A new saccharide analysis column for Charged Aerosol Detector

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Abstract

Charged Aerosol Detector (CAD) is a relatively new near-universal detection method for HPLC analysis. Previously, only refractive Index (RI) detectors could be used for universal detection independent of chemical structure; RI detectors are relatively insensitive relying on the difference of refractive index measured between the analyte and eluent. RI has significant limitations in sensitivity for analysis of saccharides as well as gradient method incompatibility. As a result, the frequency of using CAD is increasing in saccharide analysis.

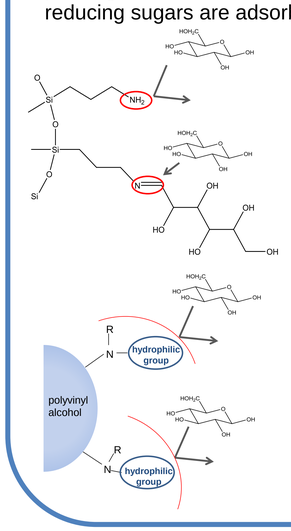
However, one drawback to the use of CAD is due to the high sensitivity. Bleeding of packing materials from the HPLC column are detected as a noise and cause an unstable base line. Generally speaking, bleeding occurs with silica-based columns and are not suitable for use with a CAD detector.

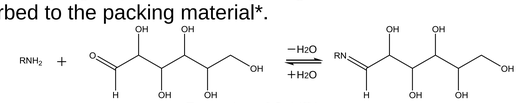
A polymer-based amino hydrophilic interaction chromatography (HILIC) column has been widely used for saccharides analysis with RID, and has demonstrated minimal bleeding in comparison to its silica-based counterpart by CAD. We made full use of the advantage of polymer-based packing material which is polyvinyl alcohol, and developed a new HILIC column with a different functional group especially for CAD. The new Shodex VG-50 was improved in the recovery ratio of saccharides and it has led to the higher sensitivity analysis needed for saccharides.

In the presentation, the newly developed applications for saccharides with Shodex VG-50 and CAD detection are introduced.

New saccharide analysis column, Shodex VG-50

In saccharide analysis, amino column is often used because basicity of amino group prevent anomer separation. But the amino column causes low recovery ratio of reducing sugar, mannose, galactose, etc. Since the amino group of packing material and carbonyl group of reducing sugar form Schiff base, reducing sugars are adsorbed to the packing material*.





Formation of Schiff base

Silica-based amino columns cause formation of a Schiff base between packing material and reducing sugars. Therefore, the recovery ratio of reducing sugars is supposed to be low.

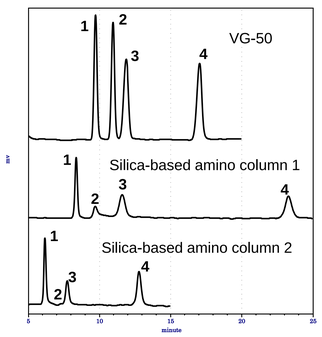
Packing material of Shodex VG-50 is modified by tertiary amino groups, and the amino groups are linked with hydrophilic group. The bulky tertiary amino groups are not able to synthesize a Schiff base to the carbonyl group of reducing sugars. Due to this function, the recovery ratio of reducing sugars is improved.

The base material of Shodex VG-50 is polyvinyl alcohol. Since polyvinyl alcohol is tolerant to alkaline conditions, it is possible to use not only acidic eluent conditions but also alkaline conditions as well.

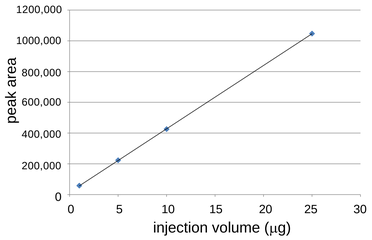
*Brons, C.; Olieman, C. *J. Chromatogr. A* **1983**, 259, 79

Analysis of saccharides

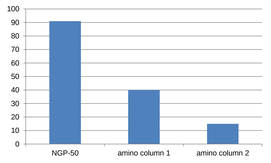
Shodex VG-50 shows high recovery ratio of reducing sugars, such as mannose. Mannose can be detected at a lower concentration using VG-50 in comparison to other amino columns.



Eluent:CH_3CN/H_2O=80/20
Flow rate:(VG-50)0.4mL/min
(silica-based amino column) 1.0mL/min
Column temp.:40℃ Detector:RI
Column size:4.6mm I.D.×150mm
Sample:1.fructose 2.mannose 3.glucose 4.sucrose
5mg/mL each 5μL



Comparison Shodex VG-50 and amino column 1 and 2 in terms of the recovery ratio of mannose.



Recovery ratio =(peak area of mannose)/(peak area of sucrose*)
**Sucrose doesn't form Schiff base. (Note: NGP-50 → VG-50)

Saccharide analysis with CAD

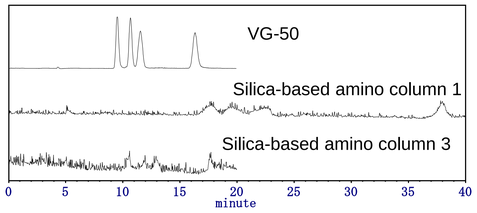
Corona Charged Aerosol Detector(CAD)
CAD charges aerosol analyte particles using nitrogen gas and corona discharge and measures the charged analyte particles by a high sensitivity electrometer. The response is independent of chemical structure like RI detector, hence CAD is more sensitive than RI. In addition, gradient methods are incompatible with RI detection; however gradient methods can be used with CAD. These features make CAD a better method of detection for in saccharides analysis.

Comparison of CAD and RI

	sennsitivity	gradient method	universality
CAD	○	○	○
RI	△	×	○

CAD detects nonvolatile and semivolatile analytes eluted from the column with high sensitivity. So, in the case of that the packing materials of the column has bleeding, the stability of the baseline is influenced by it.

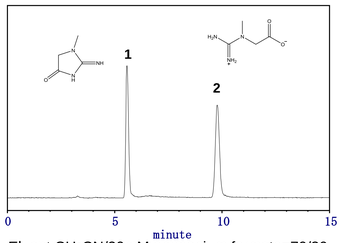
Since the functional group of new Shodex VG-50 is chemically bonded to the polyvinyl alcohol strongly, the bleeding of the packing material is minimized. As the result, new Shodex VG-50 shows more stable baseline at CAD detection.



Eluent:CH_3CN/H_2O=80/20 Flow rate:0.4mL/min
Column temp.:40℃ Detector: Corona CAD Column size:4.6mm I.D.×150mm
Sample:1.fructose 2.mannose 3.glucose 4.sucrose 40μg/mL each 5μL

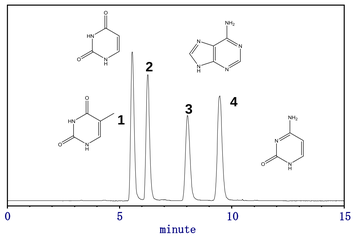
Analysis of other hydrophilic compounds

Creatine & Creatinine



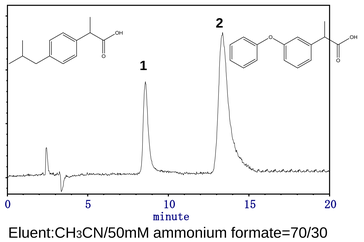
Eluent:CH_3CN/30mM ammonium formate=70/30
Flow rate:0.4mL/min Column temp.:40℃
Detector: Corona CAD
Sample: 1.creatinine 2.creatine 10μg/mL each 5μL

Nucleobases



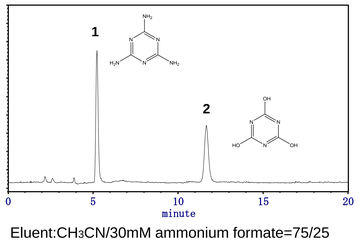
Eluent:CH_3CN/30mM ammonium formate=80/20
Flow rate:0.4mL/min Column temp.:40℃
Detector: Corona CAD
Sample:1.thymine 2.uracil 3.adenine 4.cytosine 20μg/mL each 2μL

Ibupurofen & Fenoprofen



Eluent:CH_3CN/50mM ammonium formate=70/30
Flow rate:0.6mL/min Column temp.:40℃
Detector: Corona CAD
Sample: 1.ibuprofen 1000μg/mL
2.fenoprofen 250μg/mL 5μL

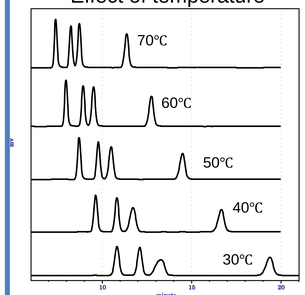
Melamine & Cyanuric acid



Eluent:CH_3CN/30mM ammonium formate=75/25
Flow rate:0.6mL/min Column temp.:40℃
Detector: Corona CAD
Sample: 1.melamine 2.cyanuric acid 5μg/mL each 5μL

Shodex VG-50 Features

Effect of temperature



Eluent:CH_3CN/H_2O=80/20 Flow rate:0.4mL/min
Detector: RI Column size:4.6mm I.D.×150mm
Sample:1.fructose 2.mannose 3.glucose 4.sucrose 5mg/mL each 5μL

Envisioned specification

Column type	Column size (I.D.×L,mm)	Particle size (μm)	Flow rate (mL/min)	Max. Pres. (Mpa)	pH range
VG-50 4D	4.6×150	5	0.2～1.0	10	2～11
VG-50 4E	4.6×250	5	0.2～1.0	12	2～11

Durability against alkaline solution

5mM NaOH solution flowed to the new column for 80 hours at room temperature.

	before testing		after testing	
	retention time(min)	plate number	retention time	plate number
fructose	8.7	8500	8.6	9100
mannose	10.4	8200	10.2	9100
glucose	11.6	7000	11.4	6200
sucrose	20.4	7200	20.0	7500

Eluent:CH_3CN/H_2O=85/15 Flow rate:0.6mL/min
Detector: RI Column size:4.6mm I.D.×150mm
Sample:1.fructose 2.mannose 3.glucose 4.sucrose 5mg/mL each 5μL

Summary

Shodex VG-50 is our new HILIC column which shows high recovery ratio of reducing sugars, such as mannose. Since reducing sugars can be detected at low concentration with Shodex VG-50, it is possible to determine quantity of reducing sugars.

Shodex VG-50 is suitable for saccharide analysis with CAD and shows a more stable baseline using CAD in comparison to other amino columns. So it is possible to quantify small amounts of reducing sugars with CAD.