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Application News

Nexera™ Method Scouting System High Performance Liquid Chromatograph

High Efficiency in Investigation of Polymer Separation Based on Composition Using Gradient Polymer Elution Chromatography (GPEC)

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User Benefits

- Unlike GPC, GPEC enables separation of polymers independent of their molecular weight, supporting multifaceted polymer analysis.
- The Nexera Method Scouting System can realize significant labor savings and high efficiency in method development when investigating the separation conditions for GPEC, in which selection of the mobile phase composition to be used and adjustment of the gradient conditions are particularly important.

■ Introduction

In polymer materials, analysis and confirmation of the polymer composition in the processes from research and development to quality control are extremely important because the composition has a large influence on the physical properties of the material. Among these polymers, copolymers are polymers which are composed of multiple types of monomers, and the properties of copolymer materials are also known to differ due to differences in the composition and arrangement of their constituent monomers. Therefore, identification and isolation of those components in copolymers are important.

Size exclusion chromatography (SEC)/gel permeation chromatography (GPC), in which separation is conducted based on polymer size, is generally used as a polymer separation technique. Because the constituent polymers can be separated by molecular weight, it is possible to obtain information such as the molecular weight distribution, but when the sample contains a mixture of polymers with similar molecular weights and different compositions, separation is not possible. The technique called gradient polymer elution chromatography (GPEC) is useful in such cases, as the polymers are separated according to differences in their composition, independent of the size of the polymer. In the GPEC separation technique, the sample material is injected in a mobile phase with a composition (poor solvent) in which the target polymer is insoluble, intentionally precipitated in the flow path, for example, at the end of the column, and then redissolved and eluted in the flow path by applying a gradient to the mobile phase composition to a composition (good solvent) that easily dissolves the target polymers. Since applying a gradient to the mobile phase composition makes it possible to separate polymers based on differences in their solubility in the solvent, the polymers can be separated according to their compositions. However, because investigation of the GPEC analytical conditions is frequently based on experience, the analytical conditions must be investigated individually for each sample. Thus, the time and trouble required in investigating the separation conditions for GPEC has been a drawback of this technique.

The Nexera Method Scouting System can achieve high efficiency in method development work, because the analytical conditions can be screened by automatically changing the type and composition of solvents for the mobile phase, the column to be used, and other analytical conditions. This article introduces an example in which the conditions for a GPEC analysis were investigated efficiently by using the Nexera Method Scouting System and dedicated software Method Scouting Solution, and an analysis of a copolymer and its constituent homopolymers (polymers consisting of only one monomer) was carried out by the GPEC separation technique.

■ Samples

Table 1 shows the copolymer used in the analysis and its constituent homopolymers. The weight-average molecular weights (Mw) of all of the polymers were around 1.0 x 10^4. The samples were all dissolved in THF and adjusted to a concentration of 0.2 %.

Table 1 Sample Materials

Polymer Name	Name
Polystyrene	Homopolymer-A
Poly(*tert*-Butyl Acrylate)	Homopolymer-B
Poly(*tert*-Butyl Acrylate)-*block*-Poly(styrene)	Copolymer (10:11)

■ GPC Analysis

Table 2 shows the analytical conditions used in the GPC analysis of the samples shown in Table 1, and Fig. 1 shows the GPC chromatograms. Because both the homopolymers and the copolymer have weight-average molecular weights of around 1.0 × 10^4, complete separation of the peaks was not possible by GPC.

Table 2 GPC Analytical Conditions

Column	: Shim-pack™ GPC-8025*1 (300 mm × 8.0 mm I.D., 6 µm)
Mobile phase	: THF (without stabilizer)
Flow rate	: 1.0 mL/min
Column temp.	: 40 ˚C
Injection vol.	: 10 µL
Vial	: SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
Detection	: Refractive index (RID-20A)
	Polarity : +
	Cell temp. : 40 ˚C
	Response : 1.5 sec

*1 P/N: 228-20805-91
*2 P/N: 227-34001-01

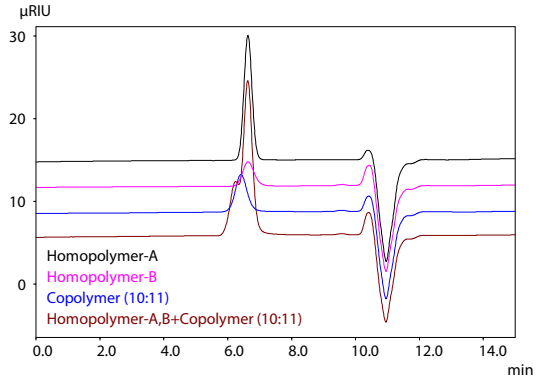


Fig. 1 GPC Chromatograms of Single Samples of Homopolymers and Copolymer and Mixed Sample

■ Improvement of Efficiency of Method Development in GPEC

In method development for GPEC, investigation of the analytical conditions requires considerable time and work because various conditions such as the type of solvent used to precipitate the target polymer when injected, the type of solvent and profile of the gradient for gradient redissolution, the column used, and the column temperature and flow rate, are changed and examined based on the experience of skilled analyst.
Therefore, in this article, efficient GPEC method development was conducted by using the Nexera Method Scouting System and dedicated software Method Scouting Solution. Fig. 2 shows the setting screen of Method Scouting Solution. Analytical conditions can be investigated by automatically changing the column and mobile phase, and automatic preparation of the mobile phase by online is also possible by mixing the solvents at any desired ratio utilizing the mobile phase blending function of the Method Scouting System, using only a small number of pre-prepared solvents. System operation is intuitive, and because it is possible to create methods with automatically changed conditions, including the flow rate, oven temperature, and gradient time program, it is not necessary to create a new method for each condition under investigation.
For more information on the features of this system and software, please see Application News 01-00018.

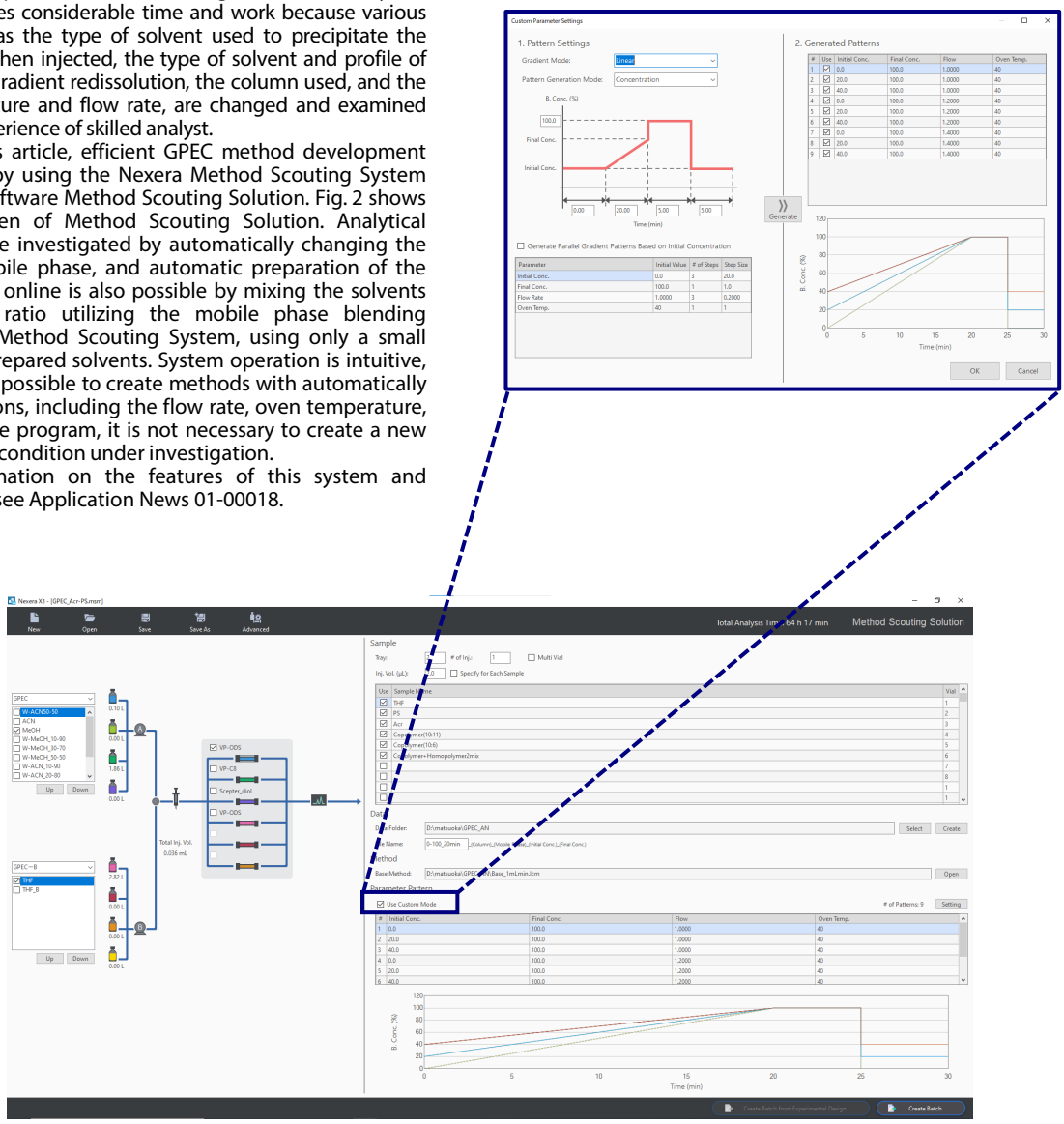


Fig. 2 Setting Screen of Method Scouting Solution

■ Investigation of GPEC Conditions

Here, the GPEC analytical conditions for the 3 sample materials shown in Table 1 were investigated by using the Nexera Method Scouting System. First, the mobile phase and column conditions were investigated, and the flow rate of the mobile phase and the gradient conditions were then investigated using the optimum mobile phase and column. Under all conditions, the column was washed with a good solvent every 5 minutes after completion of gradient elution, and the column was equilibrated for the next analysis.

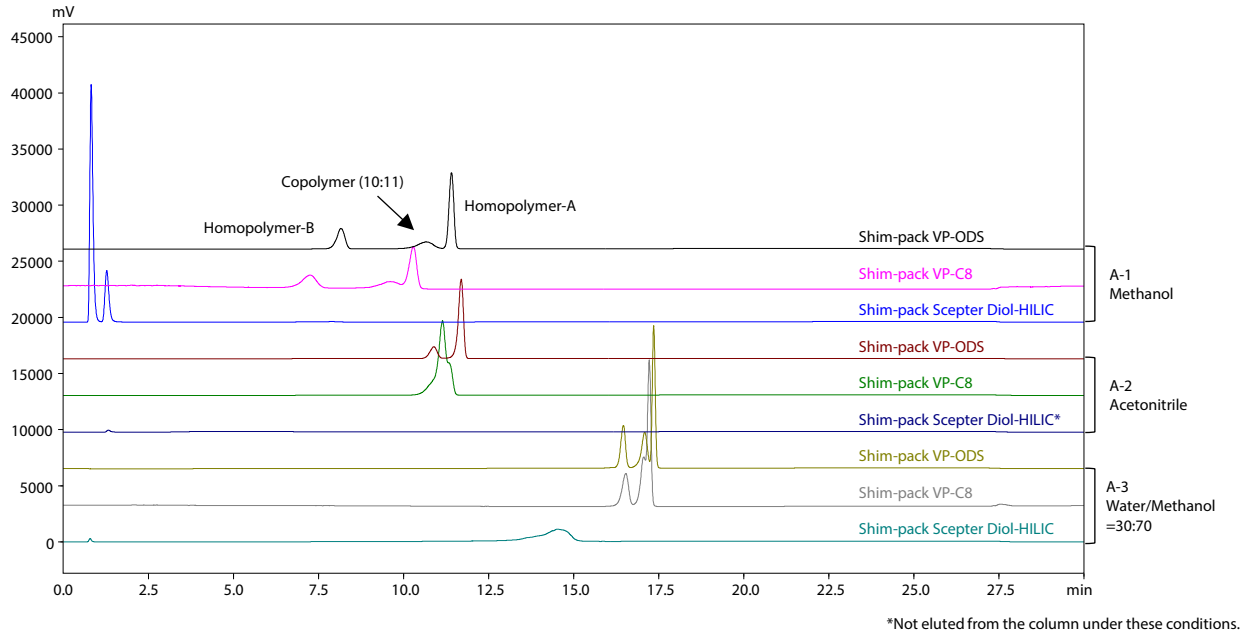
• Investigation of Mobile Phase and Column

Table 3 shows the analytical conditions studied here, and Fig. 3 shows the chromatogram of the mixed sample of the 3 polymers in Table 1. In the mobile phase, the selection of the poor solvent for use in the mobile phase A solution was investigated based on use of the good solvent THF in mobile phase B. Since 3 columns and 3 mobile phase A solutions were considered, a total of 9 combinations were examined. For the mobile phase condition (A-3), the solution was prepared automatically online by using the mobile phase blending function of the Nexera Method Scouting System. As gradient separation is necessary in GPEC, an evaporative light scattering detector (ELSD) was used in detection rather than a refractive index detector (RID).

Table 3 HPLC Conditions

Column	: 1) Shim-pack VP-ODS*1 (150 mm × 4.6 mm I.D., 5 µm)	
	: 2) Shim-pack VP-C8*2 (150 mm × 4.6 mm I.D., 5 µm)	
	: 3) Shim-pack Scepter™ Diol-HILIC-120*3 (150 mm × 4.6 mm I.D., 5 µm)	
Mobile phase	: A-1) Methanol	
	: A-2) Acetonitrile	
	: A-3) Water/Methanol=30:70	
	: B) THF (without stabilizer)	
Time program	: B. Conc. 0 % (0 min)→100 % (20-25 min) →0 % (25.01-30 min)	
Flow rate	: 1.0 mL/min	
Column temp.	: 40 ˚C	
Injection vol.	: 2 µL	
Vial	: SHIMADZU LabTotal for LC 1.5 mL, Glass*4	
Detection	: ELSD-LT III	
	Gain	: Wide
	Filter	: 40 ˚C
	Drift tube temp.	: 40 ˚C
	Nebulizer gas	: N_2
	Gas pressure	: 350 kPa

*1 P/N: 228-34937-91 *2 P/N: 228-55927-91
*3 P/N: 227-31051-05 *4 P/N: 227-34001-01



*Not eluted from the column under these conditions.

Fig. 3 Chromatograms of Mobile Phase/Column Investigation

Use of GPEC made it possible to separate polymers that could not be separated in the conventional GPC analysis, as separation in GPEC is based on differences in the polymer compositions. Considering separation and analysis time, the combination of methanol as mobile phase A and a Shim-pack VP-ODS as the column was judged to be the most suitable combination of mobile phase A and the column for the conditions investigated here.

• Investigation of Flow Rate and Gradient Conditions

Table 4 shows the analytical conditions examined in this investigation, and Fig. 4 shows the chromatograms of the mixed samples of the 3 polymers in Table 1. Here, 3 flow rate conditions and 3 time programs were considered. In the time programs, the initial concentration at the start of the gradient was changed without changing the gradient time. At the initial concentration of 40 %, the initially-eluted peaks were divided into two subpeaks regardless of the flow rate. This is thought to have occurred because part of the Copolymer (10:11) that elutes next after Homopolymer-B was not completely precipitated due to the excessive polymer solubility of the initial mobile phase solvent, resulting in early dissolution and elution. Table 5 shows the resolution between the peaks of Homopolymer-A and Copolymer (10:11). The resolution of Homopolymer-A showed its largest value at (1), and that value was no less than 1.5. Based on this result, the optimum analytical conditions for the copolymer and its constituent homopolymers in Table 1 are considered to be those of the chromatogram obtained under HPLC condition (1).

Table 4 HPLC Conditions

Column	: Shim-pack VP-ODS*1 (150 mm × 4.6 mm I.D., 5 µm)	
Mobile phase	: A) Methanol	
	: B) THF(without stabilizer)	
Time program	: 1) B. Conc. 0 % (0 min)→100 % (10-15 min) →0 % (15.01-30 min)	
	: 2) B. Conc. 20 % (0 min)→100 % (10-15 min) →20 % (15.01-30 min)	
	: 3) B. Conc. 40 % (0 min)→100 % (10-15 min) →40 % (15.01-30 min)	
Flow rate	: 1) 1.0 mL/min	
	2 1.2 mL/min	
	3) 1.4 mL/min	
Column temp.	: 40 ˚C	
Injection vol.	: 2 µL	
Vial	: SHIMADZU LabTotal for LC 1.5 mL, Glass*4	
Detection	: ELSD-LT III	
	Gain	: Wide
	Filter	: 40 ˚C
	Drift tube temp.	: 40 ˚C
	Nebulizer gas	: N_2
	Gas pressure	: 350 kPa

*1 P/N: 228-34937-91
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Table 5 Evaluation of Analytical Conditions

HPLC Conditions			Resolution (JP)
No.	Flow Rate	Time Program	
(1)	1	1	1.55
(2)	1	2	1.50
(3)	1	3	1.26
(4)	2	1	1.47
(5)	2	2	1.35
(6)	2	3	1.16
(7)	3	1	1.34
(8)	3	2	1.32
(9)	3	3	1.14

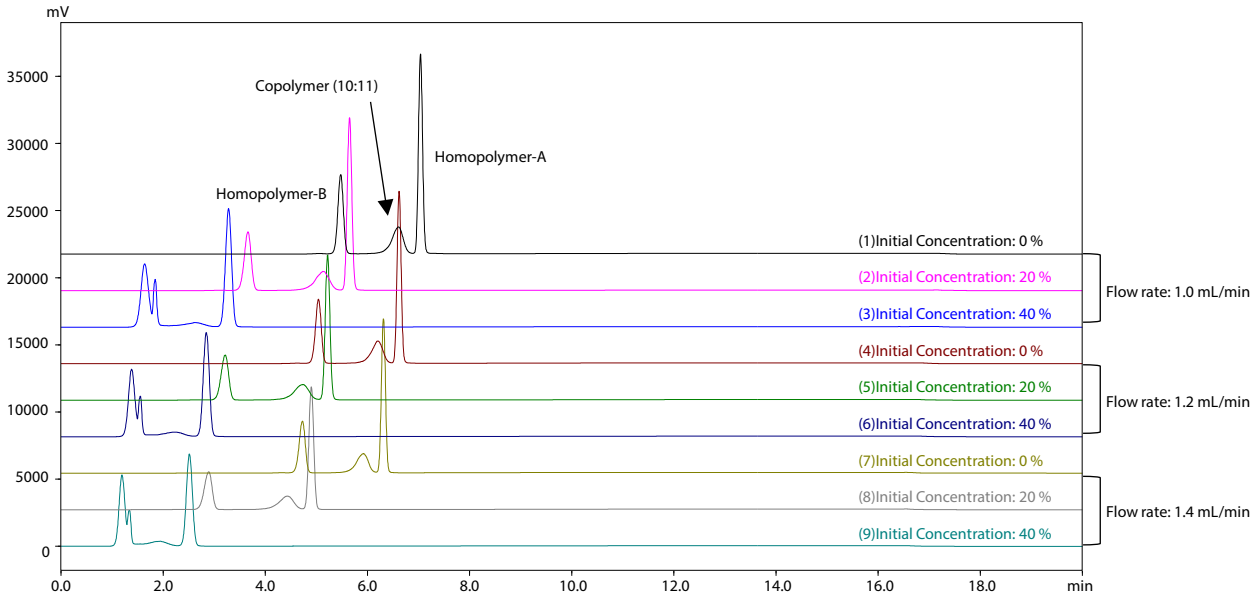

Fig. 4 Chromatograms of Flow Rate/Gradient Condition Investigation
(Elution time: 30 min)

■ Repeatability

Table 6 shows the analytical conditions obtained as a result of this investigation, and Fig. 5 shows the chromatogram obtained by analyzing a mixture of Copolymer (10:11) and Homopolymer-A and B using the conditions shown in Table 5. Table 7 shows the results of the verification of repeatability and resolution for 6 repeated analyses. The relative standard deviations (%RSD, n=6) for the retention times and areas of all compounds were no more than 0.12 and 2.25, respectively, indicating that good repeatability was obtained. Furthermore, the resolution of all polymer peaks was no less than 1.5, showing that the copolymer and its constituent homopolymers could be separated satisfactorily.

Table 6 HPLC Conditions

Column	: Shim-pack VP-ODS*1 (150 mm × 4.6 mm I.D., 5 µm)	
Mobile phase	: A) Methanol	
	: B) THF (without stabilizer)	
Time program	: B. Conc. 0 % (0 min)→100 % (10-15 min) →0 % (15.01-30 min)	
Flow rate	: 1) 1.0 mL/min	
Column temp.	: 40 ˚C	
Injection vol.	: 2 µL	
Vial	: SHIMADZU LabTotal for LC 1.5 mL, Glass*2	
Detection	: ELSD-LT III	
	Gain	: Wide
	Filter	: 40 ˚C
	Drift tube temp.	: 40 ˚C
	Nebulizer gas	: N_2
	Gas pressure	: 350 kPa

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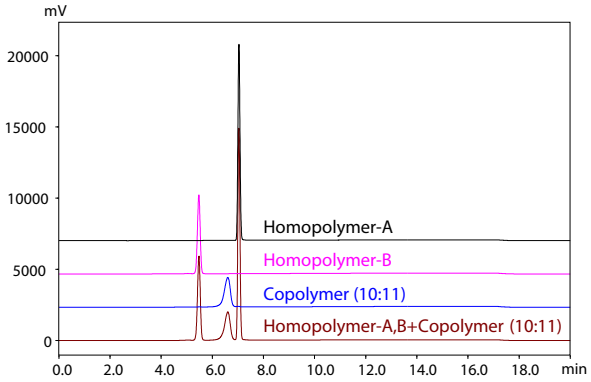

Fig. 5 Chromatograms of Mixture of Homopolymer-A and B and Copolymer (10:11) (Results of Investigation)

Table 7 Results of 6 Repeated Analyses

	Retention Time (%RSD)	Area (%RSD)	Resolution* (JP)
Homopolymer -B	0.02	1.22	--
Copolymer (10:11)	0.12	1.45	3.62
Homopolymer-A	0.01	2.25	1.54

* Average value of 6 repeated analyses.

■ Conclusion

High efficiency was achieved in an investigation of the conditions for polymer separation by GPEC using the Nexera Method Scouting System and its dedicated software Method Scouting Solution. The GPEC separation conditions were investigated for a total of 3 polymers, consisting of a copolymer and its 2 constituent homopolymers, which had similar molecular weights and could not be separated by GPC analysis, and satisfactory separation was obtained.

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