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SEC Analysis of Hyaluronic Acid, Raffinose, and Rhamnose Using Advanced Polymer Chromatography

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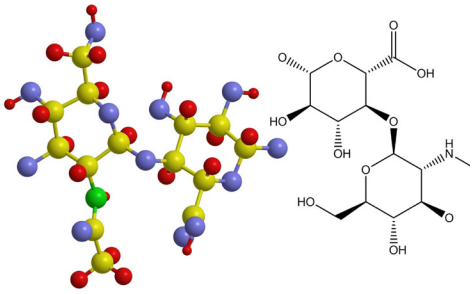

GOAL

To demonstrate the ability of Waters™ ACQUITY™ Advanced Polymer Chromatography™ (APC™) System with ACQUITY UPLC™ Protein BEH Columns for the analysis of low molecular weight species of hyaluronic acid (HA).

BACKGROUND

Hyaluronic acid (HA) polymers have a high biocompatibility, which makes them very useful in creating hydrogels for medical research.[1] Problems arise in the use of this polymer, as it easily degrades into low molecular weight species.[2] The HA hydrogels are chemically modified for medical applications, and the degradation products need to be monitored. Ruffinose and Rhamnose are two more examples of materials used for synthesis of biopolymers.[3] One technique for monitoring and characterizing biopolymers is is size exclusion chromatography (SEC) for molecular weight distribution. This distribution may contain low molecular weight species from an incomplete polymerization or from polymer degradation.

Molecular weight analysis and the accompanying method development is time consuming. An SEC method avoids interaction of the polymer with the stationary phase or itself through optimizing the mobile phase. The ideal SEC assay would include a significantly faster analysis time and flexible mobile phase capabilities.

Have you considered increasing your SEC throughput and shortening your method development time by using APC for your polymer analysis?

Table 1. Instrument conditions.

LC system:	ACQUITY APC
Flow rate:	0.8 mL/min, 0.6 mL/min, 0.4 mL/min
Mobile phase:	12 mM Ammonium acetate, pH=6.8
Sample conc:	2 mg/mL
Sample temp.:	10 °C
Standards:	Raffinose (504 g/mole), Rhamnose (164 g/mole)
Samples:	Hyaluronic acid
Injection volume:	5 µL
Column temp.:	45 °C
Columns:	ACQUITY UPLC Protein 125Å, 4.6 mm x 150 mm (p/n: 186006505)
Detector 1:	ACQUITY UPLC Tunable UV, 232 nm (p/n: 176015098)
Detector 2:	ACQUITY UPLC Refractive Index (RI) 45 °C (p/n: 176015063)
Data analysis:	Empower 3 CDS

THE SOLUTION

An analysis method for this low molecular weight HA polymer was developed using the conditions listed in Table 1 above. The HA polymer was dissolved in the mobile phase and analyzed with Waters™ ACQUITY APC System and an ACQUITY UPLC Protein BEH Column, p/n: 186006505. Various buffered mobile phases were trialed, until the ideal buffer yielded chromatography consistent with a completely dissolved sample and a column interaction free separation. The robust BEH column technology and APC's mobile phase flexibility enabled the optimization of the flow rate and mobile phase quickly as compared to traditional SEC: a few of hours versus days.

The chromatogram in Figure 1 was created using the RI data from the analysis of two low molecular weight reference sugar samples: raffinose and rhamnose. These two well characterized sugar samples were used for proof of concept in this molecular weight region and to be compared to the unknown HA sample. Three different flow rates were tested for demonstration of flow rate flexibility and no substantial effect on the chromatographic resolution: 0.8 mL/min was chosen for the speed of analysis.

The HA polymer chromatogram was compared to the low molecular weight reference sugars for resolution and sensitivity. Both detectors were used for a fit-for-purpose determination. The chromatogram in Figure 2 was completed using the photo diode array (PDA) data. The inset of the zoomed-in data reveals resolution of the smallest peak at a scale of less than 0.002 AU units.

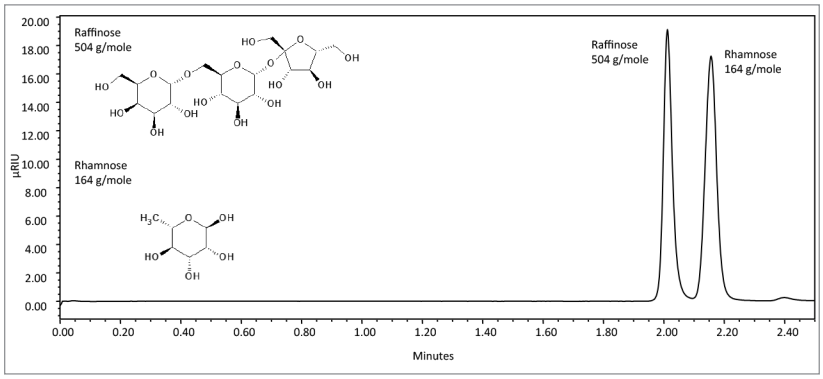

Figure 1. Chromatogram of Raffinose and Rhamnose.

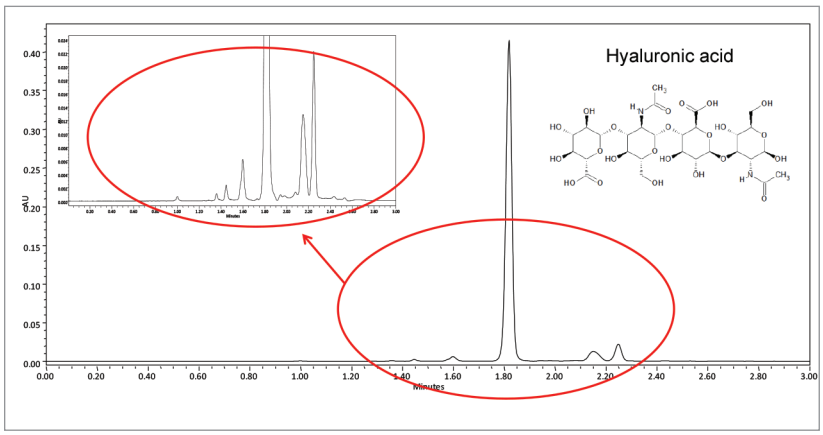

Figure 2. Chromatogram of a low molecular weight hyaluronic acid polymer.

SUMMARY

The ACQUITY APC System was able to chromatographically resolve the separation of the HA polymer, raffinose, and rhamnose. The robust mobile phase flexibility of the ACQUITY UPLC Protein BEH SEC Column enabled shorter equilibration times between flow rates as compared to traditional SEC: a couple of hours versus days. Empower 3's instrument control software enabled the use of three flow rates in one sample set.

Two columns of the same pore size can be used for increased resolution. Other options for method optimization are using various pH mobile phases and various column/detector temperature settings.

For absolute molecular weight or conformational information, advanced detectors are necessary.

The latest low dispersion detectors are now compatible with the ACQUITY APC System as highlighted by Malvern and Wyatt.[4, 5]

References

1. Lee HY, Hwang CH, Kim HE, Jeong SH. Enhancement of bio-stability and mechanical properties of hyaluronic acid hydrogels by tannic acid treatment. *Carbohydr Polym*. 2018 Apr 15: 186:290-298. doi: 10.1016/j.carbpol.2018.01.056. Epub 2018 Jan 31.
2. Tesar BM, Jiang D, Liang J, Palmer SM, Noble PW, Goldstein DR. The role of hyaluronan degradation products as innate alloimmune agonists. *Am J Transplant*. 2006 6 (11): 2622–2635. doi:10.1111/j.1600-6143.2006.01537.x. PMID 17049055.
3. Roca C, Alves VD, Freitas F, Reis MAM. Exopolysaccharides enriched in rare sugars: bacterial sources, production, and applications. *Frontiers in Microbiology*. 2015 6:288. doi:10.3389/fmicb.2015.00288.
4. https://www.waters.com/webassets/cms/events/docs/GPC%20Event/2014/GPC_SEC/Investigating%20the%20Relationship%20Between%20the%20Rheological%20Properties.pdf
5. https://www.wyatt.com/files/literature/app-notes/sec-mals-biopolymers/polysaccharide-hyaluronic-acid.pdf

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