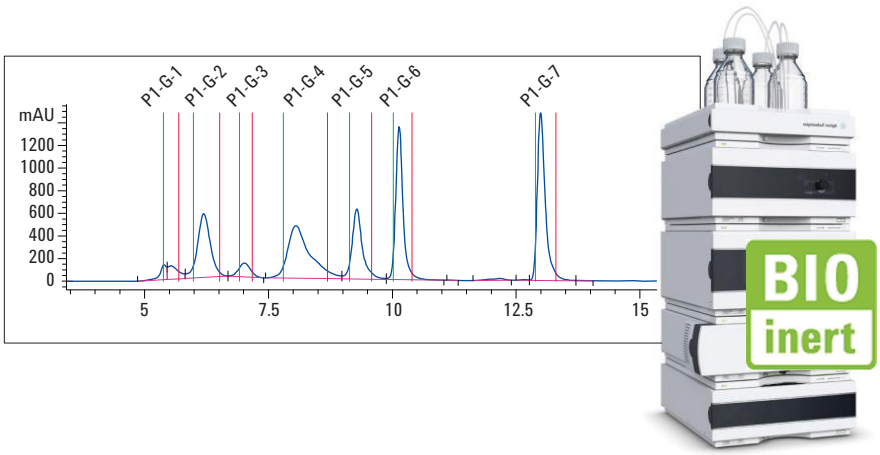
Peak-based fraction collection of proteins with the Agilent 1260 Infinity Bio-inert Quaternary LC

Versatile purification and re-analysis using automated column switching

Technical Overview



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Abstract

This Technical Overview shows the peak-based fractionation of a gel filtration standard sample with the Agilent 1260 Infinity Bio-inert Quaternary LC in combination with the Agilent 1260 Infinity Bio-inert Fraction Collector. The re-analysis of the fractions on a C8 reversed phase column confirmed the exact fractionation procedure after size exclusion chromatography. Automated peak-based fraction collection and column switching facilitate the workflow to a great extent.

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Introduction

Automated bio-purification and semi-preparative work represent major challenges in analytical liquid chromatography. Low flow rates and time-based fraction collection lead to considerable limitations in the field of small-scale preparation. Time-based fractionation is prone to errors due to mistimed fractionation time points resulting in potential split fractions within a single peak. In addition, many fractions which do not contain the substance of interest are collected. The 1260 Infinity Bio-inert Quaternary LC in combination with the 1260 Infinity Bio-inert Fraction Collector enables the user to perform highly accurate peak-based fraction collection in a completely metal-free environment.

In addition to the peak-based fractionation option, automated measurements can be facilitated by inserting a bio-inert 2-position/6-port valve. With this valve, dual column selection is possible, for example, for re-analysis of the resulting fractions.

Experimental

Instruments

The Agilent 1260 Infinity Bio-inert Quaternary LC System consisted of the following modules:

- Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)
- Agilent 1260 Infinity High Performance Bio-inert Autosampler (G5667A)
- Agilent 1290 Infinity Thermostat (G1330B) for sample cooling
- Agilent 1290 Infinity Thermostat (G1330B) for fraction cooling
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) with bio-inert solvent heat exchangers and a bio-inert 2-position/6-port valve (G5631A)
- Agilent 1260 Infinity DAD VL (G1315D with bio-inert standard flow cell, 10 mm)
- Agilent 1260 Infinity Bio-inert Analytical-scale Fraction Collector (G5664A)

Columns

- Agilent Bio SEC-3, 300Å, 7.8 × 300 mm, 3 µm
- Agilent ZORBAX 300SB-C8, 4.6 × 50 mm, 5µm

Software

OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.03 [32]

Solvents and samples

Buffer A: 50 mM sodium phosphate buffer + 150 mM NaCl, pH 6.8

Buffer B: ACN + 0.09% TFA

Buffer C: H_2O_{dd} + 0.1% TFA

Sample

Gel Filtration standard (Bio-Rad Laboratories, Inc., Hercules, CA, USA), containing:

- Protein aggregates (void peak)
- Thyroglobin (bovine) – MW 670,000 Da
- γ-globulin (bovine) – MW 158,000 Da
- Ovalbumin (chicken) – MW 44,000 Da
- Myoglobin (horse) – 17,000 Da
- Vitamin B_{12} – 1,350 Da

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). Sodium phosphate monobasic, dibasic and TFA were purchased from Sigma-Aldrich, St. Louis, USA. NaCl was purchased from VWR, Radnor, PA, USA.

Chromatographic conditions

	SEC and fraction collection	Re-analysis - reversed phase C8
Flow rate:	1 mL/min	1 mL/min
Gradient:	isocratic	0 min 5 % B, 95% C 10 min 95% B, 5% C Runtime: 10 min Stoptime: 10 min
Injection volume:	30 µL	100, 50 and 10 µL
Thermostat autosampler and FC:	8 °C	8 °C
Temperature TCC:	RT	70 °C
DAD:	280 nm/4 nm Ref.: OFF	280 nm/4 nm Ref.: OFF
Peak width:	>0.05 minute (1.0 second response time) (5 Hz)	>0.05 minute (1.0 second response time) (5 Hz)

Results and discussion

A gel filtration standard containing thyroglobulin, γ-globulin, ovalbumin, myoglobin, and Vitamin B12 was separated using SEC (Figure 1).

Figure 2 shows the fractionation options for Fraction Trigger Mode and Peak Detection. In addition to the time-based fractionation mode, peak-based fractionation can be optimized for slope and threshold adjustments to gain utmost efficiency in fractionation of the desired peaks. The peak detection mode enables the user to choose between three modes: peak detection using threshold, slope, or threshold and slope.Threshold was used for peak detection in this Technical Overview.

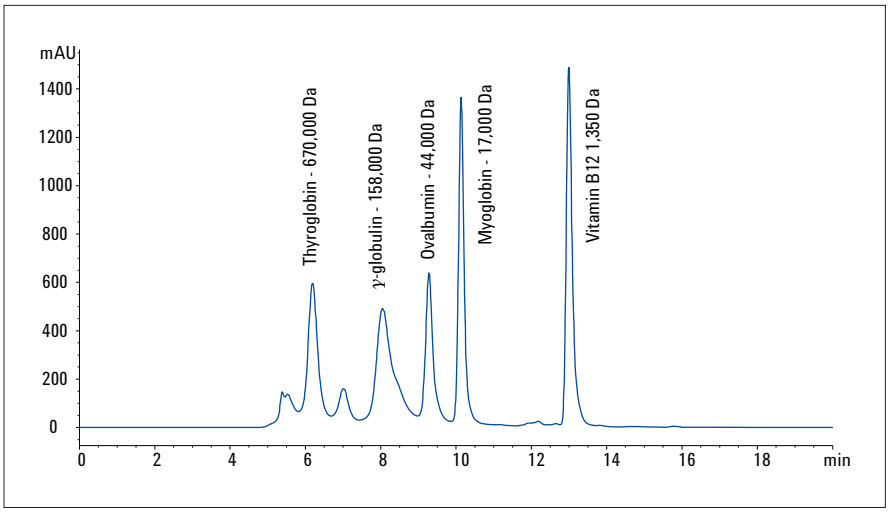

Figure 1
Size exclusion separation of gel filtration standard.

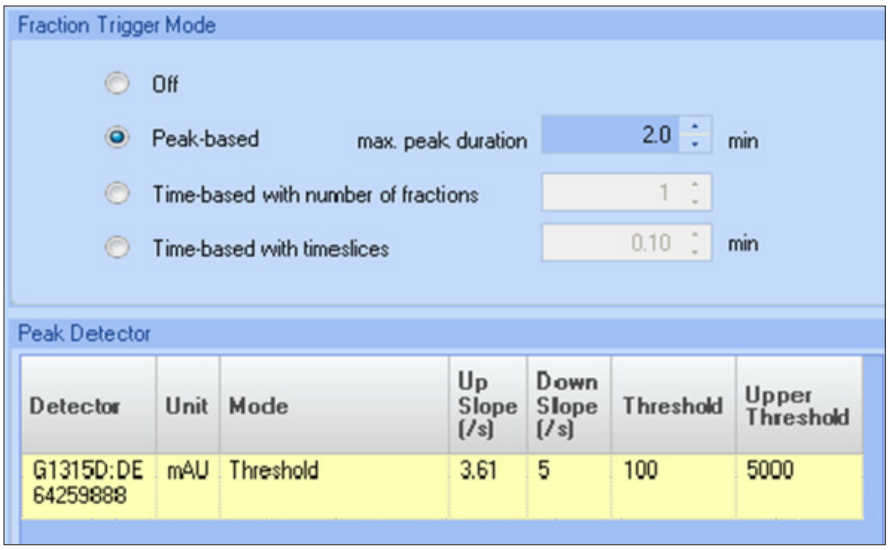

Figure 2
Fraction Trigger Mode and peak detection.

The peak fractionation can be further optimized regarding slope and threshold by loading a previous run into the fraction collector method setup of the ChemStation. The software automatically calculates the resulting fractions (Figure 3). Therefore, unnecessary fractions can be prevented, which accelerates the workflow. Furthermore, patented fraction delay calibration prior to analysis enables the user to gain superior recovery and purity[1].

After SEC, the fractions were automatically collected using peak-based fraction trigger mode into ascertained wells of a deep well plate. Afterwards, the fractions were re-analyzed using a reversed phase C8 column (Figure 4). The re-analysis of the fractions confirms the exact fractionation procedure using peak-based fraction trigger mode. With the use of the 2-position/6-port valve in the column compartment, automated column switching was enabled for re-analysis.

Conclusion

The Agilent 1260 Infinity Bio-inert Quaternary LC together with the Agilent 1260 Infinity Bio-inert Fraction Collector provides automated peak-based (in addition to time-based) fraction collection within a metal-free environment. The patented fraction delay calibration prior to analysis provides superior recovery and purity. In combination with a 2-position/6-port valve in the thermostatted column compartment, further automation is possible due to automated column switching for re-analysis of the fractions.

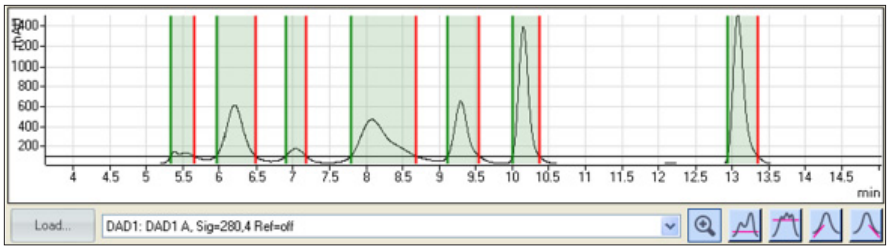

Figure 3
Adapting peak detection within the method setup of the ChemStation.

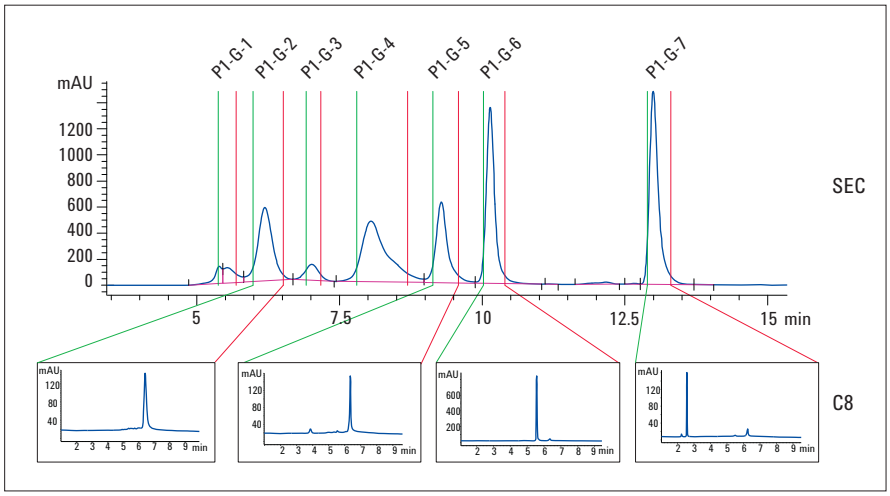

Figure 4
Peak based fractionation.

Reference

1.
"Principles in preparative HPLC";
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www.agilent.com/chem/bio-inert

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