Application Note 46

Separating Homologs and Polymers by HPLC

Many products contain homologous series of components that must be accurately quantitated to ensure product uniformity. Molecular weight determines which method to use in separating homologs and polymers.

Key Words:
- homologs • polymers • reversed phase HPLC
- normal phase HPLC • size exclusion chromatography

Homologs of many industrial polymers, with molecular weights of up to 10,000 daltons, can be separated by weight on reversed phase or normal phase HPLC columns. For example, Dow Corning® 710 fluid, a polymeric 50% phenyl silicone used as a lubricant or heat exchange fluid, contains a series of homologs of less than 10,000 daltons. The homologs are resolved according to increasing weight by using a 100Å pore reversed phase column, SUPELCOSIL™ LC-18 (octadecylsilyl bonded phase), and a mild gradient. The chromatogram shown in Figure A is typical of reversed phase separations of these homologs.

Normal phase HPLC also can be used to separate polymers with molecular weights of less than 10,000 daltons. This is shown for polyethoxylated octylphenol and nonylphenol surfactants in Figure B. The sample of Triton® X-100, containing an average of 9-10 ethoxy groups, was resolved to baseline on a 25cm SUPELCOSIL LC-Diol column. The surfactant containing 30 ethylene oxide units was analyzed within 40 minutes on the same column.

Homologs with molecular weights greater than 10,000 daltons are usually separated on high performance size exclusion chromatography (SEC) columns. The exclusion limits of SEC columns depend on the size of the pores in the packing particles, with larger pores admitting larger molecules into these particles. By combining, in series, columns having different pores sizes, analysts can expand the range of molecular weights that can be separated at one time. In Figure C, polystyrene standards having molecular weights from less than 1000 to over 1 x 10^6 daltons were sharply separated on a 100Å pore and a 300Å pore column (trimethylsilyl bonded phase). Similarly, these SUPELCOSIL 100Å and 300Å pore columns in series (always install the smaller pore size column in front of the larger pore size column) will satisfactorily separate other polymers with molecular weights between 10^3 and 10^6 daltons. Figure D depicts a typical calibration curve for this pair of columns.

SUPELCOSIL size exclusion chromatography columns contain 5 micron spherical silica particles with 100Å or 300Å diameter pores. For SUPELCOSIL LC-1 and LC-301 columns, the silica is bonded with a trimethylsilyl bonded phase. The dimensions of these HPSEC columns, 25cm x 6.2mm, provide a good compromise between large sample capacity and the most rapid analysis time. Fittings for 1/16" OD tubing are included with each column.

Figure A. Phenyl Silicone Homologs (<10,000 daltons mol. wt.) by Reversed Phase HPLC

Column: **SUPELCOSIL LC-18, 15cm x 4.6mm, 5µm particles**
Cat. No.: **58230**
Mobile Phase: methanol (A)
methanol:tetrahydrofuran, 80:20 (B), 60:40 (C)
100% A to 100% B in 1 min, then to 100% C in 20 min
Flow Rate: 1mL/min
Det.: UV, 254nm
Inj.: 20µL, 100mg DC-710/mL

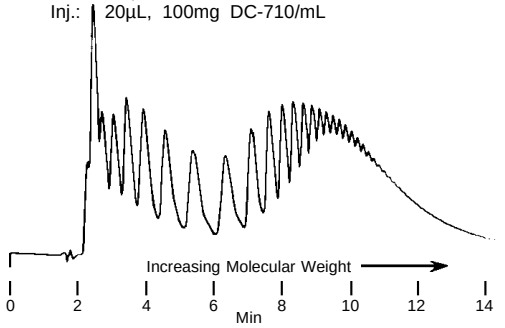

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Figure B. Nonionic Surfactants by Normal Phase HPLC

Column: **SUPELCOSIL LC-Diol, 25cm x 4.6mm, 5µm particles**
Cat. No.: **58201**
Mobile Phase: hexane:methylene chloride, 95:5 (A),
hexane:methylene chloride:methanol, 50:40:10 (B)
70% A to 50% A in 8 min, to 40% A in 7 min, to 20% A in 10 min, to 0% A in 10 min
Flow Rate: 1mL/min
Temp.: 35°C
Det.: UV, 280nm
Inj.: 10µL methylene chloride containing 20µg nonionic surfactant

Triton X-100: 9-10 Ethoxy Groups (Octylphenol)

Tergitol: 30 Ethoxy Groups (Nonylphenol)

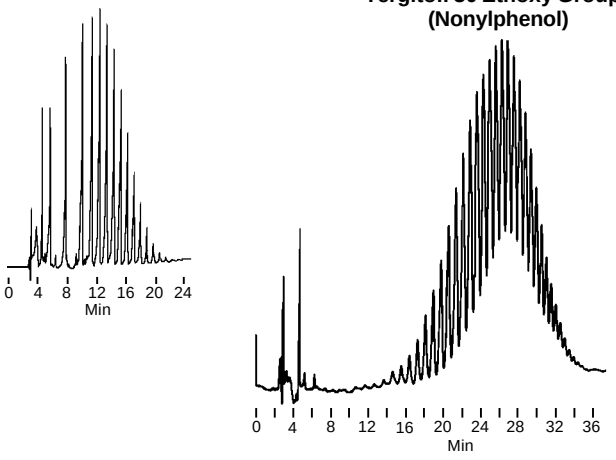

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Figure C. Polystyrene Standards Using Size Exclusion Columns in Series

Columns: **SUPELCOSIL LC-1, 25cm x 6.2mm, and SUPELCOSIL LC-301, 25cm x 6.2mm, in series**
Cat. Nos.: **58961** **58966**
Mobile Phase: tetrahydrofuran
Flow Rate: 1mL/min
Temp.: 45°C
Det.: UV, 254nm
Inj.: 10µL tetrahydrofuran, amounts given on figure

1. 1,800,000 (3.3µg)
2. 300,000 (3.3µg)
3. 100,000 (3.3µg)
4. 35,000 (5.0µg)
5. 17,500 (6.7µg)
6. 9,000 (6.7µg)
7. 2,000 (10.0µg)
8. Toluene (1.7µg) Mol. Wt. = 92

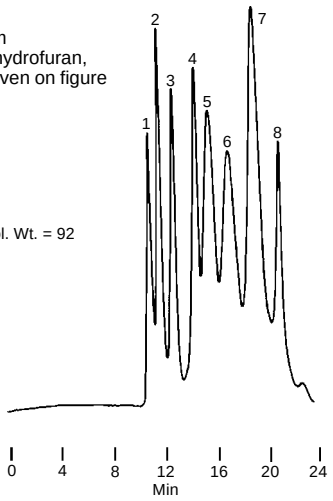

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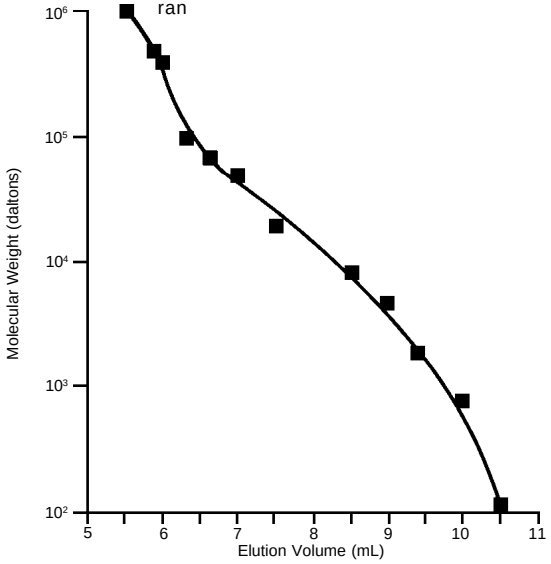
Figure D. 100Å Pore and 300Å Pore HPSEC Columns in Series Separate Homologs Having a Wide Molecular Weight Range

Columns: **SUPELCOSIL LC-1, 25cm x 6.2mm, and SUPELCOSIL LC-301, 25cm x 6.2mm, in series**
Cat. Nos.: **58961** **58966**
Mobile Phase: tetrahydrofuran
Flow Rate: 1mL/min
Temp.: 45°C
Det.: UV, 254nm
Inj.: 20µL each, 2mg/mL polystyrene standards in tetrahydrofuran



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Size Exclusion Columns (25cm x 6.2mm ID, 5µm packings)

SUPELCOSIL Column	Pore Diameter	Cat. No.
Silica		
LC-Si	100Å	**58963**
LC-3Si	300Å	**58965**
Trimethylsilyl Bonded Phase		
LC-1	100Å	**58961**
LC-301	300Å	**58966**
Diol Bonded Phase		
LC-Diol	100Å	**58967**
LC-3Diol	300Å	**58968**

SUPELCOSIL LC-18 and LC-Diol Columns (5µm packing, 100Å Pores)

Dimensions	Cat. No.
SUPELCOSIL LC-18 Modular Column	
25cm x 4.6mm	**58298**
Supelguard LC-18 Columns, pk. of 2	**59564**
SUPELCOSIL LC-Diol Modular Column	
25cm x 4.6mm	**58201**
Supelguard LC-Diol Columns, pk. of 2	**59569**
Cartridge Guard Column Holder	**55205**
(connects guard column directly to modular column)	

Mono-Disperse Polystyrene Standards (500mg bottles)

Nominal Molecular Wt.	M_w/M_n	Cat. No.
800	1.30	**45700**
4,000	1.06	**45702**
35,000	1.06	**45705**
50,000	1.06	**45706**
100,000	1.06	**45707**
233,000	1.06	**45708**
300,000	1.06	**45709**
600,000	1.10	**45710**
900,000	1.10	**45711**

Polystyrene Standards Kits, 250mg of each polymer

Low Molecular Weight Standard Kit **48937**

2500 MW	17,500 MW
5000 MW	30,000 MW
9000 MW	50,000 MW

High Molecular Weight Standard Kit **48938**

110,000 MW	600,000 MW
220,000 MW	900,000 MW
400,000 MW	1,800,000 MW

Note 46

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