TOSOH

A New Paradigm in Light Scattering Technology

LENS™ 3

Multi-Angle Laser Light Scattering

TOSOH BIOSCIENCE

LenS3 Multi-Angle Light Scattering Detector

A Revolutionary Technology for Macromolecular Characterization

The LenS3 Multi-Angle Light Scattering Detector offers a revolutionary approach for the measurement of molecular weight (MW) and radius of gyration (R_g) of synthetic polymers, polysaccharides, proteins, and biopolymers. LenS3 is accompanied by SECview Software, offering seamless versatility from data acquisition to processing. Highlighted in *Figure 1* are the features and benefits of this truly innovative detector.

Figure 1. Performance and highlights of the LenS3 Multi-Angle Light Scattering detector

Highest Sensitivity	• A unique patent pending optics design • Green light source: ~2.7× higher scattering intensity
Extended R_g Measurement Range	• A new calculation method using the Angular Dissymmetry Plot • Patent pending revolutionary normalization procedure • Better signal-to-noise ratio with improved electronics and optics
HPLC/UHPLC Compatibility	• High acquisition rate (up to 50 Hz) • Allows usage of semi-micro HPLC columns and narrow-bore UHPLC columns
Powerful and Intuitive Software	• Simultaneous multi-method execution and analysis • Simple calibration procedures • MW and R_g in a few clicks • *dn/dc* and UV extinction coefficient measurement

Get Started

Additional resources are available for helping you implement the LenS3 Multi-Angle Light Scattering Detector into your laboratory.

Web

Visit **tosohbioscience.de** for product information and ordering.

Email

Our technical service staff is ready to answer questions: techsupport.tbg@tosoh.com

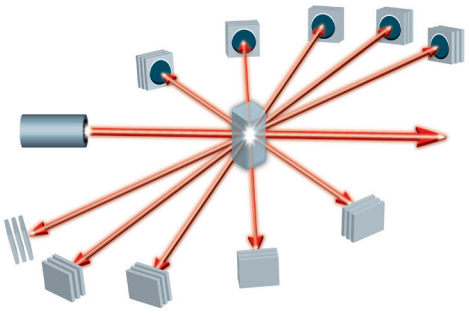
In Person

A technical seminar can be arranged on-site or via the web. Request via sales-marketing.tbg@tosoh.com

Revolutionary Detector Design

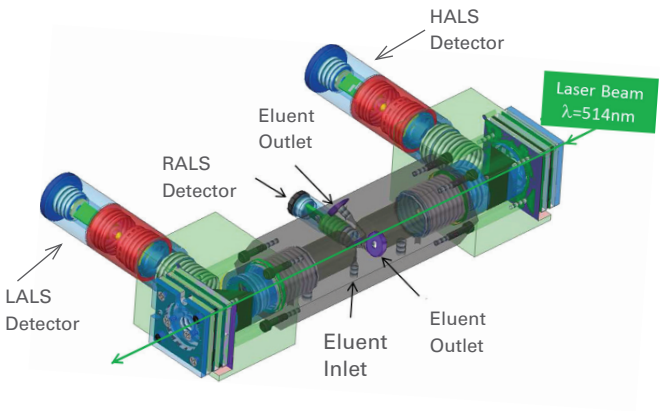
Multi-Angle Light Scattering (MALS) detectors have become a common tool to determine MW and size of macromolecules (Figure 2). The theory is based on the Rayleigh equation, where the intensity of the scattered light, R_θ, is directly related to molecular weight of macromolecules. Using the traditional Zimm method, one can determine molecular weight, radius of gyration and the second virial coefficient of large scattering molecules on the basis of angular and concentration dependence measurements of the intensity of scattered light from dilute solutions.

Figure 2. Typical MALS detector design



Alternatively, MW can be obtained accurately from a Low Angle Light Scattering (LALS) detector directly without angular extrapolation. The LenS3 Multi-Angle Light Scattering detector is a revolutionary technology that combines the best of both MALS and LALS detectors. It does not contain a conventional cell and offers an extended flow path that uses 3 angles to provide MALS and LALS analysis, as depicted in Figure 3.

Figure 3. LenS3 MALS detector design



The angles are fixed at 10° (LALS), 90° (RALS) and 170° (HALS), while the inlet flow is split into two at the entrance of the measurement path and exits at two separate outlets.

Additionally, a green laser (λ = 505 nm) provides approximately 2.7 times higher scattering intensity than a conventional red laser (λ =660 nm). Greater sensitivity is also provided by the unique design of the light path, as opposed to a conventional flow cell, which allows maximum interaction with solute molecules and a more effective light collection mechanism with lower noise (see Figure 4).

Figure 4. Cell block assembly and flow path of the LenS3 MALS detector

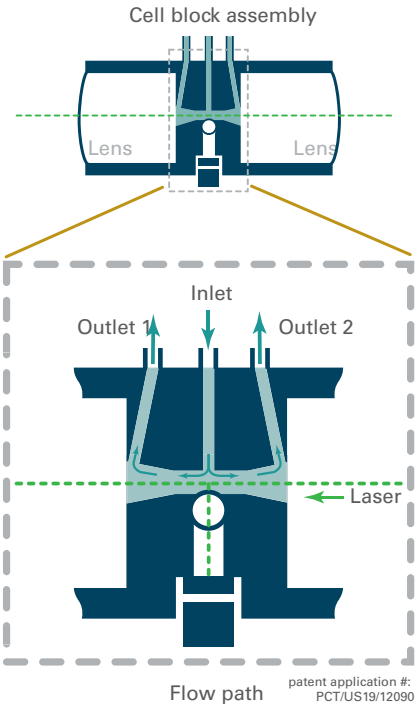
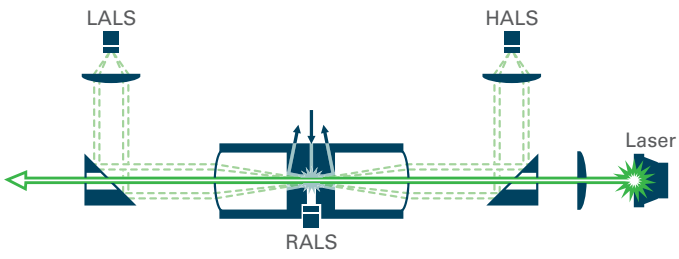


Figure 5. Optics design of LenS3 MALS detector



Revolutionary Calculation Method

The LenS3 MALS detector's number and the positions of the detector angles enable a unique and unmatched capability to offer users multiple calculation options for MW and Rg.

MW determination options:

- *Direct measurement using 10°*
- *Direct measurement using 90°*
- No longer necessary are Zimm plot extrapolations using multi-angle measurement

Rg determination options*:

- *A patent pending method using a novel Angular Dissymmetry Plot (no concentraition information necessary)*
- Replaces the historic assumption of total isotropic scattering for lower molecular size

The revolutionary calculation method for Rg measurement using the angular dissymmetry plot addresses the two major limitations of the Zimm method: enhanced signal to noise allows lower Rg measurements (Rg < 10 nm) without requiring solute concentration. This approach uses a proprietary normalization procedure in addition to lower wavelength light a and enhanced optics to accomplish lower size measurements. It requires a minimum of 3 angles with one at the lowest and the other at the highest possible angle positions to provide Rg data from below 10 nm to over 50 nm.

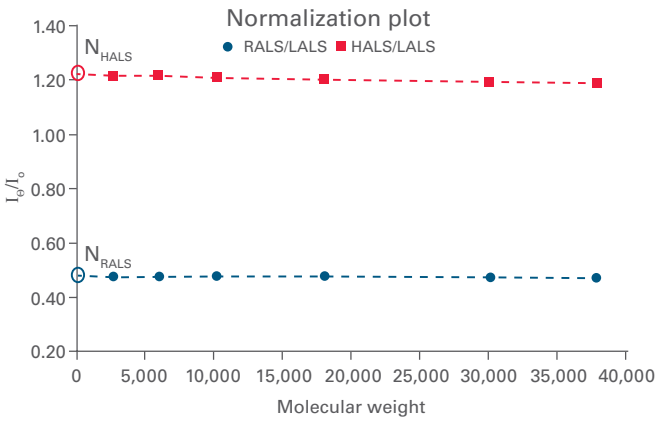
* patent application #: PCT/US19/12095: Light Scattering Detectors and Methods for the Same

Rouzeau, S.; A New Paradigm in Light Scattering Technology in Decades, 2019.

"True" Normalization Factors

Historically, the angular normalization factors are determined around a so-called "isotropic" standard for which the existing MALS technology cannot detect angular dissymmetry. With the new level of sensitivity and the strategic position of the LenS3 detectors (the extreme low at 10° and the extreme high at 170°), the angular dissymmetry can now be measured at a much lower range. The new angular normalization process considers the detectors' responses to varying sizes or molecular weights of a series of known standards. This relationship provides a whole new insight into the "true" angular normalization factors that were undetectable up to now!

Using the underlying assumption that $I_0 \approx I_{LALS}$, the normalization factors can be obtained from the below extrapolation plot independent of the standard type:



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Accurate Molecular Weight and R_g Determination

Molecular weight and radius of gyration are measured accurately across the entire distribution with the LenS3 detector.

System & Columns

- EcoSEC® GPC System with RI detector
- LenS3 Multi-Angle Light Scattering detector
- 1 × TSKgel® GMHHR-H (7.8 mm ID × 30 cm) column

Samples & Conditions

- NIST SRM706a broad polystyrene (PS) standard
- THF at 1.0 mL/min; T = 35 °C (pumps, column and RI)
- Concentration = 0.77 mg/mL; injection volume = 20 µL

Figure 6. LALS (red), RALS (blue), HALS (green) and RI (purple) signals of the NIST SRM706a standard

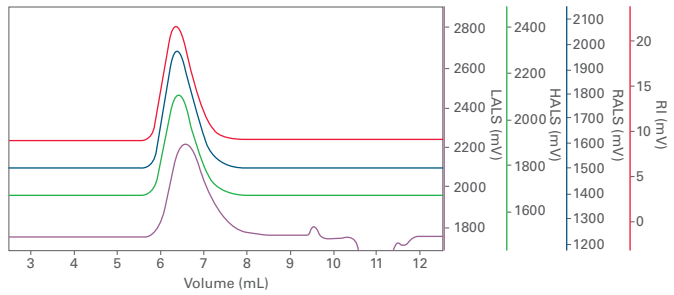

Figure 7. Molecular weight (green) and R_g (blue) distributions overlaid with RI and LALS signals

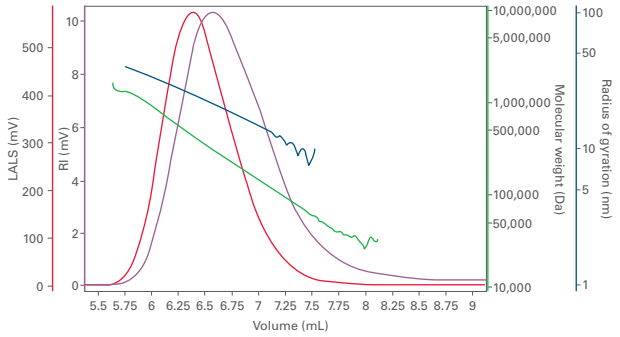

Table 1. Measured values of molecular weight and radius of gyration of the NIST SRM706a PS standard by light scattering

Parameter	From LenS3 MALS	Literature values
M_n (g/mol)	169,030	-
M_w (g/mol)	289,900	285,000 ± *23,000**
M_z (g/mol)	426,780	-
$PDI = M_w/M_n$	1.72	-
$R_{g,z}$ (nm)	27.8	27.8 ± *1**

* SRM 706; *Polystyrene (Broad Molecular Weight Distribution)*; National Bureau of Standards; U.S. Department of Commerce: Washington, DC (1967); available at https://www-s.nist.gov/srmors/certificates/706a.pdf (accessed Mar 2019).

Podzimek, S.; *Light Scattering, Size Exclusion Chromatography and Asymmetric Flow Field Flow Fractionation*; Wiley, 2011; p 233.

Radius of Gyration Below 10 nm

R_g values of small polymers below 10 nm are reliably measured by light scattering for the first time in history with the LenS3 detector!

System & Columns

- EcoSEC GPC System with RI detector
- LenS3 Multi-Angle Light Scattering detector
- 1 × TSKgel GMH$_{HR}$-H (7.8 mm ID × 30 cm) column

Samples & Conditions

- Polystyrene (PS) standards below 100K
- THF at 1.0 mL/min; T = 35 °C (pumps, column and RI)
- Concentration = 1 - 3 mg/mL; injection volume = 50 µL

Figure 8. LALS (red), RALS (blue) and HALS (green) signals with baselines and integration limits for PS 30K standard

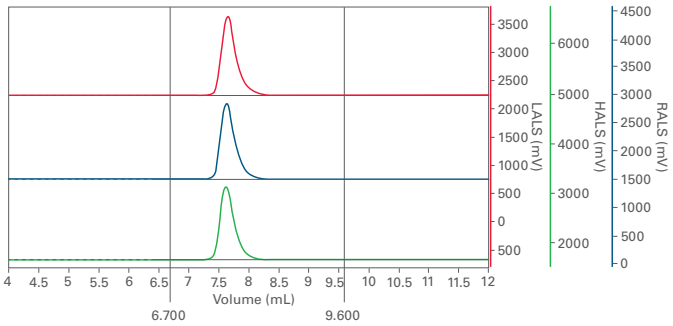

Figure 9. Angular dissymmetry plot for PS 30K

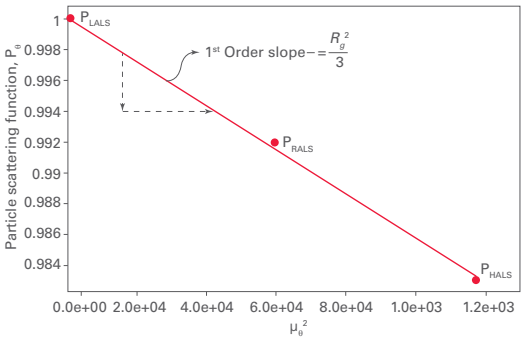

Table 2. Measured values of radius of gyration, $R_{g,z}$, of PS standards

Sample	M_w	$R_{g,z}$	Literature values*
PS100K	100,400	12.0	13.2
PS40K	40,800	7.6	8.1
PS30K	30,100	6.6	6.8
PS20K	18,600	5.5	5.3
PS10K	10,700	4.6	3.9

* Literature values calculated using the correlation $R_g = 0.0245 \cdot MW^{0.546}$

where,

$$\mu_\theta = \frac{4\pi n_0 \mathrm{Sin}\left(\frac{\theta}{2}\right)}{\lambda}$$

n_0 = refractive index of mobile phase

λ = wavelength of laser

$$P_\theta = N_\theta \cdot \frac{I_\theta}{I_0}$$

N_θ = normalization factor for angle θ

I_θ = scattering intensity for angle θ

I_0 = scattering intensity for angle 0

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Ultra Low Molecular Weight Oligomers by LALS

The Low Angle Light Scattering detector is capable of measuring molecular weight down to 200 Da!

System & Columns

- EcoSEC GPC System with RI detector
- LenS3 Multi-Angle Light Scattering detector
- 1 × G3000HXL, 1 × G2500HXL and 1 × G2000HXL (all 7.8 mm ID × 30 cm) columns

Samples & Conditions

- Tosoh A-500 polystyrene (PS) standard
- THF at 1.0 mL/min; T = 35 °C (pumps, column and RI)
- Concentration = 8.54 mg/mL; injection volume = 50 µL

Figure 10. LALS (red) and RI (purple) signals of the A-500 PS standard

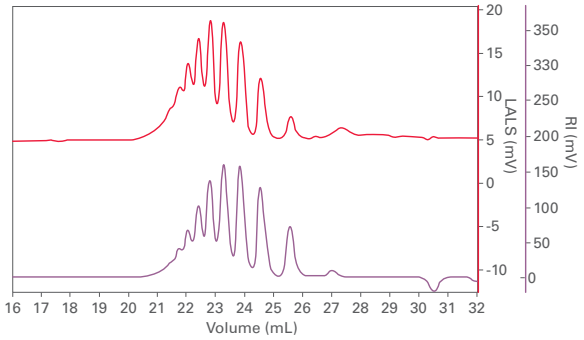

Figure 11. Molecular weight distribution (green) overlaid with RI signal

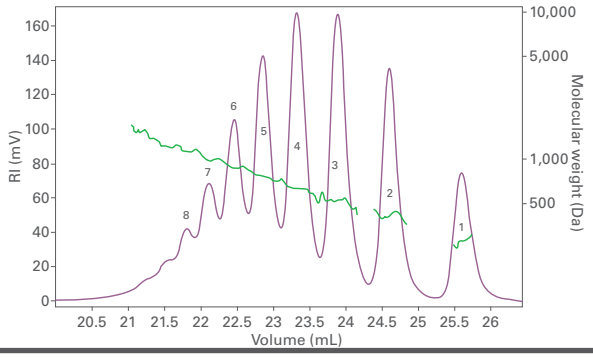

Table 3. Theoretical molecular weights of the oligomers present in A-500 and M_p values obtained from LALS (using an average *dn/dc* of 0.170)

Peak #	Theoretical MW	M_p from LALS
1	266	255
2	370	377
3	474	503
4	578	618
5	682	725
6	786	849
7	890	985
8	994	1105

Superior Sensitivity

The LenS3 Multi-Angle Light Scattering detector achieves repeatable molecular weight measurement down to 15 ng of PS 100K in THF!

System & Columns

- EcoSEC GPC System with RI detector
- LenS3 Multi-Angle Light Scattering detector
- 1 × TSKgel GMHHR-H (7.8 mm ID × 30 cm) column

Samples & Conditions

- Tosoh 100K polystyrene (PS) standard F-10
- THF at 1.0 mL/min; T = 35 °C (pumps, column and RI)
- Decreasing injected mass

Figure 12. LALS signals for decreasing injected mass of PS 100K standard F-10

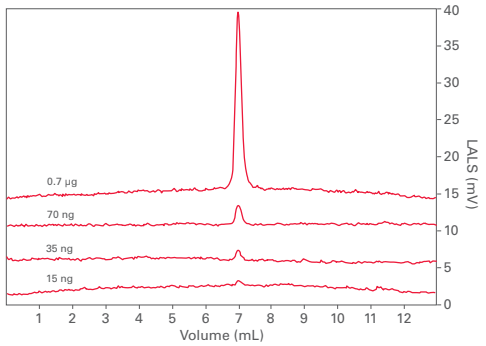

Figure 13. RALS signals for decreasing injected mass of PS 100K standard F-10

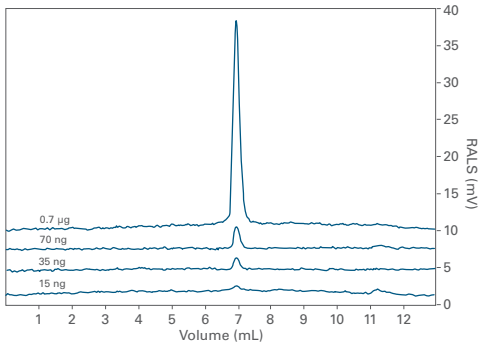

Table 4. Molecular weights by LALS and RALS for decreasing injected mass of PS 100K standard F-10

Injected mass	MW by LALS	MW by RALS
0.7 µg	102,110	101,570
70 ng	101,520	100,960
35 ng	101,150	100,870
15 ng – inj. 1	-	102,180
15 ng – inj. 2	-	100,980
15 ng – inj. 3	-	101,390
15 ng – inj. 4	-	99,800
15 ng – inj. 5	-	100,760
15 ng – Average	-	101,020 ± *780 (0.77%)*

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Protein Aggregates and Fragments Identification and Quantitation

Oligomers and fragments of BSA and monoclonal antibodies are easily detected and identified with the LenS3 detector.

System & Columns

- Thermo Fisher Dionex Ultimate® 3000 UHPLC system with UV detector @ 280 nm
- LenS3 Multi-Angle Light Scattering detector
- 1 × TSKgel UP-SW3000 (4.6 mm ID × 30 cm) column

Samples & Conditions

- Bovine Serum Albumin (BSA) and Herceptin® biosimilar
- 100 mmol/L NaH_2PO_4, pH 6.8 + 100 mmol/L Na_2SO_4
- Flow rate = 0.25 mL/min
- Concentration:
  BSA = 3.58 mg/mL, injection volume = 10 µL
  mAb = 2.75 mg/mL, injection volume = 7 µL

Figure 14. Raw chromatograms of BSA and Herceptin biosimilar samples, with light scattering and UV signals

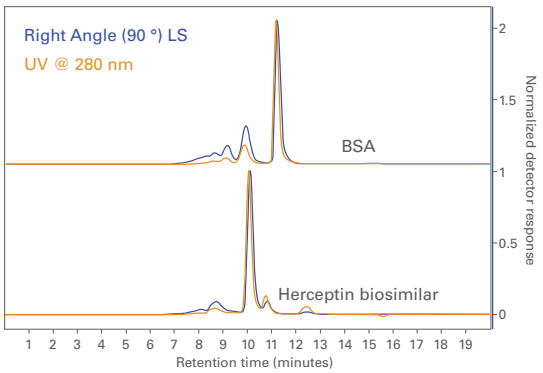
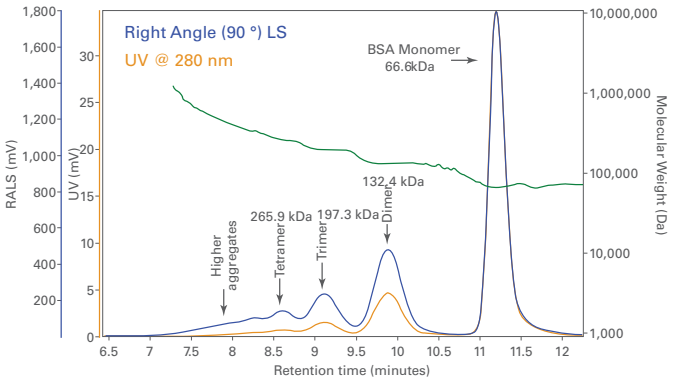
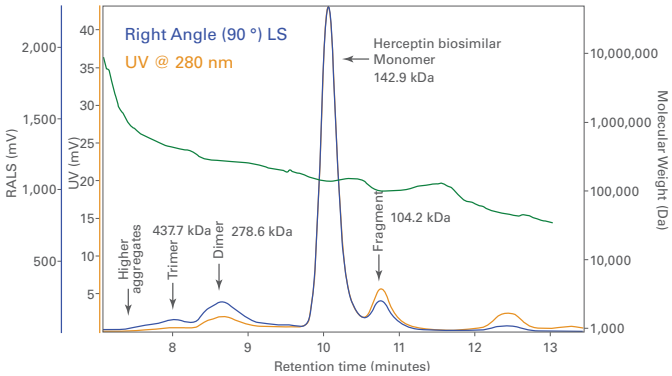


Figure 15A. Molecular weight (green) distribution curve and values of BSA oligomers overlaid with UV (orange) and RALS (blue) signals



Peak	MW (kDa)	Area (%)
Monomer	66.6	74.1
Dimer	132.4	16.7
Trimer	197.3	5.4
Tetramer	265.9	2.1
Aggregates	Up to 1,000+	1.7

Figure 15B. Molecular weight (green) distribution curve and values of Herceptin biosimilar overlaid with UV (orange) and RALS (blue) signals



Peak	MW (kDa)	Area (%)
Fragment	104.2	11.8
Monomer	142.9	69.1
Dimer	278.6	8.3
Trimer	437.7	1.3
Aggregates	Up to 5,000+	0.3

Superior Sensitivity for mAb

Superior sensitivity for mAbs can be demonstrated down to 2 ng of sample loading.

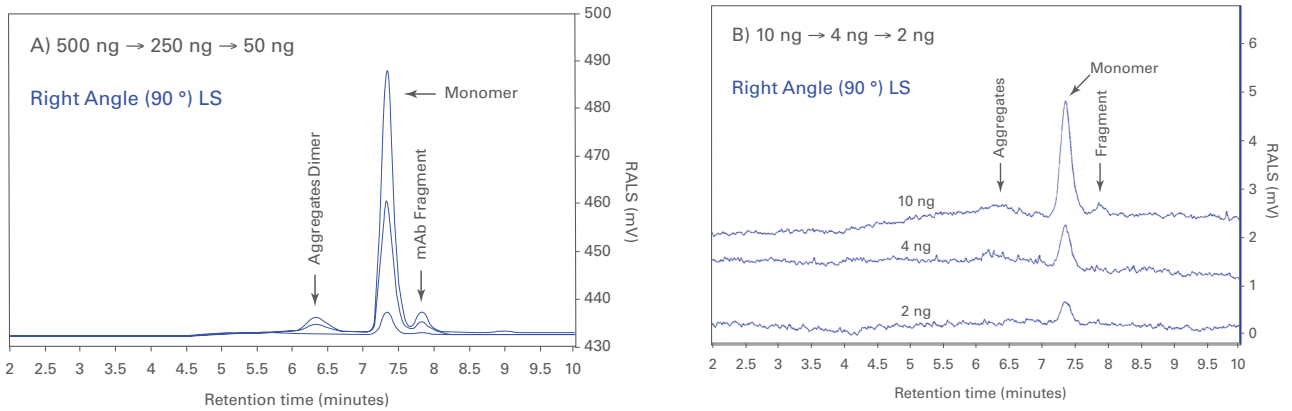
System & Columns

- Thermo Fisher Dionex Ultimate 3000 UHPLC system with UV detector @ 280 nm
- LenS3 Multi-Angle Light Scattering detector
- 1 × TSKgel UP-SW3000 (4.6 mm ID × 30 cm) column

Samples & Conditions

- Herceptin biosimilar
- 100 mmol/L NaH_2PO_4, pH 6.8 + 100 mmol/L Na_2SO_4
- Flow rate = 0.35 mL/min
- Injected mass from 500 ng down to 2 ng

Figure 16A and 16B. Light scattering signal (RALS) of Herceptin biosimilar monomer, fragment and aggregates with decreasing injected mass



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Analysis of Unpurified and Purified Oligonucleotides

Rapid and accurate molecular weight profiling of small oligonucleotides is now possible!

System & Columns

- Thermo Fisher Dionex Ultimate 3000 UHPLC system with UV detector @ 260 nm
- LenS3 Multi-Angle Light Scattering detector
- 1 × TSKgel UP-SW2000 (4.6 mm ID × 30 cm) column

Samples & Conditions

- 20-bases custom oligonucleotide with MW=6,141 Da
- 0.5 mol/L NaCl + 0.1 mol/L EDTA + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3 in 0.1 mol/L phosphate buffer, pH 7.52
- Flow rate = 0.30 mL/min
- Injection volume = 10 µL
- Concentration:
  Purified sample = 0.3 mg/mL
  Unpurified sample = 1 mg/mL

Figure 17. RALS (blue) and UV (orange) signals of the unpurified 20-mer

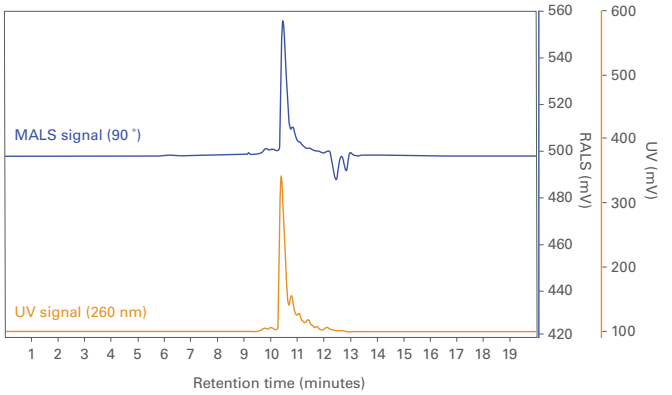

Figure 18. Molecular weight distribution (green) of the unpurified 20-mer

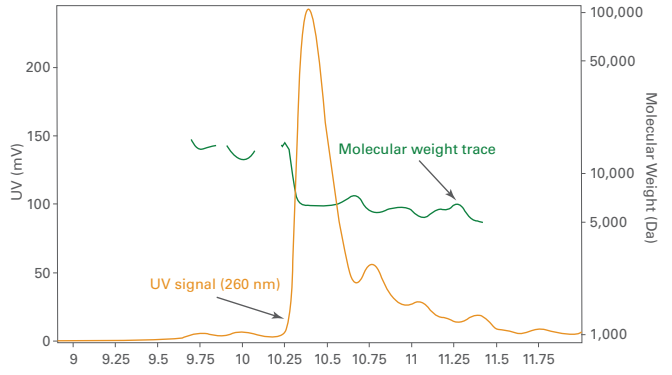

Figure 19. Peak analysis of the unpurified 20-mer

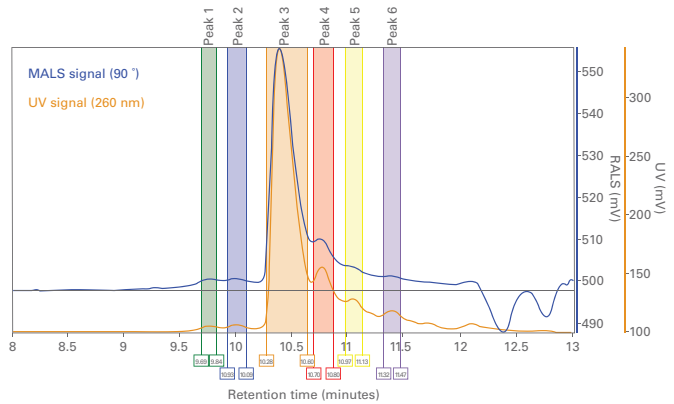

Table 5. Retention time and molecular weight of each peak (triplicate injection)

Peak	Retention time (min)	% RSD	MW (Da)	% RSD
1	9.774	0.1%	13,599	2.1%
2	10.012	0.0%	11,550	1.9%
3	10.398	0.1%	6,398	0.7%
4	10.776	0.1%	5,751	1.5%
5	11.053	0.1%	5,177	2.3%
6	11.422	0.2%	4,446	5.5%

Figure 20. Overlay of the unpurified (green) and purified (red) 20-mer UV chromatograms

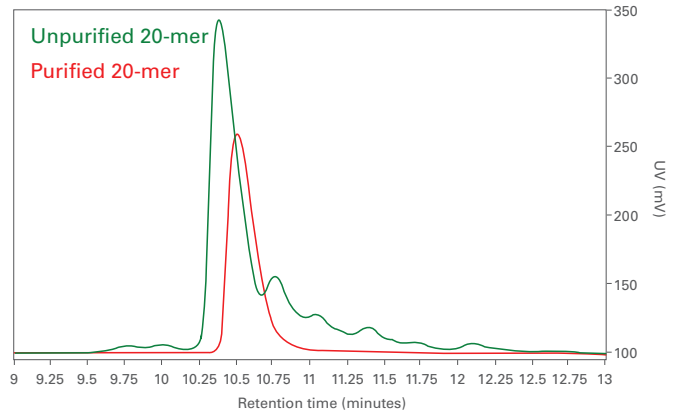

Figure 21. Molecular weight distribution (green) of the purified 20-mer

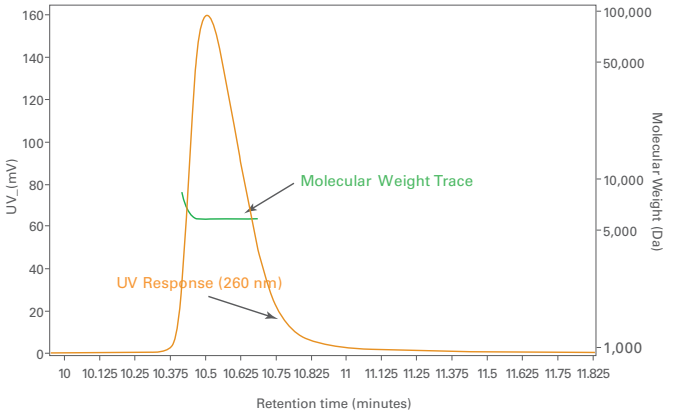

Figure 22. Peak analysis of the purified 20-mer

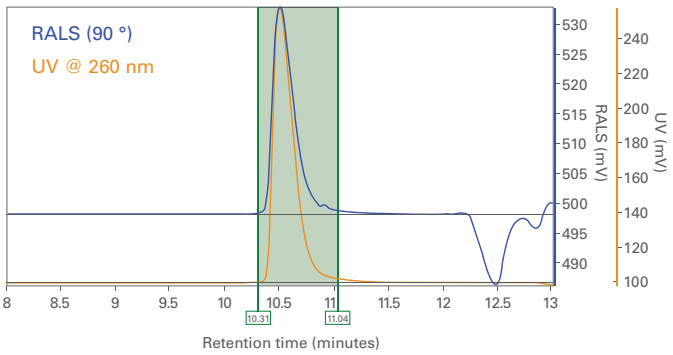

Table 6. Retention time and molecular weight of the purified 20-mer (triplicate injection)

Injection	Retention time (min)	MW (Da)
1	10.431	6,066
2	10.443	6,023
3	10.445	6,038
Average	10.440	6,042
%RSD	***0.1%***	***0.3%***

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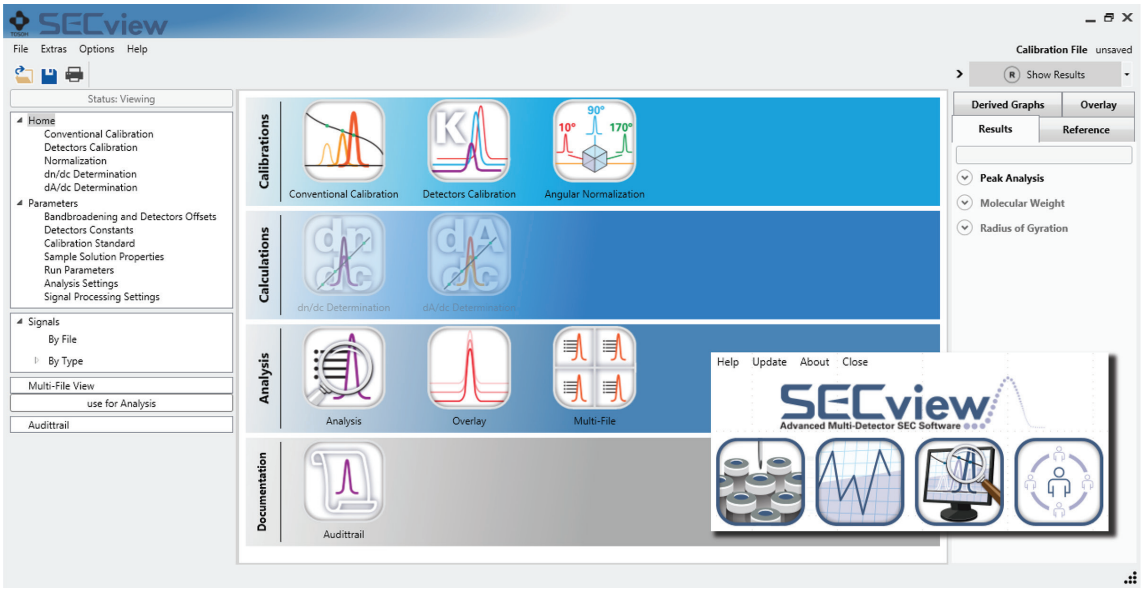
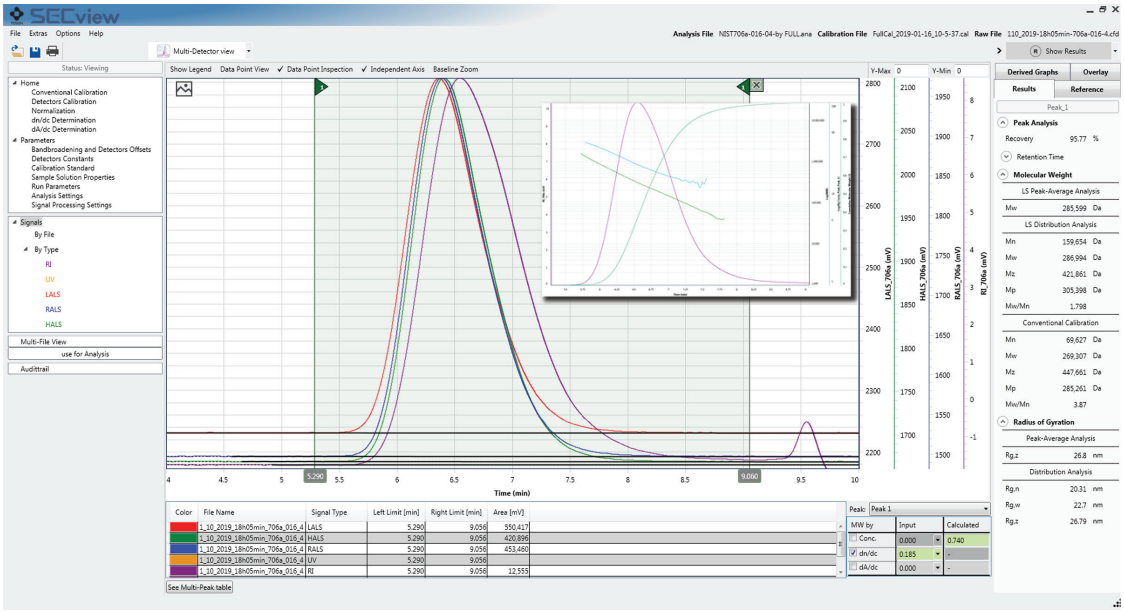
Introducing SECview: the most user-friendly multi-detector GPC/SEC software

Designed from the ground up by the industry's top experts, SECview provides the most intuitive platform, performing basic to the most advanced multi-detector GPC/SEC analysis. Totally focused around enhancing the user-interface experience, SECview is a welcoming fresh perspective that streamlines the complex calculations required by the advanced detectors. This allows users to swiftly obtain results and complete the analysis without lengthy and cumbersome steps.

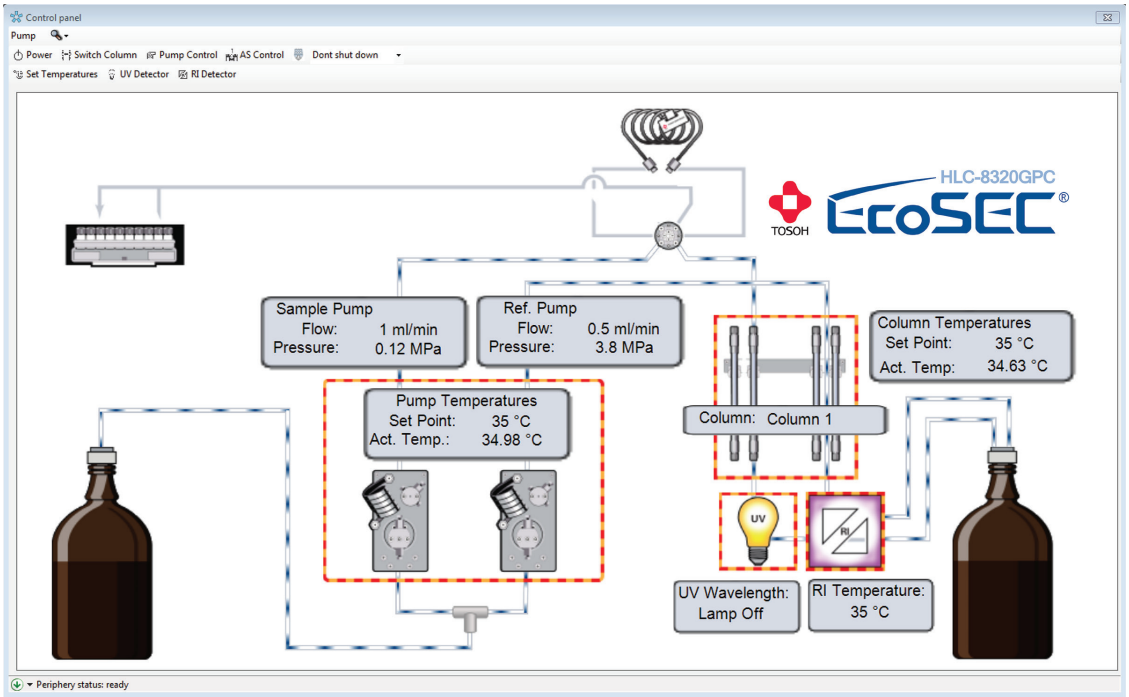


SECview is a total and complete GPC/SEC solution!

Whether the goal is to obtain Absolute Molecular Weight and Radius of Gyration data from the LenS3 MALS detector or to perform Conventional Column Calibration (CCC) for routine GPC/SEC analysis, the new software platform can easily accommodate all data processing techniques. In fact, SECview is the only GPC/SEC data processing module today that performs analysis of sample-of-interest using multiple calibration methods SIMULTANEOUSLY!



When coupling the LenS3 MALS detector with an existing EcoSEC or EcoSEC Elite GPC System, SECview is capable of GPC system/hardware control, multi-channel data acquisition and data processing and analysis. Hence, SECview is a powerful software platform that provides a total single-source solution for the most modern and advanced GPC/SEC setup in the market.



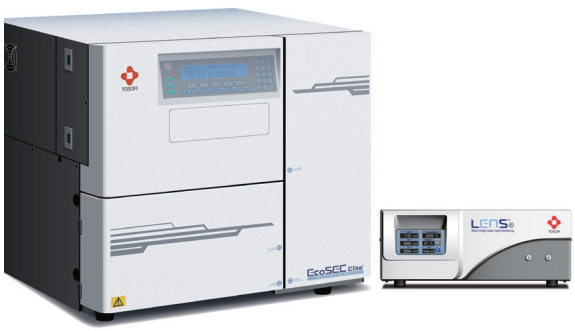
SECview is 21-CFR-Part 11 compliant and provides the necessary tools for audit trails, system validation and multi-user access for users operating in the FDA-regulated environment.

SECview Features

- Simultaneous multi-method execution and analysis
- Multi-point *dn/dc* and UV extinction coefficient determination
- Automatic peak detection for conventional column calibration method
- Multi-peak selection and “independent” data processing
- Adaptable multiple-injection overlay platform
- Advanced peak band broadening and inter-detector volume correction algorithms
- Direct access to the raw data signals while offering powerful de-spiking and smoothing options
- Access to data point cursors on chromatograms and derived graphs
- Easy export of raw data to ASCII files and graph/chromatogram to picture file

SECview Parameters

- Absolute molecular weight by low angle (10°) and right angle (90°) light scattering
- Molecular weight in peak average and distribution *(PDI)* forms
- Radius of gyration by 3 angle MALS using the patent pending angular dissymmetry plot
- Concentration, injection-mass recovery, *dn/dc*, and UV extinction coefficient



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Specifications

Number of measurement angles	3
Position of the measurement angles	LALS (10°) RALS (90°) HALS (170°)
Cell geometry	proprietary conical flow path (single inlet, dual outlets)
Laser source type	diode
Laser power	20 mW +/- 5
Laser wavelength	505 nm
Laser temperature control	yes
Wetted material	teflon, PEEK, glass, stainless steel
Maximum flow rate	2 mL/min
Inlet position	front or side
Baseline noise (in THF @ 1 mL/min)	< 1 mV
Baseline drift (in THF @ 1 mL/min)	< 1 mV / 30 min
MW range	< 200 to 10^7 Da
R_g range	< 2 nm to > 50 nm (in progress)
Acquisition rate	< 1 to 50 Hz, user selectable
A/D board channels / resolution	8 channels / 24 bits
Dynamic range	+/- 10 V
Analog inputs	RI, UV and start signal
Connection to PC	ethernet
Dimensions	36.5 (W) × 48.5 (D) × 13 (H) cm = 14.4″ × 19.1″ × 5.1″
Weight	16 kg = 35 lbs
Intellectual property	PCT/US19/12090: Light Scattering Detectors and Sample Cells for the Same PCT/US19/12095: Light Scattering Detectors and Methods for the Same

Ordering Information

Part #	Description
40000	LenS$_3$ Multi-Angle Light Scattering Detector
40001	UHPLC Conversion Service Kit for LenS$_3$ MALS Detector

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