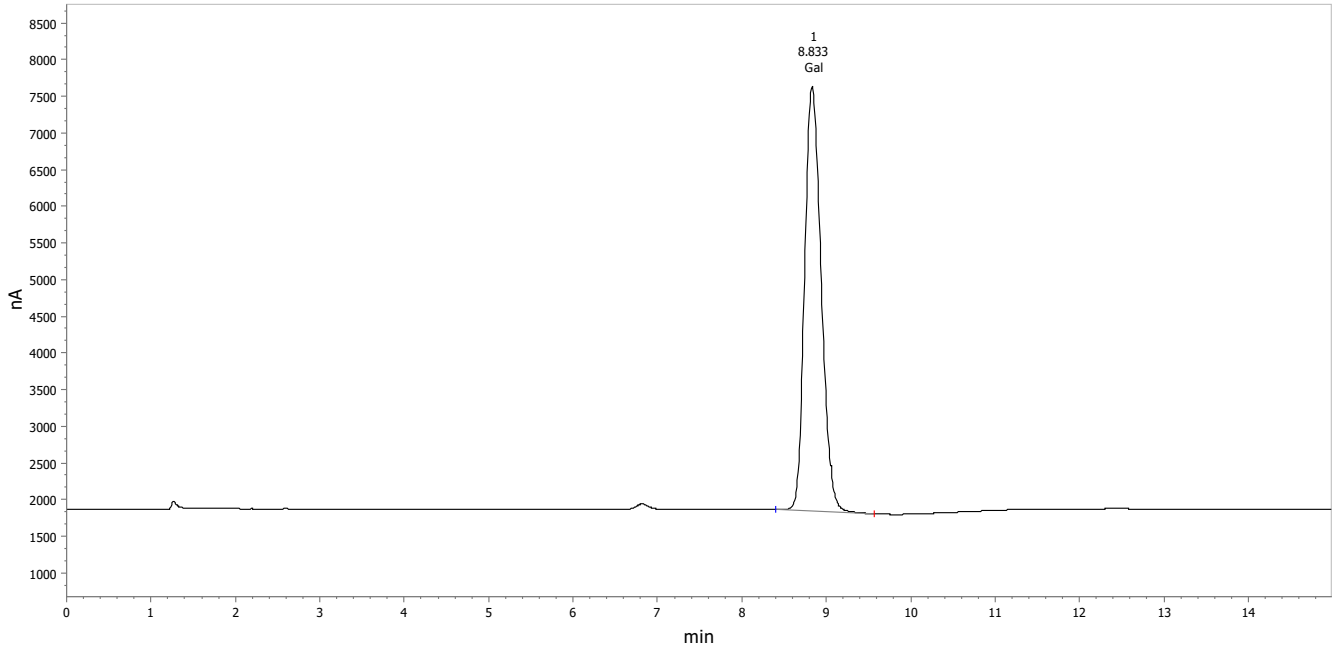
Chromatogram of 16 kinds of sugars and uronic acids



Chromatogram of the blank



Chromatogram of Glu, GlcNAc, GalNAc, Xyl, Man (30 mM NaOH)



Chromatogram of Gal (30 mM NaOH)